From: Cassandra Louis Duthil <clouisduthil@usaid.gov>
Sent: Thu, 19 Jan 2017 18:15:25 +0000
Subject: Re: Time Sensitive: PREDICT International Travel - GVP Beijing Update
To: Andrew Clements <aclements@usaid.gov>, Elizabeth Leasure <ealeasure@ucdavis.edu>
Cc: Alisa Pereira <apereira@usaid.gov>, Cara Chrisman <cchrisman@usaid.gov>, David John Wolking <djwolking@ucdavis.edu>, Jonna Mazet <jkmazet@ucdavis.edu>, Katherine Leasure <kaleasure@ucdavis.edu>

Hello Liz,

Yes, please proceed with travel arrangements. I only notify the mission of participant changes but they do not reapprove.

Travel is approved.

Best,

On Thu, Jan 19, 2017 at 12:52 PM Elizabeth Leasure <ealeasure@ucdavis.edu> wrote:

Hi Cassandra and Andrew. Just wanted to follow up on concurrence for this update to the Beijing meeting ITA, as we need to move forward with bookings for Danielle Anderson, or she may not be able to get her visa

in time due to office closures for the Chinese New Year. Would you mind following up on this or confirming that we can proceed?

Thanks,

Liz

Elizabeth Leasure

One Health Institute

University of California, Davis

530-754-9034 (office)

REDACTED

From: Andrew Clements [mailto:aclements@usaid.gov]

Sent: Friday, January 13, 2017 1:48 AM

To: Elizabeth Leasure

Cc: Alisa Pereira; Cassandra Louis Duthil; Cara Chrisman; David John Wolking; Jonna Mazet; Katherine Leasure

Subject: Re: PREDICT International Travel - GVP Beijing Update

Thanks, Liz.

Changes noted and travel approved subject to RDMA concurrence.

Andrew P. Clements, Ph.D.

Senior Scientific Adviser

Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

U.S. Agency for International Development

Mobile phone: REDACTED

Email: aclements@usaid.gov

On Jan 13, 2017, at 2:15 AM, Elizabeth Leasure <ealeasure@ucdavis.edu> wrote:

Hi Andrew. Please note that **Jaime Sepulveda** and **Nathan Wolfe** have cancelled their participation in the Beijing meeting due to scheduling conflicts.

I have also been advised of an update to the ITA of participant, Gian Luca Burci. He will depart from Geneva, Switzerland rather than Washington, DC, as that is where he is now based. Lastly, I have included a new ITA for Danielle Anderson,

who was recommended to the GVP Beijing meeting by another who was not able to attend (LinFa Wang; not included in original ITA).

1.

Burci (China): $1,700 airfare/$377 (Beijing) max daily per diem

2.

Anderson (China): $1,200 airfare/$377 (Beijing) max daily per diem

Travel requests:

1.

UC Davis would like to request approval for Gian Luca Burci to travel from

Geneva, Switzerland to Beijing, China from February 4-8, 2017 for a

Global Virome Project Working Group meeting to take place February 5-7, 2017.

Trip purpose: Mr. Burci is an invited participant of the Global Virome Project. The meeting will provide an opportunity for working groups to meet and collaborate on project strategics development. There will also be a

press event to announce the China National Virome Project.

2.

UC Davis would like to request approval for Danielle Anderso to travel from Singapore to

Beijing, China from February 4-8, 2017 for a Global Virome Project Working Group meeting to take place February 5-7, 2017.

Trip purpose: Ms. Anderson is an invited participant of the Global Virome Project. The meeting will provide an opportunity for working groups to meet and collaborate on project strategies development. There will also bc

a press event to announce the China National Virome Project.

Elizabeth Leasure

One Health Institute

University of California, Davis

530-754-9034 (office)

REDACTED

From:	Elizabeth Leasure <ealeasure@ucdavis.edu>
To:	PREDICTMGT <predictmgt@usaid.gov>
CC:	Jonna Mazet <jkmazet@ucdavis.edu>;David John Wolking <djwolking@ucdavis.edu>;Katherine Leasure <kaleasure@ucdavis.edu>
Sent:	2/17/2017 4:50:32 PM
Subject:	PREDICT International Travel Requests

Please find below international travel requests for your review and approval. Please let me know if you have any questions. Thanks!!

1. Euren (Sierra Leone): $1,797 airfare/$319 (Freetown) max daily per diem
2. Seck, Gomis (Rwanda): $700 airfare each/$294 (Kigali) max daily per diem

Travel requests:

1. Metabiota would like to request travel approval for Jason Euren, Research and Implementation Coordinator, to travel from San Francisco, California, USA to Freetown, Sierra Leone from March 12-25, 2017 to train behavioral staff on non-syndromic human surveillance activities.

Trip purpose: Jason Euren will be training behavioral staff on non-syndromic human surveillance activities. He will spend the first week working in Freetown. After conducting a 2-day refresher training, he will take the behavioral team to sites in and around Freetown to launch behavioral research in livestock settings. During the second week, Mr. Euren and the behavioral team will accompany the animal sampling team to Koinadugu and Kono to launch behavioral research in wildlife settings.

2. UC Davis would like to request travel approval for Mame Cheikh Seck and Jules Gomis, members of the PREDICT Senegal human surveillance team, to travel from Dakar, Senegal to Kigali, Rwanda from March 26-31, 2017 to attend fieldwork training with the PREDICT Rwanda human surveillance team.

Trip purpose: During the visit, Mame and Jules will attend a field sampling trip and be trained in PREDICT procedures for human surveillance, safe sampling, and data management under the mentorship of Dr. Julius Nziza. In addition, Mame will gain an insight into the roles and responsibilities of a human surveillance coordinator, including project management and stakeholder communication. Jules will be trained in project management.

From: William B. Karesh <karesh@ecohealthalliance.org>
To: PREDICTMGT <predictmgt@usaid.gov>;Predict inbox <predict@ucdavis.edu>
Sent: 8/7/2017 7:54:24 AM
Subject: [predict] Fwd: ECDC tool for the prioritisation of infectious disease threats

Begin forwarded message:

From: Preparedness <Preparedness@ecdc.europa.eu>
Subject: ECDC tool for the prioritisation of infectious disease threats
Date: August 7, 2017 at 4:04:52 AM EDT
To: Preparedness <Preparedness@ecdc.europa.eu>
Cc: Massimo Ciotti <Massimo.Ciotti@ecdc.europa.eu>, Jonathan Suk <Jonathan.Suk@ecdc.europa.eu>

Dear Colleagues,

We are pleased to announce that the ECDC tool for the prioritisation of infectious disease threats is now available at: https://ecdc.europa.eu/en/publications-data/ecdc-tool-prioritisation-infectious-disease-threats.

This qualitative tool, implemented as an Excel workbook, is based on multi-criteria decision analysis. It ranks infectious disease threats in a transparent, comparable and methodologically reproducible manner. The tool enables the relative ranking of different infectious disease threats. It is intended as a supplement to other methods that also support decision-making in preparedness planning.

Best wishes from the ECDC Preparedness Team

European Centre for Disease Prevention and Control (ECDC)
Postal address: Granits väg 8, 171 65 Solna, Sweden
Visiting address: Tomtebodavägen 11A, 171 65 Solna, Sweden
Phone +46 (0)8 58 60 10 00 / Fax +46 (0)8 58 60 10 01

From: Damien Joly <djoly@metabiota.com>
To: William B. Karesh <karesh@ecohealthalliance.org>;Jonna Mazet <jkmazet@ucdavis.edu>;David John Wolking <djwolking@ucdavis.edu>;Tracey Goldstein <tgoldstein@ucdavis.edu>
Sent: 11/15/2017 12:42:07 PM
Subject: Re: CBEP RFI for wildlife work in Cambodia, Laos, Vietnam

Thanks Billy. It seems the submission date has passed?

Damien Joly, PhD
Head, Data Research
Metabiota

Member, American College of Epidemiology
Assoc. Adjunct Professor · Dept. of Ecosystem and Public Health · Faculty of Vet. Med. · U. of Calgary
Information Management Coordinator · Emerging Pandemic Threats - PREDICT program

Unit 7, 1611 Bowen Road, Nanaimo BC V9S 1G5
djoly@metabiota.com · *tel +1 250 616 4961* · *skype* damienjoly
http://www.metabiota.com

From: William B. Karesh
Sent: November 15, 2017 11:00:50 AM
To: Jonna Mazet; David John Wolking; Tracey Goldstein; Damien Joly
Subject: CBEP RFI for wildlife work in Cambodia, Laos, Vietnam

From: Andrew Clements <aclements@usaid.gov>
To: Jonna Mazet <jkmazet@ucdavis.edu>;Jon Epstein <epstein@ecohealthalliance.org>;desmond@ecohealthalliance.org <desmond@ecohealthalliance.org>;bhbird@ucdavis.edu <bhbird@ucdavis.edu>
Sent: 2/22/2018 8:21:39 AM
Subject: WHO | Lassa Fever – Liberia

FYI

http://www.who.int/csr/don/22-february-2018-lassa-fever-liberia/en/

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

From:	Andrew Clements <aclements@usaid.gov>
To:	David J Wolking <djwolking@ucdavis.edu>
CC:	Christine Kreuder Johnson <ckjohnson@ucdavis.edu>;Jonna Mazet <jkmazet@ucdavis.edu>;PREDICTMGT <predictmgt@usaid.gov>
Sent:	1/9/2020 12:49:49 PM
Subject:	Re: Follow up (global analyses)

Thanks. Have a good trip, Chris.

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On Jan 9, 2020, at 8:02 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

Hi Andrew,

We're exploring this and options (also frantically prepping Chris for the close-out meetings in Nepal next week).

We'll get back to you this week with some ideas.

David

On Wed, Jan 8, 2020 at 5:45 AM Andrew Clements <aclements@usaid.gov> wrote:
Hi Chris,

Would sometime next week work for you all to discuss the previewing of the global analyses? If so, please suggest a day and time.

Thanks!

Andrew

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

From:	Peter Daszak <daszak@ecohealthalliance.org>
To:	Jonna Mazet <jkmazet@ucdavis.edu>;Andrew Clements <aclements@usaid.gov>
CC:	Christine Kreuder Johnson <ckjohnson@ucdavis.edu>;David J Wolking <djwolking@ucdavis.edu>;William B. Karesh <karesh@ecohealthalliance.org>;PREDICTMGT <predictmgt@usaid.gov>
Sent:	4/6/2020 8:22:36 PM
Subject:	RE: Trump ended coronavirus detection pandemic program - Los Angeles Times

Appreciate your email Andrew and I'll continue trying to get the facts out to reporters, including how this was a visionary program that USAID launched and funded for 10 yrs.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street
New York, NY 10001
USA

Tel.: +1-212-380-4474
Website: www.ecohealthalliance.org
Twitter: @PeterDaszak

EcoHealth Alliance develops science-based solutions to prevent pandemics and promote conservation

From: Jonna Mazet
Sent: Monday, April 6, 2020 6:31 PM
To: Andrew Clements
Cc: Christine Kreuder Johnson ; David J Wolking ; Peter Daszak ; William B. Karesh ; PREDICTMGT
Subject: Re: Trump ended coronavirus detection pandemic program - Los Angeles Times

Thanks, Andrew, appreciate your understanding and support during this tough time.
Stay well,
Jonna

On Mon, Apr 6, 2020 at 11:55 AM Andrew Clements <aclements@usaid.gov> wrote:

Thanks, Jonna and Peter. I really appreciate all of you continuing to be responsive to the media and trying to keep them on track which is a challenge when they have an agenda. I agree that it is not helpful when reporters are referred to USAID but don't get the information they are looking for.

Andrew

Andrew Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

On Fri, Apr 3, 2020 at 10:17 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Hi Andrew,
We are trying very hard to turn the slam pieces on the US government into positive ones, especially for USAID. We were able to help in the last two total really harsh pieces to fix a lot of the content and always send everyone to USAID for that question. That said, they pick the inflammatory headlines & push. I personally don't lament the end & both mention the new call & talk about all the positive thing Predict has done & is doing.

Maybe we should chat about how do this even better, but recognize that perfection is never possible.

Unfortunately, academic freedom at the University of California prevents me from being able to commit to not answering questions. It is also tough on us that when we refer people to USAID, the reporters are apparently not given much in the way of interviews, so it looks like we or others are hiding something from this side.

Let us know if you'd like to talk more,
Jonna

On Fri, Apr 3, 2020 at 8:08 AM Andrew Clements <aclements@usaid.gov> wrote:
Hi all,

Just read the following article:

https://www.latimes.com/science/story/2020-04-02/coronavirus-trump-pandemic-program-viruses-detection

Can I respectfully request that, from now on, all people associated with Predict resist from providing any quotes to media that have anything to do with the ending of Predict? So no public speculating on why it ended and no public lamenting that it's ended. Yes, it's unfortunate that the work will not continue, but I think the point has been made enough times now.

A more-helpful alternative (which some of you have used) is to talk about all the great things that were accomplished by Predict and how USAID was visionary in supporting this kind of work when no one else did and in the face of many critics. So more of "we had a great run for 10 years" and less of "it should have run for another 10 years.

As always, any questions about why Predict is ending or the future of the EPT program should be referred to USAID. As noted in the article, USAID is planning a new project to reduce spillover which is a natural transition of part of the portfolio. Unfortunately, many of the articles about Predict ending neglect to say there are other continuing and new investments under EPT.

Thanks for your understanding. Please let me know if you have any questions.

Andrew

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

Subject: Re: Travel arrangements
From: Carlos Morel <morel@cdts.fiocruz.br>
Sent: Thu, 12 Jan 2017 08:52:08 -0200
Cc: Dennis Carroll <dcarroll@usaid.gov>, Jonna Mazet <jkmazet@ucdavis.edu>
To: Katherine Leasure <kaleasure@ucdavis.edu>

Dear Katie,
Thanks for your email, attention and support.

The alternative I proposed yesterday - attending the PMAC meeting in Bangkok, Feb/3 - was some sort of last tentative not to miss the GVP meeting. If you approve an exception request for my travel on the same or similar itinerary as Renata, then I would not travel via Bangkok, would go directly to Beijing.

This Sunday I will travel to Geneva.

Best regards,

Carlos
--

Em 12 de jan de 2017, à(s) 01:34, Katherine Leasure <kaleasure@ucdavis.edu> escreveu:

Dear Carlos,

The reservation for Renata has been booked, and confirmation sent to her via email (both her Fiocruz and Gmail accounts). She will be traveling on the KLM itinerary as noted her email. We are happy to cover the additional nights of accommodation in Beijing in order to allow adequate time to rest and acclimate.

We are working on the exception request for your travel by business class, and will continue to make every effort to coordinate travel that will allow you to join and share your valuable contributions in Beijing.

Best regards,
Katie

From: Carlos Morel [mailto:[redacted]
Sent: Tuesday, January 10, 2017 12:57 PM
To: Dennis Carroll; Jonna Mazet
Cc: katherine Leasure; Renata Curi Hauegen; Carlos M Morel
Subject: Travel arrangements

Dcar Dcnnis an Jonna,

Thanks for your feedback to my message and also for your support.

From 1979 - when I first travelled to UCLA to start a collaborative project funded by Brazil's CNPq and US National Science Foundation - until 1998 when I was nominated Director TDR at WHO, I only flew economy, even during very long international travels. Either paid by Brazilian agencies of S&T or by WHO/TDR as a temporary adviser to TDR programs before becoming its Director. Even a travel that started in Geneva and then forced me to go to Thailand, Los Angeles, New York and back to Rio (yes, a round trip around the world) was in economy: I had to deliver a seminar in each city and I did not complain, as I was young enough and felt that as routine.

During my period as Director TDR or a member of the Board of Directors of the TB Alliance, they granted me business for long travels. Coming back to Brazil in 2004 one episode really had a big impact on me: the death of John La Montagne, deputy director of NIAID, while standing in line at the Mexico City airport (http://www.nytimes.com/2004/11/06/health/john-lamontagne-61-expert-on-development-of-vaccines-dies.html?_r=0). We were colleagues at the TB Alliance BoD, became colleagues and friends and John started

to collaborate with us at Fiocruz shaping a very big collaboration Fiocruz-NIH/NIAID. Once we invited him to come for a two day-meeting in Rio; one week before the meeting he called me to say that a new important meeting at NIAID forced him to shorten his travel to Rio to only ONE DAY - having dinner with us, participating at the meeting in the morning & early afternoon, going back to the Rio airport in the evening. I tried to convince him to cancel his trip, arguing that we could find another suitable date, etc.; no way. John was so committed to the collaboration that he came to the meeting, spending less than 24 hours in Rio. Some months later I got a phone call from Maria Freire, then the CEO of the TB Alliance, crying because of John's death in Mexico airport. John was unique as a colleague, friend and collaborator and was pushed to his limits traveling very long fights in economy, due to the NIH travel policy.

Summary of issues to consider:

- The fastest itinerary (Air France or KLM) Rio-Beijing takes **23 hours and 15 minutes** - with a cheaper option taking **27 hours**;
- I do hope that you can find at the UC Davis travel policy a way to allow business class when dealing with ***very*** long travels - at least for people of my age, 73;
- If UC Davis wants someone to arrive the same day of the meeting after traveling 23+ hours, they should be prepared to have zombies participating, sleeping during meeting hours or missing sessions due to exhaustion;
- Either flying economy or business, we (both Renata and myself) are not prepared to accept an itinerary that does not allow 1-2 days of rest after over 20 hours flight (we are prepared to cover the hotel expenses for these additional days);
- My MD colleagues as well as my private physician know well how active I am and therefore I will not ask them for a waiver due to medical reasons.

I really believe in the GVP project and really want to attend the Beijing meeting, but not at any cost. Renata has just forwarded to Katie a proposed itinerary and the information requested from her present regular passport (which expires next April). I propose the following: as soon as Renata receives her travel reservation and be able to apply for her travel license and service passport, we then move on this discussion, ok?

Best,

Carlos

From:	Jonna Mazet <jkmazet@ucdavis.edu>
To:	Jon Epstein <epstein@ecohealthalliance.org>;James Desmond <desmond@ecohealthalliance.org>
CC:	Peter Daszak <daszak@ecohealthalliance.org>;Billy Karesh <karesh@ecohealthalliance.org>;Tracey Goldstein <tgoldstein@ucdavis.edu>
Sent:	1/25/2017 4:18:43 PM
Subject:	Fwd: Jerry Garteh's Work at the FDA

FYI
J

---------- Forwarded message ----------
From: **Kendra Chittenden** <kchittenden@usaid.gov>
Date: Tue, Jan 24, 2017 at 10:07 AM
Subject: Fwd: Jerry Garteh's Work at the FDA
To: Alisa Pereira <apereira@usaid.gov>, Jonna Mazet <jkmazet@ucdavis.edu>

Just found the initial email chain

---------- Forwarded message ----------
From: **Kendra Chittenden** <kchittenden@usaid.gov>
Date: Mon, Oct 24, 2016 at 9:20 AM
Subject: Re: Jerry Garteh's Work at the FDA
To: Monica Dea <mdea@usaid.gov>

Thanks!

On Mon, Oct 24, 2016 at 9:14 AM, Monica Dea <mdea@usaid.gov> wrote:
Dear Kendra, please see the information from Adam regarding Jerry Garteh's employment by USAID US Forest Services.
thanks.
---------- Forwarded message ----------
From: **Keith Metzner** <kmetzner@usaid.gov>
Date: Wed, Oct 19, 2016 at 7:22 PM
Subject: Fwd: Jerry Garteh's Work at the FDA
To: Jennifer Tikka <jtikka@usaid.gov>
Cc: Leslie Flagg USAID <lflagg@usaid.gov>, Monica Dea <mdea@usaid.gov>

Jennifer,

You may wish to consult with Monica about how to proceed from here.

According to Monica Mr. Jerry Garteh has been working part-time with PREDICT since April or May of 2016 and has been the full-time PREDICT Liberia Country Coordinator since 1 Oct.

Something is strange here?

Keith

Keith Metzner

Natural Resources Officer
USAID LIBERIA
Office of Economic Growth
Embassy of the United States of America
502 Benson Street, Monrovia, Liberia

kmetzner@usaid.gov
Tel: (+231) 77- 677-7000 Ext. 7414
Mobile: (+231) 777-555-073

---------- Forwarded message ----------
From: **Welti, Adam J -FS** <adamjwelti@fs.fed.us>
Date: Wed, Oct 19, 2016 at 7:11 PM
Subject: RE: Jerry Garteh's Work at the FDA
To: Keith Metzner <kmetzner@usaid.gov>
Cc: Jennifer Tikka <jtikka@usaid.gov>, Leslie Flagg USAID <lflagg@usaid.gov>, "Sheridan, Kathleen A -FS" <kathleenasheridan@fs.fed.us>

Hi Keith,

Jerry is under contract with our office via METI and is full time. At this time, his contract runs through December 2016 but as we finalize our work plan for the coming months, our goal would be to extend his contract through September 2017 to support further activities. He and I are working on the draft work plan for this next phase and will submit for your review in the coming days. I am attaching here, prior documentation on the survey and follow on associated efforts (including report on 1st phase work plan as well as 2nd phase work plan).

Please let me know of any additional questions.

Kindly,

Adam Welti

Africa and Middle East Program

US Forest Service, International Programs

From: Keith Metzner [mailto:kmetzner@usaid.gov]
Sent: Wednesday, October 19, 2016 2:43 PM
To: Welti, Adam J -FS <adamjwelti@fs.fed.us>
Cc: Jennifer Tikka <jtikka@usaid.gov>; Leslie Flagg USAID <lflagg@usaid.gov>
Subject: Jerry Garteh's Work at the FDA

Hi Adam,

Can you please share something about Jerry Garteh's contract and his work at the FDA.

Is he part or full-time and for how long?

Thanks,

Keith

Keith Metzner

Natural Resources Officer

USAID | LIBERIA

Office of Economic Growth

Embassy of the United States of America

502 Benson Street, Monrovia, Liberia

kmetzner@usaid.gov

Tel: (+231) 77- 677-7000 Ext. 7414

Mobile: (+231) 777-555-073

+(231) 77 677 7000
+(231) 77 677 7226
+(231) 77 673 4677

mobile (703-209-5424) |KChittenden@usaid.gov

mobile (703-209-5424) |KChittenden@usaid.gov

From:	William B. Karesh <karesh@ecohealthalliance.org>
To:	Jonna Mazet <jkmazet@ucdavis.edu>
CC:	Jon Epstein <epstein@ecohealthalliance.org>;Jim Desmond <desmond@ecohealthalliance.org>;Peter Daszak <daszak@ecohealthalliance.org>;Tracey Goldstein <tgoldstein@ucdavis.edu>
Sent:	1/26/2017 6:55:32 PM
Subject:	Re: Jerry Garteh's Work at the FDA

Thanks.

Jon, the emails go back months but I can't see that they ever communicated their questions or concerns with us, nor do they mention that the US Forest Service Contract stipulates the Mr Garcia is free to work on other contractual agreements.

Let's discuss in person when you have chance.

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

+1.212.380.4463 (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Liaison - USAID Emerging Pandemic Threats - PREDICT 2 program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Jan 25, 2017, at 7:19 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

FYI
J

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Cc: Leslie Flagg USAID <lflagg@usaid.gov>, Monica Dea <mdea@usaid.gov>

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Keith Metzner

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To: Keith Metzner <kmetzner@usaid.gov>
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Adam Welti

Africa and Middle East Program

US Forest Service, International Programs

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Keith

Keith Metzner

Natural Resources Officer

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502 Benson Street, Monrovia, Liberia

kmetzner@usaid.gov

Tel: (+231) 77- 677-7000 Ext. 7414

Mobile: (+231) 777-555-073

AID

+(231) 77 677 7000
+(231) 77 677 7226
+(231) 77 673 4677

mobile (703-209-5424) |KChittenden@usaid.gov

mobile (703-209-5424) |KChittenden@usaid.gov

From: Andrew Clements <aclements@usaid.gov>
Sent: Tue, 21 Feb 2017 16:58:04 +0100
Subject: Fwd: CHN 21-02-17 OIE Alert - Alerta - Alerte - Highly pathogenic avian influenza - Influenza aviaire hautement pathogène - Influenza aviar altamente patógena
To: Gina Samaan REDACTED "ZHANG, Wenqing" REDACTED Jonna Mazet <jkmazet@ucdavis.edu>, William Karesh <Karesh@ecohealthalliance.org>, Christine Kreuder Johnson <ckjohnson@ucdavis.edu>, Peter Daszak <daszak@ecohealthalliance.org>

FYI. Highly pathogenic H7N9 detected in Guangdong province, China. Will be interesting to see if it's still localized or if it has already spread.

---------- Forwarded message ----------
From: <oie-info-web@oie.int>
Date: Tue, Feb 21, 2017 at 4:41 PM
Subject: CHN 21-02-17 OIE Alert - Alerta - Alerte - Highly pathogenic avian influenza - Influenza aviaire hautement pathogène - Influenza aviar altamente patógena
To: oie-info-web@oie.int

English PDF reports

Français Rapports PDF

Español informes PDF

Highly pathogenic avian influenza ,China (People's Rep. of)

Information received on 18/02/2017 from Dr Zhang Zhongqui, Director General , China Animal Disease Control Centre, Veterinary Bureau, Ministry of Agriculture, Beijing, China (People's Rep. of)

Summary

Report type	Immediate notification
Date of start of the event	10/01/2017
Date of confirmation of the event	10/01/2017
Report date	18/02/2017
Date submitted to OIE	18/02/2017
Reason for notification	Reoccurrence of a listed disease
Date of previous occurrence	2015
Manifestation of disease	Sub-clinical infection
Causal agent	Highly pathogenic avian influenza virus
Serotype	H7N9

Nature of diagnosis	Laboratory (advanced)
This event pertains to	a defined zone within the country

New outbreaks

Summary of outbreaks

Total outbreaks: 1

Outbreak Location

- GUANGDONG (Live bird markets)

Total animals affected

Species	*Susceptible*	*Cases*	*Deaths*	*Destroyed*	*Slaughtered*
Birds		**			

Outbreak statistics

Species	*Apparent morbidity rate*	*Apparent mortality rate*	*Apparent case fatality rate*	*Proportion susceptible animals lost**
Birds	**	**	**	**

* Removed from the susceptible population through death, destruction and/or slaughter;
** Not calculated because of missing information;

Epidemiology

Source of the outbreak(s) or origin of infection

- Unknown or inconclusive

Epidemiological comments

Samples from live bird markets sent to the National Reference Laboratory for Avian Influenza by the Guangdong veterinary authotities tested positive for a highly pathogenic avian influenza H7N9 virus. Affected live poultry markets have been closed and surveillance is strengthened in the whole province.

Control measures

Measures applied

- Movement control inside the country
- Screening
- Disinfection / Disinfestation
- Quarantine
- Vaccination permitted (if a vaccine exists)
- No treatment of affected animals

Measures to be applied

- No other measures

Diagnostic test results

Laboratory name and type	National Reference Laboratory for Avian Influenza (National laboratory)			
Tests and results	*Species*	*Test*	*Test date*	*Result*
	Birds	virus sequencing	10/01/2017	Positive

Future Reporting

The event is continuing. Weekly follow-up reports will be submitted.

Influenza aviaire hautement pathogène ,Chine (Rép. pop. de)

Information reçue le 18/02/2017 de Dr Zhang Zhongqui, Director General , China Animal Disease Control Centre, Veterinary Bureau, Ministry of Agriculture, Beijing, Chine (Rép. pop. de)

Résumé

Type de rapport	Notification immédiate
Date de début de l'événement	10/01/2017
Date de confirmation de l´événement	10/01/2017
Date du rapport	18/02/2017
Date d'envoi à l'OIE	18/02/2017
Raison de notification	Réapparition d'une maladie listée par l'OIE
Date de la précédente apparition de la maladie	2015
Manifestation de la maladie	Infection sub-clinique
Agent causal	Virus de l'influenza aviaire hautement pathogène
Sérotype	H7N9
Nature du diagnostic	Tests approfondis en laboratoire (i.e. virologie, microscopie électronique, biologie moléculaire, immunologie)
Cet événement	une zone définie à l'intérieur du pays

se rapporte à	

Nouveaux foyers

Récapitulatif des foyers

Nombre total de foyers : 1

Localisation du foyer

- GUANGDONG (Marchés d'oiseaux vivants)

Nombre total d'animaux atteints

Espèce(s)	*Sensibles*	*Cas*	*Morts*	*Détruits*	*Abattus*
Oiseaux		**			

Statistiques sur le foyer

Espèce(s)	*Taux de morbidité apparent*	*Taux de mortalité apparent*	*Taux de létalité apparent*	*Proportion d'animaux sensibles perdus**
Oiseaux	**	**	**	**

* Soustraits de la population sensible suite à la mort, à l´abattage et/ou à la destruction;
** Non calculé par manque de données;

Epidémiologie

Source du/des foyer(s) ou origine de l´infection

- Inconnue ou incertaine

Autres renseignements épidémiologiques / Commentaires

Des échantillons prélevés dans des marchés d'oiseaux vivants envoyés au Laboratoire national de référence pour l'influenza aviaire par les autorités vétérinaires de Guangdong se sont avérés positifs pour le virus H7N9 de l'influenza aviaire hautement pathogène. Les marchés d'oiseaux vivants concernés ont été fermés et la surveillance est renforcée dans toute la province.

Mesures de lutte

Mesures de lutte appliquées

- Restriction des déplacements à l'intérieur du pays
- Dépistage
- Désinfection / Désinfestation
- Quarantaine
- Vaccination autorisée (si un vaccin existe)
- Aucun traitement des animaux atteints

Mesures à appliquer

- Aucune autre mesure

Résultats des tests de diagnostics

Nom du laboratoire et

Laboratoire national de référence pour l'influenza aviaire (Laboratoire national)

type				
Tests et résultats	*Espèce(s)*	*Test*	*Date du test*	*Résultat*
	Oiseaux	séquençage viral	10/01/2017	Positif

Rapports futurs

Cet événement se poursuit. Des rapports de suivi hebdomadaires devront être envoyés.

Influenza aviar altamente patógena ,China (Rep. Pop. de)

Información recibida el 18/02/2017 desde Dr Zhang Zhongqui, Director General , China Animal Disease Control Centre, Veterinary Bureau, Ministry of Agriculture, Beijing, China (Rep. Pop. de)

Resumen

Tipo de informe	Notificación inmediata
Fecha del inicio del evento	10/01/2017
Fecha de confirmación del evento	10/01/2017
Fecha del informe	18/02/2017
Fecha de envio del informe a la OIE	18/02/2017
Motivo de la notificación	Reaparición de una enfermedad de la Lista de la OIE
Fecha de la anterior aparición de la enfermedad	2015
Manifestación de la enfermedad	Infección sub-clínica
Agente causal	Virus de la influenza aviar altamente patógena
Serotipo	H7N9
Naturaleza del diagnóstico	Pruebas de diagnóstico de laboratorio avanzadas (ej. virología, microscopía electrónica, biología molecular e inmunología)

Este evento concierne	una zona definida dentro del país

Nuevos focos

Resumen de los focos **Número total de focos: 1**

Localización del foco

- GUANGDONG (Mercados de aves vivas)

Número total de animales afectados

Especies	*Susceptibles*	*Casos*	*Muertos*	*Destruidos*	*Sacrificados*
Aves		**			

Estadística del foco

Especies	*Tasa de morbilidad aparente*	*Tasa de mortalidad aparente*	*Tasa de letalidad aparente*	*Proporción de animales susceptibles perdidos**
Aves	**	**	**	**

* Descontados de la población susceptible a raíz de su muerte, destrucción o sacrificio;
** No calculado por falta de datos;

Epidemiología

Fuente del o de los focos u origen de la infección

- Desconocida o no concluyente

Otros detalles epidemiológicos / comentarios

Muestras tomadas en los mercados de aves vivas enviadas al Laboratorio nacional de referencia para la influenza aviar por las autoridades veterinarias de Guangdong dieron positivo para el virus H7N9 de la influenza aviar altamente patógena. Los mercados de aves vivas afectados han sido cerrados y se ha reforzado la vigilancia en toda la provincia.

Medidas de Control

Medidas implementadas

- Restricción de los movimientos en el interior del país
- Tamizaje
- Desinfección / Desinfestación
- Cuarentena
- Vacunación autorizada (si existe vacuna)
- Ningún tratamiento de los animales afectados

Medidas para implementar

- Ninguna otra medida

Resultados de las pruebas diagnósticas

Nombre y tipo de Laboratorio nacional de referencia para la influenza aviar (Laboratorio nacional)

laboratorio				
Pruebas y resultados	*Especies*	*Prueba*	*Fecha de la prueba*	*Resultados*
	Aves	secuenciación viral	10/01/2017	Positivo

Informes futuros

El episodio continúa. Informes de seguimiento semanales serán enviados

--

Andrew Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

From:	William B. Karesh <karesh@ecohealthalliance.org>
To:	PREDICTMGT <predictmgt@usaid.gov>;predict@ucdavis.edu" <predict@ucdavis.edu>;Jonna Mazet <jkmazet@ucdavis.edu>;Peter Daszak <daszak@ecohealthalliance.org>;Eddy Rubin <erubin@metabiota.com>
CC:	Catherine Machalaba <machalaba@ecohealthalliance.org>
Sent:	2/28/2017 11:20:46 PM
Subject:	GHSA and IHR MEF

Hi Everyone,

Catherine Machalaba was able to gain some good engagement with WHO at the WHO Stakeholders Consultation on Planning Costing, and Financing for Accelerated IHR Implementation and Global Health Security in Geneva over the last two days.

The immediate action items are:

1) providing input on the JEE questions to inform the April revision (and learning more about the anticipated PVS revision). We will have an opportunity to have input on this and hopefully get more added related to environment and wildlife capabilities.

2) ensuring environment is included in the development of the costing guide (Catherine thinks here case was well received and WHO is interested if we can provide specific input when those discussions happen),

3) encouraging our country partners to participate in planned JEE missions and country planning workshops (several PREDICT and RESISSE and other project countries are in the pipeline for the next few months e.g.:

JEE
Tanzania (Zanzibar only) - March 20-24
Myanmar - May 3-9
Mongolia - May 12-19
Thailand - June 26-30

Work planning between now and June:

Liberia, Sierra Leone, Ethiopia, Bangladesh, Jordan.

We will get started on input for the JEE review and costing aspects and follow up with drafts soon.

BK

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

+1.212.380.4463 (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: Abel Ekiri <abekiri@ucdavis.edu>
To: "One Health (CDC)" <onehealth@cdc.gov>, "lkramer@usaid.gov" <lkramer@usaid.gov>, "[redacted]", "nkabir@usaid.gov" <nkabir@usaid.gov>, [redacted] "lparish@usaid.gov" <lparish@usaid.gov>, "Clements, Andrew (CDC usaid.gov)" <AClements@usaid.gov>, [redacted] gmwangoka@ihi.or.tz" <gmwangoka@ihi.or.tz>, David John Wolking <djwolking@ucdavis.edu>, Christopher kilonzo <ckilonzo@ucdavis.edu>, "predict@ucdavis.edu" <predict@ucdavis.edu>, "Clements, Andrew (CDC usaid.gov)" <AClements@usaid.gov>, "apereira@usaid.gov" <apereira@usaid.gov>, [redacted] "janetrix.amuguni@tufts.edu" <janetrix.amuguni@tufts.edu>, "irwego@umn.edu" <irwego@umn.edu>, "ayawe@ohcea.org" <ayawe@ohcea.org>, "pelicank@umn.edu" <pelicank@umn.edu>, "mcrane@usaid.gov" <mcrane@usaid.gov>, Jonna Mazet <jkmazet@ucdavis.edu>
Cc: "Barton Behravesh, Casey (CDC/OID/NCEZID)" <dlx9@cdc.gov>, "Eggers, Carrie (CDC/CGH/DGHP)" <xfy1@cdc.gov>, "Alroy, Karen (CDC/OID/NCIRD)" <nfu1@cdc.gov>, "Salyer, Stephanie J. (CDC/CGH/DGHP)" <wig9@cdc.gov>, "Gatei, Wangeci (CDC/CGH/DGHP)" <wgg3@cdc.gov>, Sarah Paige <spaige@usaid.gov>, "Ashna Kibria" <akibria@usaid.gov>, Ricardo Echalar <rechalar@usaid.gov>, "emwijarubi@usaid.gov" <emwijarubi@usaid.gov>, [redacted], "pswai@usaid.gov" <pswai@usaid.gov>, "jdavis@usaid.gov" <jdavis@usaid.gov>, "emily.s.kelley2.civ@mail.mil" <emily.s.kelley2.civ@mail.mil>, "Judd, Kelsie N CTR (US)" <kelsie.n.judd.ctr@mail.mil>
Subject: Re: Request by 3/3- Information on Zoonotic Disease Illnesses or Outbreaks in Tanzania: PREDICT Part 1
Sent: Fri, 3 Mar 2017 19:30:54 +0000
PREDICT data and resources.zip

Dear Kerri,

The PREDICT-Tanzania team has been involved in surveillance activities for emerging and unknown viral zoonotic pathogens in Tanzania over the last 7 years. We sharing two compressed files containing materials related to PREDICT surveillance activities and viral findings as well as select publications on what we consider to be critical zoonotic disease threats to Tanzania, including endemic diseases and transboundary threats from the greater East/Central Africa region and beyond. We would like to emphasize the importance of considering both known and emerging (including newly discovered or introduced) zoonotic diseases in the workshop and are proponents of broadening the discussion beyond specific pathogens to potentially include groups of dangerous zoonotic disease threats with similar signs and symptoms, for example viral hemorrhagic fevers.

We hope these resources will contribute to and inform the discussion at the upcoming OHZDP Workshop.

Contents of the two compressed folders are detailed below. Due to large file size, will send these in two parts (Part 1 & 2) so you can download them easily.

PREDICT Data and Resources folder:

1) The PREDICT-1 Tanzania Final Report along with a map and list of viral findings from the first phase of the project (2009-2014). This information is also publically available in an interactive platform at http://data.predict.global/

2) A selection of publications from PREDICT Consortium partners and from joint work conducted by the collaborative Sokoine University of Agriculture, Ifakara Health Institute, and UC Davis Health for Animals and Livelihood Improvement (HALI) project (haliproject.org). These publications were selected as they describe work on known and emerging zoonotic disease threats in Tanzania and the greater region.

Emerging viral threats and viral hemorrhagic fevers (VHFs) – East-Central Africa region folder:

A selection of publications demonstrating the risk of influenza and viral hemorrhagic fevers with particular emphasis on zoonotic disease threats from wildlife (e.g., Ebola, Marburg, CCHF, HPAI, RVF, and novel viral threats such as arenavirues and henipaviruses). These publications were selected as they provide evidence for these threats within Tanzania's borders and in the greater region.

Please don't hesitate to contact us if we can provide further information that may be useful for the workshop.

Sincerely,

Abel B. Ekiri, BVM, MS, PhD, DACVPM

Research Scientist

USAID PREDICT project

One Health Institute

University of California, Davis

aekiri@ucdavis.edu

From: One Health (CDC) <onehealth@cdc.gov>
Sent: Wednesday, February 22, 2017 7:29:04 AM
To: lkramer@usaid.gov; [redacted] nkabir@usaid.gov; [redacted] lparish@usaid.gov; Clements, Andrew (CDC usaid.gov); [redacted] gmwangoka@ihi.or.tz; David John Wolking; Abel Ekiri; Christopher kilonzo; predict@ucdavis.edu; Clements, Andrew (CDC usaid.gov); apereira@usaid.gov; [redacted] janetrix.amuguni@tufts.edu; irwego@umn.edu; ayawe@ohcea.org; pelicank@umn.edu; mcrane@usaid.gov
Cc: One Health (CDC); Barton Behravesh, Casey (CDC/OID/NCEZID); Eggers, Carrie (CDC/CGH/DGHP); Alroy, Karen (CDC/OID/NCIRD); Salyer, Stephanie J. (CDC/CGH/DGHP); Gatei, Wangeci (CDC/CGH/DGHP); Sarah Paige; Ashna Kibria; Ricardo Echalar; emwijarubi@usaid.gov; [redacted] pswai@usaid.gov; jdavis@usaid.gov; emily.s.kelley2.civ@mail.mil; Judd, Kelsie N CTR (US)
Subject: Request by 3/3- Information on Zoonotic Disease Illnesses or Outbreaks in Tanzania

Dear partners,

CDC and USAID are collaborating with the government of Tanzania to conduct a One Health Zoonotic Disease

Prioritization (OHZDP) workshop. CDC has developed a One Health Zoonotic Disease Prioritization Tool (OHZDPT) which allows a country to bring together multisectoral, One Health representatives to prioritize endemic and emerging zoonoses of greatest national concern using equal input from all represented sectors including human, animal (livestock and wildlife), and environmental health. Having a list of prioritized zoonoses allows a country to focus limited financial and personnel resources to build laboratory capacity, strengthen surveillance in humans and animals, develop joint outbreak response plans, and to create joint prevention and control strategies. Specific details are in the attachment titled, "CDC One Health Zoonotic Disease Prioritization Workshop Overview." The prioritization of zoonotic diseases is a key component of GHSA and GHSA roadmaps and helps to set priorities for further systems capacity building in line with GHSA and JEE. Tanzania's National One Health Platform is hosting the workshop.

Beginning about 60 days before the desired workshop date, trained CDC workshop facilitators work with in country partners to develop a list of endemic and emerging zoonoses of concern for prioritization during the workshop and to identify multisectoral partners, both voting members and observers, for participation in the workshop.

The OHZDP tool is semi-quantitative and relies on facilitators, in collaboration with stakeholders, generating a list of zoonoses in advance of workshop discussions. Before the stakeholders come together, developing a list of endemic and emerging zoonotic diseases that each ministry would like to discuss during the prioritization workshop is needed. This list is developed from both published and unpublished information sources. A team approach should be used to develop the list of about 30-40 endemic and emerging zoonotic diseases for prioritization during the workshop. The CDC One Health Office has started with a list of 32 endemic and emerging zoonotic diseases that have been identified either as reportable diseases in Tanzania's National One Health Strategy Plan, and/or were deemed important in the report of the expert group selected to prioritize zoonotic diseases in Tanzania, October-December 2016. Please find the list of 32 diseases attached.

CDC facilitators coordinate the creation of this list and literature review by working with in-country partners, including CDC and USAID staff. We would like to give partners the opportunity to share information and data on zoonotic diseases in Tanzania that may be beneficial to have during the workshop. We are asking partners to share any available information such as reports, publications, or other materials regarding zoonotic disease illnesses or outbreaks in Tanzania. If you have any additional information you'd like to share on any of the zoonoses on the existing list, or on zoonoses that are not listed, but are present in Tanzania, please let us know by **Friday, March 3rd. Send all information to onehealth@cdc.gov and CDC facilitators Karen Alroy (nfu1@cdc.gov) and Carrie Eggers (xfy1@cdc.gov).**

Thank you for your partnership in preparing for Tanzania's One Health Zoonotic Disease Prioritization Workshop. If you have any questions, please let us know.

Kerri Simone, MPH *on behalf of*

CDC One Health Office

cdc.gov/onehealth

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PLoS NEGLECTED TROPICAL DISEASES

Re-Emergence of Crimean-Congo Hemorrhagic Fever Virus in Central Africa

Gilda Grard[1]*, Jan Felix Drexler[2], Joseph Fair[3], Jean-Jacques Muyembe[4], Nathan D. Wolfe[3,5], Christian Drosten[2], Eric M. Leroy[1,6]

1 Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon, **2** Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, **3** Global Viral Forecasting, San Francisco, California, United States of America, **4** Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, **5** Department of Epidemiology, School of Public Health, University of California Los Angeles, Los Angeles, California, United States of America, **6** Institut de Recherche pour le Développement (IRD), UMR 224 (MIVEGEC), IRD/CNRS/UM1, Montpellier, France

Abstract

Background: Crimean-Congo hemorrhagic fever (CCHF) is a severe tick-borne disease well recognized through Europe and Asia where diagnostic tests and medical surveillance are available. However, it is largely neglected in Africa, especially in the tropical rainforest of Central Africa where only sporadic human cases have been reported and date back to more than 30 years. We describe here an isolated human case that occurred in the Democratic Republic of the Congo in 2008 and performed phylogenetic analysis to investigate whether it resulted from a regional re-emergence or from the introduction of a novel virus in the area.

Methods and Findings: Near complete segment S and partial segment M sequences were characterized. Bayesian phylogenetic analysis and datation were performed to investigate the relationship between this new strain and viral strains from Africa, Europe and Asia. The new strain is phylogenetically close to the previously described regional genotype (II) that appears to be specific to Central Africa. Phylogenetic discrepancy between segment S and M suggested genetic exchange among local sublineages, which was dated back to 130–590 years before present.

Conclusions: The phylogenetic analyses presented here suggest ongoing CCHF virus circulation in Central Africa for a long time despite the absence of reported human cases. Many infections have most probably been overlooked, due to the weakness of healthcare structures and the absence of available diagnostic procedure. However, despite the lack of accurate ecological data, the sporadic reporting of human cases could also be partly associated with a specific sylvatic cycle in Central Africa where deforestation may raise the risks of re-emergence. For these reasons, together with the high risk of nosocomial transmission, public health authorities' attention should be drawn to this etiological agent.

Citation: Grard G, Drexler JF, Fair J, Muyembe J-J, Wolfe ND, et al. (2011) Re-Emergence of Crimean-Congo Hemorrhagic Fever Virus in Central Africa. PLoS Negl Trop Dis 5(10): e1350. doi:10.1371/journal.pntd.0001350

Editor: A. Desiree LaBeaud, Children's Hospital Oakland Research Institute, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: gildagrard@gmail.com

Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV, family *Bunyaviridae*, genus *Nairovirus*) is a tick-borne virus. It causes severe illness throughout Africa, Asia, Southeast Europe and the Middle East, with case fatality rates ranging from 3% to 30%. Its worldwide distribution closely matches that of its main arthropod vector, ixodid ticks belonging to the genus *Hyalomma*. Human infection occurs through tick bites, contact with infected livestock, or nosocomial transmission. The CCHFV negative-stranded RNA genome is divided into a small (S), medium (M) and large (L) segment.

Previous phylogenetic analysis of the S segment clustered strains into 6 to 7 distinct phylogeographic groups: West Africa in group I, Central Africa (Uganda and Democratic Republic of Congo (DRC)) in group II, South Africa and West Africa in group III, Middle East and Asia (that may be split into 2 distinct groups Asia 1 and Asia 2 [4]) in group IV, Europe and Turkey in group V, and finally Greece in group VI [1–5]. However, some of these phylogenetic lineages include strains separated by large spatial distances (such as South Africa and West Africa) suggesting viral migration, most likely via migratory birds transporting infected ticks, or secondary introductions following importation of commercial livestock. Comparative phylogenetic analysis revealed, with a few exceptions, parallel clustering of the S and L segments, while M segment reassortment seems more frequent [1,4–6].

During the last 60 years, CCHFV outbreaks have been described in Asia, the Middle East and the Balkans, where the virus has become endemic and caused several thousand human cases. During the last decade, CCHFV has caused human disease

Author Summary

Crimean-Congo hemorrhagic fever virus (CCHFV) is transmitted to humans through tick-bite or contact with infected blood or tissues from livestock, the main vertebrate hosts in a peri-domestic natural cycle. With numerous outbreaks, a high case fatality rate (3%–30%) and a high risk for nosocomial transmission, CCHFV became a public health concern in Europe and Asia. However virus surveillance in Africa is difficult due to the limited sanitary facilities. Especially, CCHFV occurrence in Central Africa is very poorly described and seems highly in contrast with the temperate to dry environments to which the virus is usually associated with. We described a single human infection that occurred in Democratic Republic of the Congo after nearly 50 years of absence. The phylogenetic analysis suggests that CCHFV enzootic circulation in the area is still ongoing despite the absence of notification, and thus reinforces the need for the medical workers and authorities to be aware of the outbreak risk. The source of infection seemed associated with a forest environment while no link with the usual agro-pastoral risk factors could be identified. More accurate ecological data about CCHFV enzootic cycle are required to assess the risk of emergence in developing countries subjected to deforestation.

in previously unaffected countries (Turkey 2002, Iran 2003, Greece 2008, Georgia 2009) and has re-emerged in countries located southwest of the Russian Federation after an absence of nearly 30 years [7]. By contrast, fewer than 100 cases have been recorded in Africa [8], most of them in South Africa [9,10]. In East and West Africa, enzootic CCHFV circulation has been shown by serological surveys of cattle and virus isolation from ticks since the 1970s [11,12] but until the outbreaks in Mauritania in 2004 [13] and Sudan in 2008 [14], only sporadic human cases had been reported. In Central African Republic (CAR), limited serological evidences of CCHFV circulation in Zebu cattle has been provided [15] and three viral strains were isolated from ticks between 1973 and 1976, one of which lead to accidental infection of a laboratory worker [11]. Subsequent isolations from ticks were performed in the 80's [16] but no human case was reported. Despite the early identification of human CCHFV infection in DRC (Kisangani, 1956), CCHFV occurrence in Central Africa has not been much described and only sporadic human cases have been reported. One month after having isolated the first CCHFV strain (strain Congo 3011) in newborn mice, Dr. Courtois became infected and this was the last notified case in DRC, from which the strain Congo3010 was isolated [17,18]. The virus was next identified in Uganda between 1958 and 1978. Fifteen CCHFV strains were isolated from febrile patients, of which nearly half were laboratory workers having handled infectious samples [11,17,18]. From the geographic information associated with the other patients, it can be inferred that CCHFV was present both in the Entebbe area and in the Arua district (previous West Nile district) located 350 km North, near the border of Sudan. Three CCHFV strains were also isolated from ticks and an early serological survey suggested cattle infection [11]. No other epidemiological or ecological information is available on CCHFV in Central Africa or its borders, and no further cases have been recorded.

In 2008, CIRMF (Centre International de Recherches Médicales de Franceville, Gabon) identified CCHFV in a serum sample received for etiological diagnosis of a case of hemorrhagic fever in DRC. This is the only identification of CCHV in DRC for more than 50 years. To determine whether it was due to introduction of a novel virus or to re-emergence of a local genotype, we determined the phylogenetic relationships between this virus (hereafter referred to as Beruwe-2008) and previously described isolates. Phylogenetic analysis showed that the Beruwe-2008 strain belonged to the genotype previously identified in this area and thus suggested that it had re-emerged. Local CCHFV persistence may have been supported by a sylvatic natural cycle specific to Central Africa, indicating that countries subject to major deforestation may note an increasing number of human infections.

Methods

Ethics statement

Laboratory investigations were performed subsequently to the WHO request for surveillance and early alert of hemorrhagic fever outbreak in Central Africa. Because of the emergency settings associated with the suspicion of such acute illnesses, no ethics committee approval or written consent was deemed necessary. The blood sample was taken by national healthcare workers of the Lubutu hospital where the patient came for medical care. He was informed that his blood sample will be further used for diagnostic investigation and gave his verbal consent. The patient described here is anonymous. The blood sample was next sent to the Institut National de Recherche Biomédicale (Kinshasa, DRC) and then to CIRMF upon WHO authorities. The study was approved by the scientific committee of CIRMF.

Case description

The patient was a 26-year-old man living in Beruwe (Nord Kivu province) in DRC, 325 km from Kisangani (Figure 1). He became ill in the mining area where he worked. He complained of fever and headache on day 1 and developed moderate bloody diarrhea on day 2. Epistaxis, oral bleeding and hematuria occurred on day 3. He treated himself with ibuprofen and paracetamol during the first three days. On day 4 after onset he additionally took quinine and finally presented with severe asthenia and persistent bleeding to Lubutu hospital, where the serum sample was taken. At this stage the patient was subicteric, with bleeding at the venipuncture site, but had only low-grade fever (37.6°C). He declared no contact with wild animals during the previous three weeks but he had slept in the forest. No further information on his outcome was available.

Molecular diagnosis

The patient's serum was manipulated in biosafety level 4 (BSL-4) conditions. The serum was first tested for Ebola and Marburg viruses. As results were negative, investigations were next performed for CCHFV. RNA was extracted with the QIAamp viral RNA mini kit (Qiagen, Courtaboeuf, France) according to manufacturer's instructions. Reverse transcription (RT) and real-time PCR amplification were performed with the High Capacity cDNA RT kit and Taqman universal PCR master mix (Applied Biosystems - Life Technologies Corporation, Carlsbad, California), and previously reported primers and probes [19]. Conventional one-step RT-PCR was performed with CCHFV primers as previously reported [20] and with SuperScript III one-step RT-PCR system and Platinum Taq DNA polymerase (Invitrogen -Life Technologies Corporation, Carlsbad, California). This yielded a 536-nucleotide fragment in the S segment, sequencing of which confirmed CCHFV identification.

Genetic characterization

As viral isolation on Vero cells was unsuccessful, viral RNA was extracted from the patient's serum as described above, and was used for RT-PCR amplification with Platinum Taq DNA

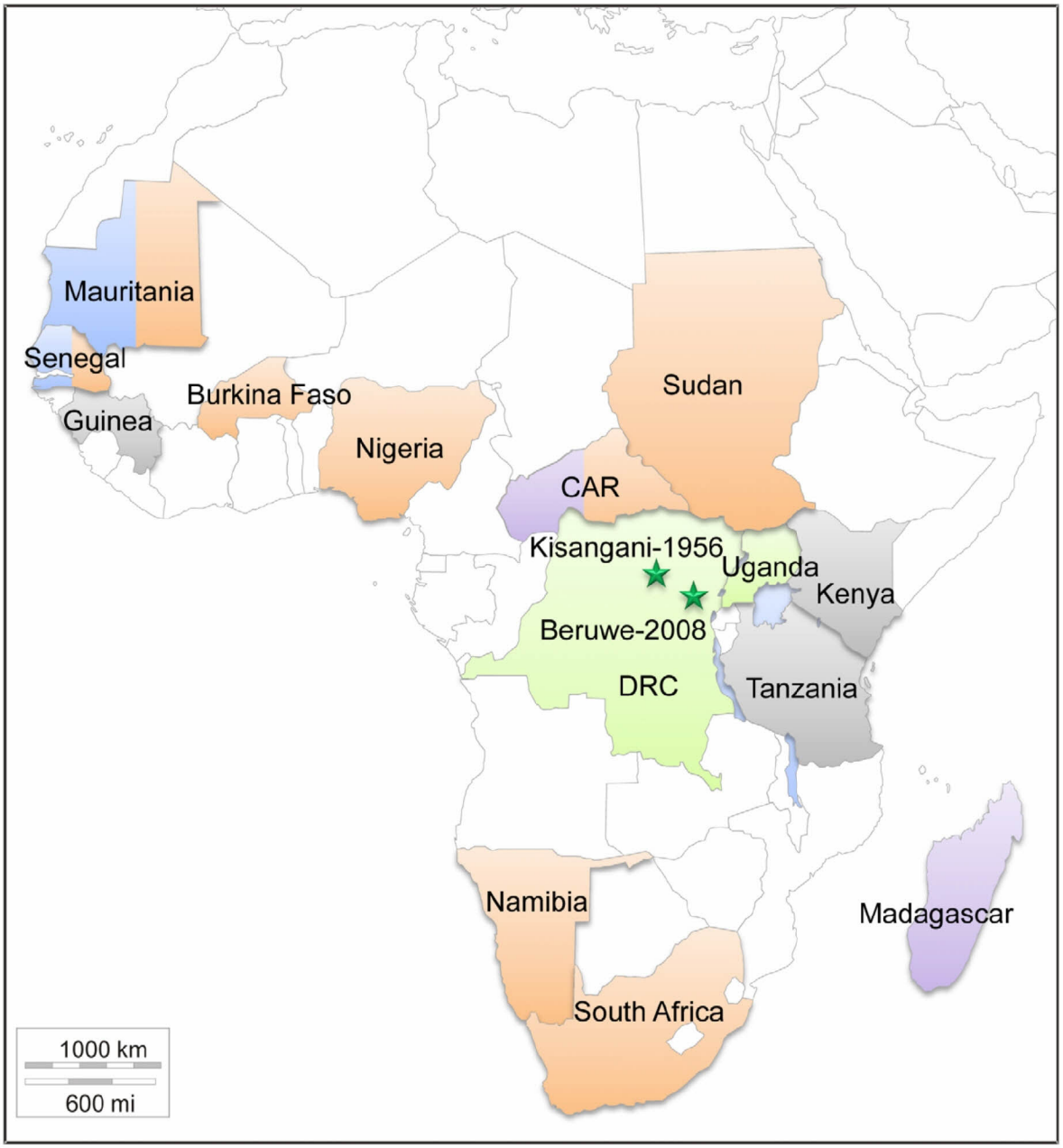

Figure 1. Geographic distribution of CCHFV in Africa. Genotypes I, II, III and IV are indicated in blue, green, orange and mauve, respectively. Undetermined genotypes are in grey. Stars represent human infections diagnosed in Democratic Republic of the Congo (DRC). CAR: Central African Republic.
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polymerase (Invitrogen). Primers were derived from nucleotide alignments (Table 1). Three overlapping PCR products allowed near-complete characterization of the S segment coding sequence (GenBank accession number HQ849545) and partial characterization of the M segment (GenBank accession number HQ849546). Amplification of the L segment was unsuccessful, being limited by the sample quantity.

Phylogenetic analysis

A total of 44 complete sequences for segment S and 38 complete sequences for segment M were retrieved from GenBank (Table S1 in online supporting information). Nucleotides were aligned according to the amino-acid profile using the Translation Align algorithm implemented in Geneious software [21]. Initial phylogenetic analyses were performed with MrBayes V3.1 [22,23] using a GTR+gamma+invariant site substitution model for 4 million MCMC chain iterations sampled every 100 generations, corresponding to 40 000 trees (data not shown). Following confirmation of the tree topology from MrBayes, the tip-dated coding alignments were submitted to Bayesian inference of node ages by using BEAST V1.4.7 [24] under the assumption of a codon-based substitution model (SRD06) and an uncorrelated

Table 1. Primers used for CCHFV genetic characterization of the S and M segments.

Segment S		
Fragment 1 (590 bp)		
1st round	CCHF-MU: TCTCAAAGATATCGTTGCC	CCHF-ER7: GAATTAGGGAAGCAACCAAG
Fragment 2 (550 bp)		
1st round	CCHF-F2b: AAAGAGATGTTGTCAGACATGAT	CCHF-R2b: GTTTCTTTCCCCACTTCATTGG
2nd round	CCHF-F3b: GAAGAAGGAACTTGATCCTCAA	CCHF-R2b
Fragment 3 (536 bp) [19]		
1st round	CCHF-F2: TGGACACCTTCACAAACTC	CCHF-R3: GACAAATTCCCTGCACCA
Segment M		
Fragment 1 (280 bp)		
1st round	CCHF-M1F: AATGCAATAGATGCTGAAATGCA	CCHF-M2R: TTGYTTGCYTC1AYRGTYGC
2nd round	CCHF-M1F	CCHF-M1R: GAYTGRACTGG1GAYAWYGAAAC
Fragment 2 (420 bp)		
1st round	CCHF-M2F: CAAGTRTCRGAGTCAACRGG	CCHF-M2R: TTGYTTGCYTC1AYRGTYGC
2nd round	CCHF-M3F: TGGCTCTRAAGAGRAGYTGYTGGATRA	CCHF-M3R: TTRCARACRGCYAGCATRACATT
Fragment 3 (510 bp)		
1st round	CCHF-M4F: GAGTC1CAYAAATGCTAYTGYAGTCT	CCHF-M4R: ACTGAACTCCAGCTAAGTGCTA
2nd round	CCHF-M4F	CCHF-M5R: GTTGAYTGRACATTRATTGCYCCCCA

Sequences are reported in 5′-3′ orientation.
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relaxed lognormal molecular clock and expansion, exponential and constant population growth models. The Expansion model yielded the best results, as indicated by ESS statistics and Bayes factor analysis of the posterior probability trace in TRACER. Sixty million generations were sampled every 1000 states, corresponding to 60 000 trees, that were annotated with TreeAnnotator and visualized with FigTree V1.3.1 from the BEAST package.

Results and Discussion

In 2008 we received a serum sample for etiological diagnosis of a case of hemorrhagic fever in DRC. The patient' serum was handled under BSL-4 facilities for RNA purification and tested positive for CCHFV by real-time PCR and conventional amplification with previously described detection systems [19,20]. The patient became ill in Beruwe, approximately 325 km from Kisangani, where the only 2 previously reported cases of CCHFV in DRC occurred in 1956 (Figure 1). The patient worked in a mining area near a forest environment and didn't seem linked to agro pastoral activities. As this was the only identified case of CCHV in DRC for more than 50 years, we performed a phylogenetic analysis to determine whether it was due to introduction of a novel virus or re-emergence of a local genotype.

Virus isolation in Vero cells was unsuccessful, presumably owing to virus degradation subsequently to difficulties and delays of transportation. Genetic characterization was thus based on RT-PCR of RNA extracted from the patient's serum. As reassortment usually affects the M segment, priority was given to sequencing segments S and M, while segment L amplification was limited by sample quantity and was unsuccessful. Near-complete characterization of the segment S coding sequence was achieved, yielding 1501 contiguous nucleotides; the 5′ end was missing, presumably owing to RNA degradation. A 1001-nucleotide fragment was generated for segment M, corresponding to nucleotide positions 2382 to 3380 of the Congo3010-1956 glycoprotein coding sequence (DRC strain).

Pairwise nucleotide comparison of the Beruwe-2008 segment S sequence with those of the most closely related strains Congo3010-1956 (DRC) and Semunya-1958 (Uganda) – showed 92.4% and 92.0% similarity, respectively. In segment M the pairwise identities were 96.1% and 93.8% respectively. Identity between the Beruwe-2008 strain and strains belonging to other genetic groups ranged from 82.2% to 87.6% in segment S and from 72.5% to 81.3% in segment M (Table 2).

Bayesian phylogenetic analysis with a molecular clock assumption was applied to segment S (Figure 2A) and M (Figure 2B) datasets. Both methods yielded tree topologies largely matching the phylogeographic groups previously defined from complete segments S and M [1–3]. In both segments, and with posterior probabilities reaching 1, the Beruwe-2008 sequence grouped with the aforementioned DRC and Uganda strains forming lineage II

Table 2. P-distances between the Beruwe-2008 sequences and other sequences included in the phylogenetic analysis.

	P-distance range (%)	
	Segment S	**Segment M**
Group 1: West Africa	[16.1–16.4]	[18.7–19.5]
Group 2: Central Africa	[7.6–8]	[3.9–6.2]
Group 3: South & West Africa	[14.1–15]	[24.6–27]*
Group 4: Asia-Middle East	[12.4–14.8]	
Group 5: Europe-Turkey	[13.2–13.8]	[24.4–25.1]
Group 6: Greece	[17.8]	[27.5]

*In segment M, phylogeographic groups III and IV are combined and the reported p-distances include both groups.
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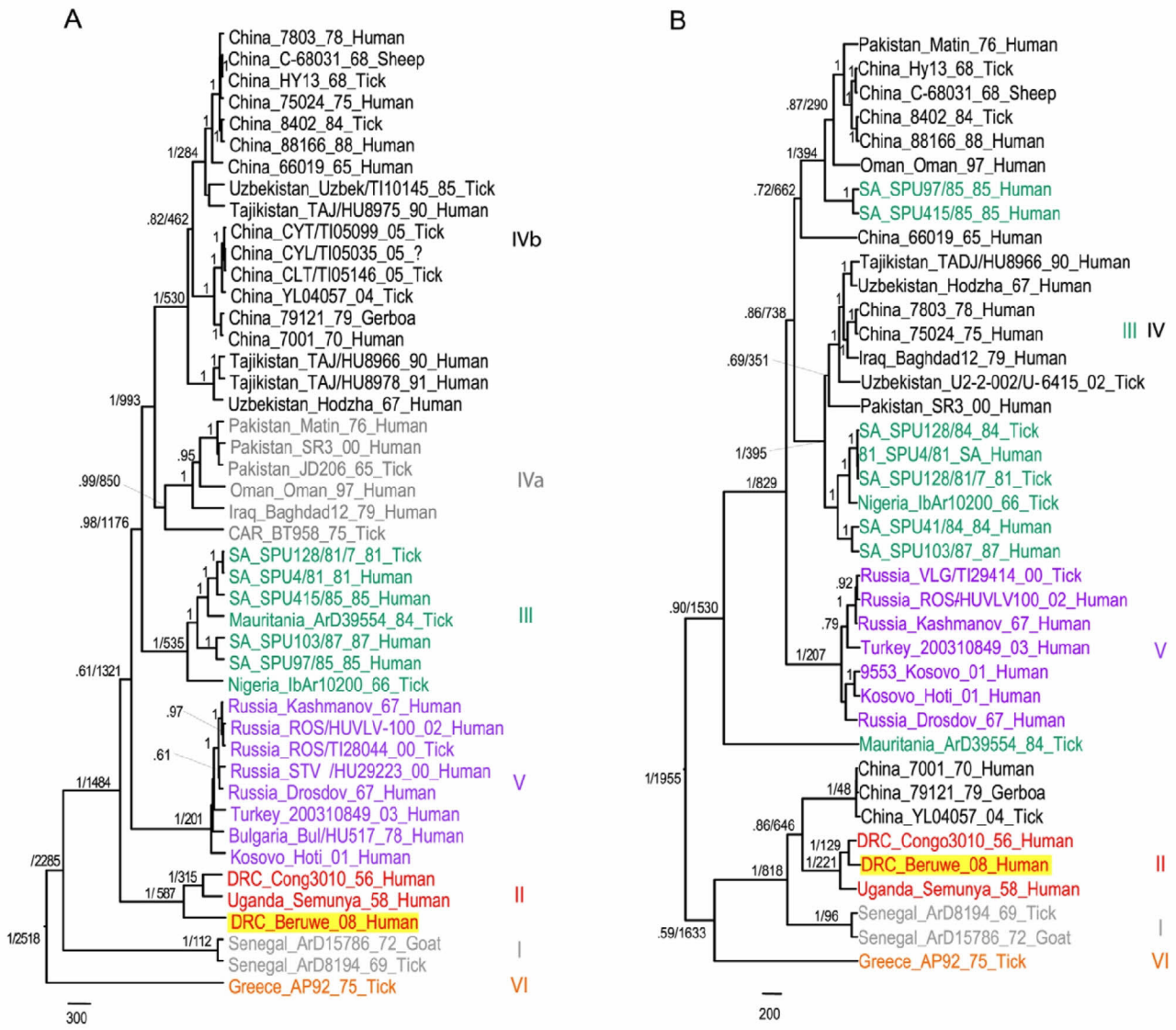

Figure 2. Phylogenetic analysis of CCHFV in segments S and M. Caption A: analysis performed on 1501 nucleotides of segment S (near complete sequence). Caption B: analysis performed on partial segment M sequence, 1001 nucleotides long. Genotypes are indicated in roman numerals, named according to Caroll *et al.* [2] with the equivalent group nomenclature according to Chamberlin et al. [4] indicated in brackets: I – West Africa (Africa 1); II – Central Africa (Africa 2); III – South and West Africa (Africa 3); IV – Middle East/Asia, divided into groups IVa and IVb respectively corresponding to groups Asia 1 and Asia 2 acoording to [4]; V – Europe/Turkey (Europe 1); VI- Greece (Europe 2). The sequence from this study is highlighted in gold. Sequences are named as follows: country_strain_sampling year_host. Posterior probabilities are indicated above branches, followed by node age estimations. For clarity, posterior probability values below 0.6 and date estimates for the terminal branches were removed. The scale bar corresponds to evolutionary distance in years.
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(Central Africa group). Although we cannot rule out the possibility of segment L reassortment, the Beruwe-2008 strain most likely belongs to the genotype previously identified in Central Africa, thus representing viral re-emergence rather than introduction of another genotype. In addition, the phylogenetic position of the Beruwe-2008 strain inside this Central African clade differed between the two segments, lying at the most ancestral branch in segment S while sharing a more recent common ancestry with the Congo3010-1956 strain from Kisangani in segment M. This is highly suggestive of intra-genotypic reassortment, thus implying co-circulation of these two DRC sub-lineages at this time. However, though it may be less probable, we cannot exclude definitively recombination between the two strains.

Our dating analysis of the S segment resulted in time estimations slightly more recent than previously reported, but nonetheless within the same range and in keeping with an ancient origin of CCHF viruses [2]. The MRCA (most recent common ancestor) for the whole CCHFV species was estimated to have arisen 2518 years before present (BP) (95% High Posterior Density (HPD): 820-5281), the lineage II split-off was dated to 1484 years BP (95% HPD: 583-3389) and the MRCA of the three Central African strains was estimated at 587 years BP (95% HPD: 200-1327). In the M segment, the MRCA estimates were slightly more recent, most probably owing to the use of partial rather than complete coding sequences and to different evolution of the two genes. This resulted in MRCA estimates of 1955 years BP for the

whole species (95%HPD: 886-3844), 221 years BP for the three Central African strains (95%HPD: 114-407) and 129 years BP (95% HPD: 75-228) for the two DRC strains. The genotype II split-off was estimated to have occurred 646 years BP, but the differences in the tree topologies prevented a true node age comparison with segment S.

CCHV genotype II has been identified only in DRC and Uganda, while different CCHV lineages have been identified in neighboring countries to the north. Multiple genotypes have been identified in CAR, belonging to groups IV and III [2,20], the latter also being encountered in Sudan [14]. By contrast no other genotype has been identified in Central Africa, for which reports on CCHFV are scarce and date back to 30 years. Hence, the data currently available suggest that genotype II is specific to central Africa. In DRC, CCHV has been reported only once, 50 years ago, but our data strongly suggest that the same genotype is still actively circulating.

Of note, the MRCA estimates presented here are in agreement with ancient divergence of this lineage (around 1000 years ago), but whether or not this split-off was linked to virus adaptation to Central Africa cannot be assessed. However, as the MRCA of the three strains was dated back to 683 to 243 years BP (Figure 2A and B respectively), one might reasonably assume that the association of genotype II with this area goes back to this time period and thus did not result from very recent introduction. In addition, the co-circulation of different sub-lineages supports the possibility that ongoing CCHFV circulation occurred in the same area for some time. However, as the reassortment event would have taken place approximately 120 years BP, there is no evidence that CCHFV has been permanently circulating inside the Beruwe microhabitat, and we cannot exclude the possibility that this virus was very recently (re)introduced.

In addition to the CCHFV genotypic specificity for Central Africa, its occurrence in the tropical rainforest contrasts strongly with the ecological characteristics of other areas in which CCHFV has been isolated [11]. Indeed, the enzootic distribution of CCHFV mostly coincides with temperate to dry or semi-dry climates in the forests, steppes and savannahs of Eurasia and West, East and South Africa. In these environments, domestic animals and their associated ticks are major agents of rural enzootic cycles affecting nearby human populations [11,25]. Despite the lack of accurate ecological data, the occurrence of CCHFV in Central Africa and its apparent genotypic specificity may suggest a distinctive sylvatic natural cycle in the deep tropical forest characterized by high rainfall, specific wildlife species, and a low density of domestic animals. Interestingly, co-speciation or long-term association with specific tick species has been previously suggested to explain the geographical distribution of CCHV genetic variants in Russia and Central Asia [26]. Such a sylvatic cycle, involving specific vectors and hosts with few contacts with human populations, could partly explain the lack of outbreaks and the sporadic nature of recorded human cases. In addition, as CCHFV is known to have been present in Central Africa for decades, and as human populations often live in isolated villages, many human infections may have been overlooked. However increasing invasion and destruction of rainforest habitats may lead to a higher risk of human CCHFV cases in future.

Hence, despite 30 years without a single reported case, the data presented here suggest that CCHFV continues to circulate in Central Africa. More information on the epidemiology and the natural cycle of CCHFV in this ecosystem is required to assess its potential for emergence, notably in Gabon and Republic of the Congo. However health authorities and medical staff should be aware of the possibility of viral (re)emergence and of the high risk of nosocomial transmission.

Supporting Information

Table S1 GenBank accession numbers for the sequences used in this study. Countries, strains, date of sampling and hosts are reported along with the associated GenBank accession numbers for segment S and segment M.
(DOC)

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Author Contributions

Conceived and designed the experiments: GG EML. Performed the experiments: GG. Analyzed the data: GG JFD CD EML. Contributed reagents/materials/analysis tools: J-JM JFD CD. Wrote the paper: GG EML JFD CD JF NDW.

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A Novel Rhabdovirus Associated with Acute Hemorrhagic Fever in Central Africa

Gilda Grard[1,2☯], Joseph N. Fair[3☯], Deanna Lee[4,5☯], Elizabeth Slikas[6], Imke Steffen[6], Jean-Jacques Muyembe[7], Taylor Sittler[4,5], Narayanan Veeraraghavan[4,5], J. Graham Ruby[8,9], Chunlin Wang[10], Maria Makuwa[7], Prime Mulembakani[7], Robert B. Tesh[11], Jonna Mazet[12], Anne W. Rimoin[13], Travis Taylor[3], Bradley S. Schneider[3], Graham Simmons[6], Eric Delwart[6], Nathan D. Wolfe[3], Charles Y. Chiu[4,5,14]*, Eric M. Leroy[1,2]*

1 Viral Emergent Diseases unit, Centre International de Recherches Médicales de Franceville, Franceville, Gabon, **2** MIVEGEC, UMR (IRD 224 - CNRS 5290 - UM1 - UM2), Institut de Recherche pour le Développement, Montpellier, France, **3** Global Viral Forecasting, Incorporated, San Francisco, California, United States of America, **4** Department of Laboratory Medicine, University of California, San Francisco, California, United States of America, **5** UCSF-Abbott Viral Diagnostics and Discovery Center, San Francisco, California, United States of America, **6** Blood Systems Research Institute, San Francisco, California, United States of America, **7** Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, **8** Howard Hughes Medical Institute, Chevy Chase, Maryland, United States of America, **9** Department of Biochemistry, University of California, San Francisco, California, United States of America, **10** Department of Biochemistry, Stanford University, Stanford, California, United States of America, **11** Department of Pathology, University of Texas Medical Branch, Galveston, Texas, United States of America, **12** Department of Epidemiology, University of California at Davis, Davis, California, United States of America, **13** Department of Epidemiology, University of California at Los Angeles, Los Angeles, California, United States of America, **14** Department of Medicine, Division of Infectious Diseases, University of California, San Francisco, San Francisco, California, United States of America

Abstract

Deep sequencing was used to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with a 2009 outbreak of 3 human cases of acute hemorrhagic fever in Mangala village, Democratic Republic of Congo (DRC), Africa. The cases, presenting over a 3-week period, were characterized by abrupt disease onset, high fever, mucosal hemorrhage, and, in two patients, death within 3 days. BASV was detected in an acute serum sample from the lone survivor at a concentration of 1.09×10^6 RNA copies/mL, and 98.2% of the genome was subsequently *de novo* assembled from ~140 million sequence reads. Phylogenetic analysis revealed that BASV is highly divergent and shares less than 34% amino acid identity with any other rhabdovirus. High convalescent neutralizing antibody titers of >1:1000 were detected in the survivor and an asymptomatic nurse directly caring for him, both of whom were health care workers, suggesting the potential for human-to-human transmission of BASV. The natural animal reservoir host or arthropod vector and precise mode of transmission for the virus remain unclear. BASV is an emerging human pathogen associated with acute hemorrhagic fever in Africa.

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Competing Interests: The authors have filed a patent application related to BASV. This does not alter the authors' adherence to all PLOS Pathogens policies on sharing data and materials.

* E-mail: eric.leroy@ird.fr (EML); charles.chiu@ucsf.edu (CYC)

☯ These authors contributed equally to this work.

Introduction

Viral hemorrhagic fever (VHF) encompasses a group of diseases characterized by fever, malaise, bleeding abnormalities, and circulatory shock [1,2,3]. Quality research on these infections is hindered by the fact that they are sporadic and often occur in geographically remote and politically unstable regions of the developing world. Most VHF diseases are associated with a short incubation period (2–21 days), abrupt onset, rapid clinical course, and high mortality, placing VHF agents amongst the most virulent human pathogens [4]. All known VHFs are zoonoses, and to date have been attributed to only four families of enveloped, single-stranded RNA viruses – *Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. Viruses from these families have caused major deadly outbreaks on the African continent (Fig. 1). Lassa fever virus (*Arenaviridae*) causes an estimated 500,000 cases each year in West Africa [5]. Crimean-Congo hemorrhagic fever (CCHF) and Rift Valley Fever viruses (*Bunyaviridae*) are associated with outbreaks in

Author Summary

We used deep sequencing, a method for generating millions of DNA sequence reads from clinical samples, to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with a 2009 outbreak of 3 human cases of acute hemorrhagic fever in Mangala village, Democratic Republic of Congo (DRC), Africa. The cases, presenting over a 3-week period, were characterized by abrupt disease onset, high fever, bloody vomiting and diarrhea, and, in two patients, death within 3 days. BASV was present in the blood of the lone survivor at a concentration of over a million copies per milliliter. The genome of BASV, assembled from over 140 million sequence reads, reveals that it is very different from any other rhabdovirus. The lone survivor and a nurse caring for him (with no symptoms), both health care workers, were found to have high levels of antibodies to BASV, indicating that they both had been infected by the virus. Although the source of the virus remains unclear, our study findings suggest that BASV may be spread by human-to-human contact and is an emerging pathogen associated with acute hemorrhagic fever in Africa.

West, South and East Africa [6]. Ebola and Marburg viruses (*Filoviridae*) have caused several sporadic human outbreaks with high mortality (50–90%) in Central Africa, where they have also decimated local great ape populations [7]. Yellow fever and dengue viruses (*Flaviviridae)* are widely distributed throughout Sub-Saharan Africa where they cause both endemic and sporadic epidemic diseases in human populations [8].

Rhabdoviruses are members of the family *Rhabdoviridae* and order *Mononegavirales* and are enveloped viruses with single-stranded, negative-sense RNA genomes [9]. Their genomes encode at least five core proteins in the following order: 3′-nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large protein, or RNA-dependent RNA polymerase (L)-5′ (N-P-M-G-L). Rhabdoviruses are currently divided into six genera, with the two genera *Ephemerovirus* and *Vesiculovirus*, together with about 130 unclassified viruses, forming the dimarhabdovirus supergroup (“dipteran mammal-associated rhabdovirus”) [10]. Notably, although rhabdoviruses span all continents and exhibit a wide host range, infecting plants, invertebrates, vertebrate animals, and humans, relatively few are known to cause human infections. Rabies virus (RABV) and related viruses from the *Lyssavirus* genus and Chandipura virus (CHPV) from the *Vesiculovirus* genus are known to cause acute encephalitis syndromes [11,12]. Other viruses from the genus *Vesiculovirus* cause vesicular stomatitis (mucosal ulcers in the mouth) and “flu-like” syndromes in both cattle and humans [13].

Unbiased next-generation or “deep” DNA sequencing is an emerging method for the surveillance and discovery of pathogens in clinical samples [14]. Unlike polymerase chain reaction (PCR), deep sequencing does not rely on the use of target-specific primers. Thus, the technique is particularly useful for the identification of novel pathogens with high sequence divergence that would elude detection by conventional PCR assays. Deep sequencing has been used previously to discover a new hemorrhagic fever-associated arenavirus from southern Africa, Lujo virus [15], as well as a new polyomavirus in human Merkel cell carcinoma [16]. With the depth of sequence data now routinely extending to >100 million reads, *de novo* genome assembly of novel viruses directly from primary clinical samples is feasible, as demonstrated by assembly of the 2009 pandemic influenza H1N1 virus genome from a single patient’s nasal swab without the use of a reference sequence [17]. Here we report the critical role of deep sequencing in the discovery of a novel rhabdovirus associated with a small outbreak of fulminant hemorrhagic fever in the remote village of Mangala, Bas-Congo province, Democratic Republic of Congo (DRC), between May 25 and June 14, 2009.

Results

Case Reports from an Acute Hemorrhagic Fever Outbreak

Patient 1. The first case was a 15-year-old boy who presented to the health center in Mangala village (Boma Bungu Health Zone) on May 25, 2009 with malaise, epistaxis (nose bleeding), conjunctival injection, gingival bleeding, hematemesis (vomiting with blood), and watery diarrhea with blood (Table 1). No fever or respiratory symptoms were noted. Hemorrhagic symptoms initially appeared on May 24, and the patient died 2 days later from sudden circulatory collapse. The patient lived in the Tshela neighborhood of Mangala village and attended the local public school. All close contacts were monitored for 21 days, and none developed any signs of illness.

Patient 2. The second case was a 13-year-old girl. She attended the same public school as Patient 1 but was in a different class. She also lived in the Tshela neighborhood of Mangala village, about 50 meters from Patient 1′s house. They knew each other but had no known face-to-face contact during the previous weeks. This patient presented to the health center on June 5, 2009 with headache, fever, abdominal pain, epistaxis, conjunctival injection, mouth bleeding, hematemesis, and diarrhea with blood. She was examined by a nurse and received acetaminophen and dipyrone for fever and quinine for possible malaria. Symptoms appeared on June 4, and the patient died suddenly on June 7, three days after onset. None of her close contacts developed symptoms during the 21 days of monitoring after her death.

Patient 3. The third case was a male nurse aged 32 years working in the health center visited by Patients 1 and 2. His disease appeared suddenly on June 13, 2009 with epistaxis, ocular and oral hemorrhage, hematemesis, and diarrhea with blood. Two days after the onset of hemorrhagic symptoms, he developed fever, anorexia, headache, fatigue, and abdominal pain. He was transferred to the regional general hospital of Boma (Fig. 1), a city of about 200,000 inhabitants, where a serum sample was obtained on June 15, just prior to treatment with fluid resuscitation, blood transfusion, and empiric antibiotics. Laboratory tests for malaria, tuberculosis, dengue, and bacterial sepsis were negative, and the patient recovered spontaneously a few days later. All persons in Mangala and Boma who had contact with Patient 3 were monitored for 21 days, and none became ill. Patient 3, like the two other patients, lived in the Tshela neighborhood of Mangala village, about 50 meters from Patients 1 and 2. Importantly, patient 3 was directly involved in the care of Patients 1 and 2 when they presented to the health center with hemorrhagic symptoms.

No disease outbreaks had been reported in the past in Boma Bungu Health Zone with the exception of a cholera diarrheal outbreak in 2006, and, notably, no cases of hemorrhagic disease had previously been reported. In addition, although DRC is a country endemic for filovirus infection (Fig. 1), no outbreaks of Ebola or Marburg fever have ever been described in Bas-Congo province. No animal die-offs or other unusual events in association with these cases were noted.

Initial Sample Collection and Diagnostic Testing

A cluster of three human cases of typical acute hemorrhagic fever occurred between May 25 and June 13, 2009 in Mangala village,

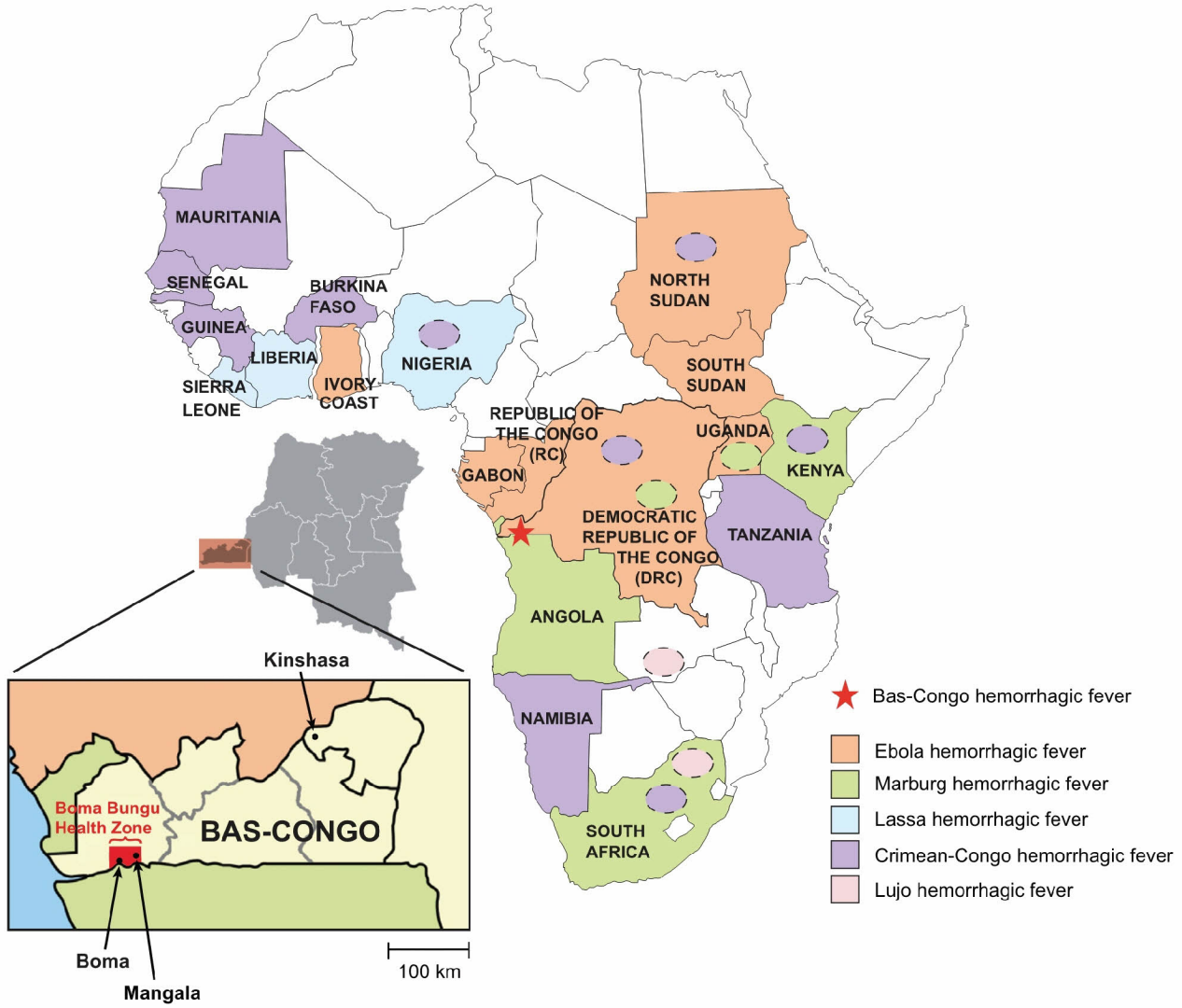

Figure 1. Map of Africa showing countries that are affected by viral hemorrhagic fever (VHF) outbreaks. Ebola VHF is pictured in orange, Marburg VHF in green, Crimean-Congo HF in violet, Lujo VHF in pink, and Lassa VHF in blue. Yellow fever and dengue VHF, which exhibit a wide geographic distribution throughout Sub-Saharan Africa, are not shown. Mangala village, located in the Bas-Congo province in DRC, is represented by a red star.
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located in a remote tropical forest region in Central Africa. Cases were characterized by abrupt disease onset, high fever of >39°C when present, overt hemorrhagic symptoms with epistaxis, conjunctival injection, mouth and gastrointestinal bleeding, followed by death within 3 days of symptom onset in two patients (Table 1). The first patient, who died <48 hours after presentation, exhibited hemorrhagic symptoms without a documented fever, and only the third adult patient recovered from his illness. All three patients lived within a 2500-m^2 area in the same neighborhood of Mangala, a remote village in Bas-Congo province of DRC (Fig. 1). The first two patients died rapidly in Mangala village, and no blood samples were collected. A blood sample was collected from the third surviving patient three days after symptom onset and sent to Centre International de Recherches Médicales de Franceville (CIRMF) for etiological diagnosis. The sample tested negative by TaqMan real-time PCR assays for all viruses known to cause acute hemorrhagic fever in Africa (data not shown).

Discovery and Genome Assembly of the BASV Rhabdovirus

To identify a potential causative pathogen in the third surviving patient with unknown hemorrhagic fever, RNA extracts from the serum sample were analyzed using unbiased deep sequencing (Fig. 2). The initial Roche 454 pyrosequencing library yielded a total of 4,537 sequence reads, of which only a single 220 bp read (0.022%) aligned with any annotated viral protein sequence in GenBank. The translation product showed similarity to a segment of the L protein, or RNA-dependent RNA polymerase, from Tibrogargan and Coastal Plains rhabdoviruses, with 41% identity to Coastal Plains virus (GenBank ADG86364; BLASTx E-score of 2×10^{-6}). This finding suggested the presence of a novel, highly divergent rhabdovirus in the patient's serum. Attempts to extend the initial sequence by primer walking or PCR using rhabdovirus consensus primers failed due to limited sample availability; thus, we resorted to ultra-deep sequencing on an Illumina HiSeq 2000.

Table 1. Demographics of and clinical symptoms developed in the three patients suspected to be infected by Bas-Congo virus (BASV).

	Patient 1	Patient 2	Patient 3
Sex	**Male**	**Female**	**Male**
Age	15	13	32
Village	Mangala	Mangala	Mangala
Neighborhood	Tshela	Tshela	Tshela
Occupation	Schoolboy	Schoolgirl	Nurse
Disease onset	May 24	June 4	June 13
Time until death	2 days	3 days	survived
Fever (T>39°C)	No	Yes	Yes
Weakness	No	No	Yes
Malaise	Yes	No	No
Headache	No	Yes	Yes
Abdominal pain	No	Yes	Yes
Epistaxis (nose bleeding)	Yes	Yes	Yes
Ocular hemorrhage/conjunctival injection (eye bleeding)	Yes	Yes	Yes
Oral hemorrhage (mouth bleeding)	Yes	Yes	Yes
Hemorrhagic vomiting	Yes	Yes	Yes
Hemorrhagic diarrhea	Yes	Yes	Yes

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Out of the 140,164,344 reads generated from Illumina sequencing, 4,063 reads (0.0029%) had nucleotide or protein homology to rhabdoviruses with an E-score of $<10^{-5}$. These reads were used as "seeds" for iterative *de novo* assembly, resulting in construction of an estimated 98.2% of the genome of the novel rhabdovirus. We provisionally named this rhabdovirus BASV, or Bas-Congo virus, referring to the province from which the outbreak originated.

The coverage of BASV achieved by deep sequencing was at least 10-fold across nearly the entire genome and included 29,894 reads out of ~140 million (0.021%) (Fig. 2). The viral load in the patient's serum was 1.09×10^6 RNA copies/mL by quantitative RT-PCR. The only moderately high titer is consistent with the fact that the sampled patient was a survivor of BASV infection and would thus be anticipated to have relatively lower viral titers in the blood, as also seen for survivors of Ebola virus infection [18].

Cultivation of the patient's serum in Vero, BHK, LLC-MK_2 (rhesus monkey kidney), CCL-106 (rabbit kidney) and C6/36 (*Aedes albopictus* mosquito) cell cultures failed to show cytopathic effect, and serial quantitative BASV RT-PCR assays on primary and passaged cell culture supernatants turned negative. Subsequent electron microscopy of inoculated cell cultures was negative for viral particles. In addition, no illnesses or deaths occurred in suckling mice inoculated intracerebrally with the BASV-positive serum and observed over 14 days.

Phylogenetic Analysis of BASV and Comparison with other Rhabdoviruses

Phylogenetic trees reveal that BASV belongs to the *dimarhabdoviridae* supergroup and is distantly related to members of the Tibrogargan group and the *Ephemerovirus* genus, although it clusters separately from other rhabdoviruses in an independent deeply rooted branch (Figs. 3 and 4; Fig. S1). Comparative analysis of the concatenated BASV proteins with representative dimarhabdoviruses reveals very low overall amino acid pairwise identity of 25.0 to 33.7%, depending on the virus (Fig. 5). Notably, BASV diverges significantly from either of the two main recognized human pathogens among rhabdoviruses, rabies virus or Chandipura virus.

The sequence divergence of BASV relative to other rhabdoviruses is also correlated with differences in genome structure (Fig. 5). The prototype genome organization of rhabdoviruses, found in lyssaviruses, is N-P-M-G-L. However, molecular analysis of novel rhabdoviruses has often revealed more complex genomes, with up to 10 additional open reading frames (ORF) located within an existing gene or interposed between the five core genes [19,20,21]. Rhabdoviruses from the Tibrogargan group (TIBV and CPV) share a distinctive genome structure with three additional genes, two between M and G (U1 and U2) and one between G and L (U3) [22]. Interestingly, BASV also has these three additional genes (U1–U3), confirming the phylogenetic relationship and overall structural similarity to the Tibrogargan group viruses. Based on their size, the U3 proteins of TIBV, CPV, and presumably BASV are candidate viroporins [22]. BASV is more distant structurally and phylogenetically from the Ephemero and Hart Park Group rhabdoviruses (Figs. 3 and 4), which do not contain U1 or U2 genes, but rather an additional two or three genes between G and L (including a putative U3 viroporin in BEFV referred to as the alpha-1 protein) (Fig. 5, asterisk). Moussa virus (MOUV), another rhabdovirus recently discovered in Africa (Fig. 4), does not contain any accessory genes but instead, shares the prototype N-P-M-G-L rhabdovirus structure [23].

BASV Serological Testing of the Case Patient and Close Contacts

To confirm that BASV is infectious to humans, convalescent sera were collected in early 2012 from surviving Patient 3 as well as five additional health care workers from Mangala identified as close contacts and tested in a blinded fashion for the presence of neutralizing antibodies to BASV (Fig. 6). Two of the six sera tested strongly positive with 50% protective doses between 1:1,000 and

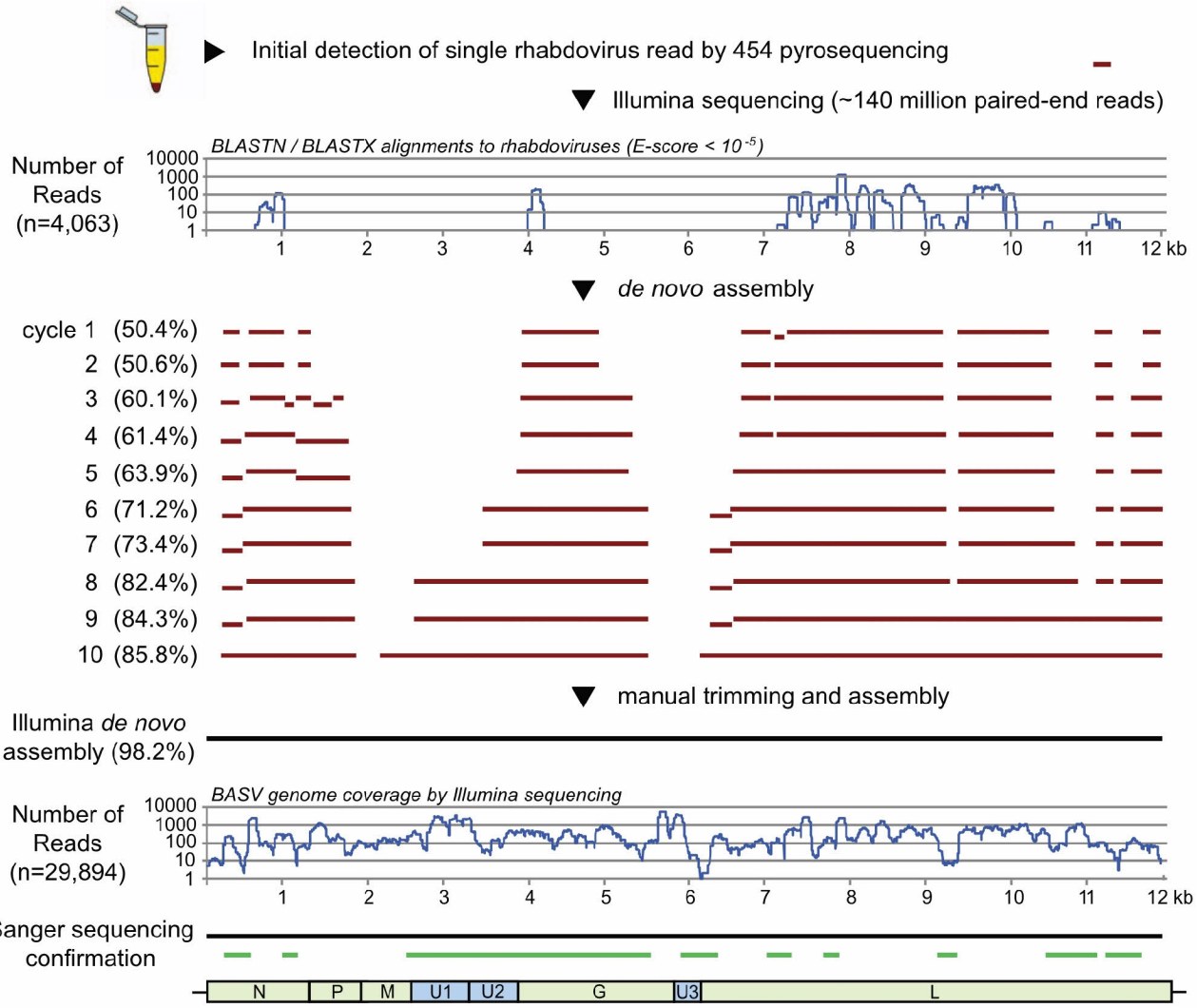


Figure 2. Deep sequencing and whole-genome *de novo* assembly of BASV. After initial discovery of BASV from a single 454 pyrosequencing read, 98.2% of the BASV genome was assembled *de novo* from >140 million paired-end Illumina reads. The horizontal lines (red) depict regions of the genome successfully assembled at the end of each cycle. PCR and Sanger sequencing were performed to confirm the assembly and genomic organization of BASV (green lines).
doi:10.1371/journal.ppat.1002924.g002

1:5,000 (Figs. 6A and 6F). Moreover, the observed neutralization was highly specific for BASV-G, since no neutralization was observed with pseudoviruses harboring the vesicular stomatitis virus glycoprotein (VSV-G). One of the neutralizing sera had been collected from surviving Patient 3 (Fig. 6A, "Patient 3"), whereas the other serum sample, containing even higher titers, corresponded to an asymptomatic nurse directly caring for Patient 3 during his period of acute hemorrhagic illness (Fig. 6F, "Contact 5"). Specifically, Contact 5 was the primary health care provider to Patient 3 at the health center and during his transfer to the general hospital at Boma. All 6 individuals, including Patient 3, tested negative for BASV viremia by specific RT-PCR (data not shown).

Epidemiological Screening for BASV in the DRC

BASV was not detected by PCR in 43 serum samples from other unknown cases or outbreaks of hemorrhagic fever reported in the DRC from 2008–2010 (Fig. 7A, pink). Five of these 43 samples originated from the Bas-Congo outside of Mangala village and the Boma Bungu Health Zone. In total, the unknown hemorrhagic cases/outbreaks spanned 9 of the 11 provinces in the DRC, and all 43 samples also tested negative by PCR for the known hemorrhagic fever viruses circulating in Africa (data not shown). Fifty plasma samples collected from randomly selected blood donors in the Kasai-Oriental province of DRC (Fig. 7A, star; Table S2) were also screened and found to be negative for BASV-neutralizing antibodies (Fig. 7B).

Discussion

Among more than 160 species of rhabdoviruses identified to date, fewer than 10 have been isolated from humans [24]. In addition, while human infection by rhabdoviruses has previously been associated with encephalitis, vesicular stomatitis, or "flu-like" illness, the discovery of BASV is the first time that a member of the *Rhabdovirus* family has been associated with hemorrhagic fever in humans with a fulminant disease course and high fatality rate. To our knowledge, this is also the first successful demonstration of

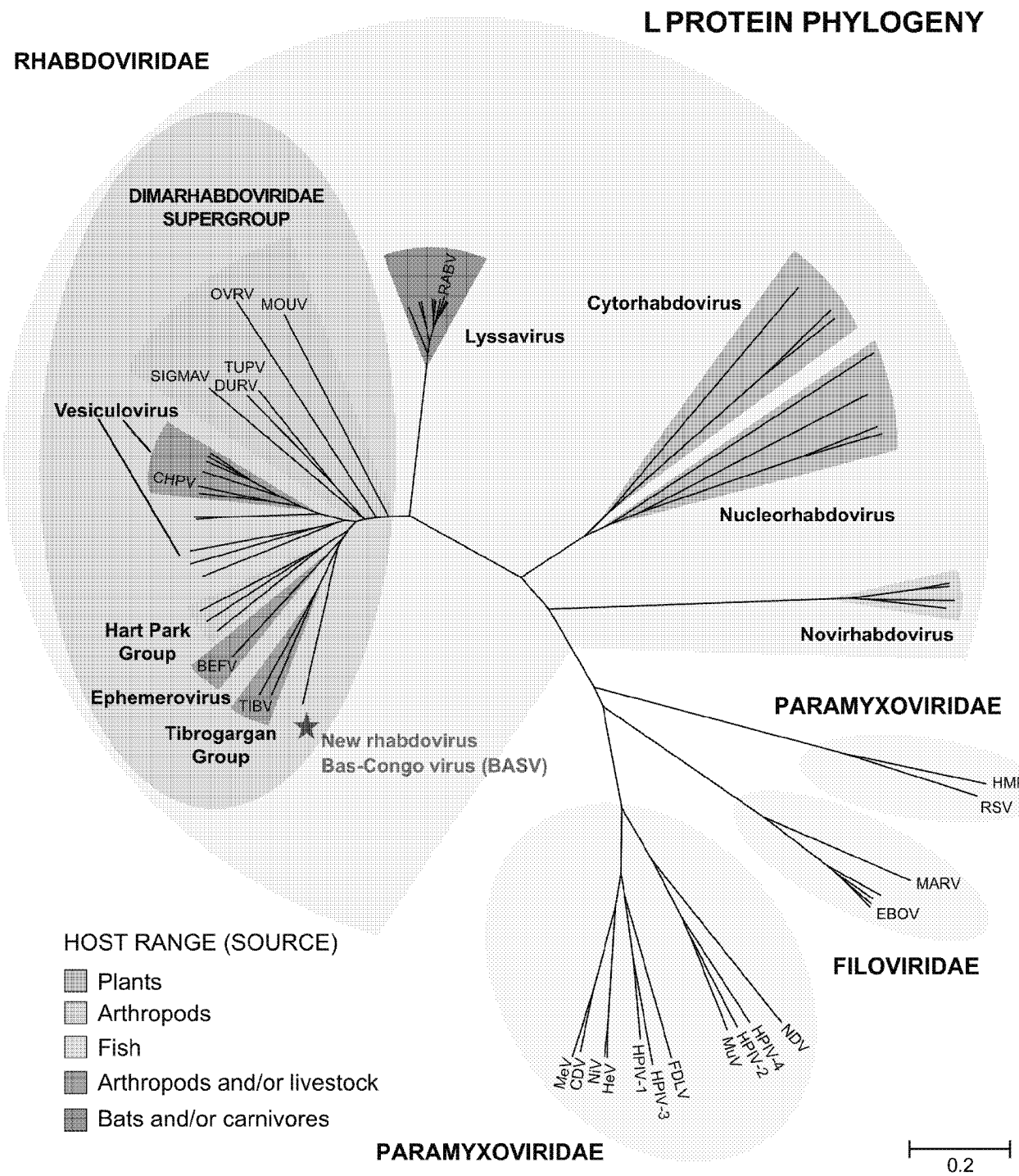


Figure 3. Phylogenetic analysis of the L proteins of BASV and other viruses in the order *Mononegavirales*. The host from which each virus was isolated is represented by a specific color. To generate the *Mononegavirales* (*Rhabdoviridae*, *Filoviridae* and *Paramyxoviridae*) phylogeny trees, all complete sequences of the large (L) protein, or RNA-dependent RNA polymerase (2000–2300 amino acids in length) were downloaded from GenBank. Abbreviations and accession numbers used for the phylogenetic analysis are provided in Methods.
doi:10.1371/journal.ppat.1002924.g003

de novo assembly of a novel, highly divergent viral genome in the absence of a reference sequence and directly from a primary clinical sample by unbiased deep sequencing.

Several lines of evidence implicate BASV in the hemorrhagic fever outbreak among the 3 patients in Mangala. First, this virus was the only credible viral pathogen detected in the blood of the lone survivor during his acute hemorrhagic illness by exhaustive deep sequencing of over 140 million reads. Analysis of the Illumina deep sequencing reads for the presence of other viral pathogens yielded only endogenous flora or confirmed laboratory contaminants (Table

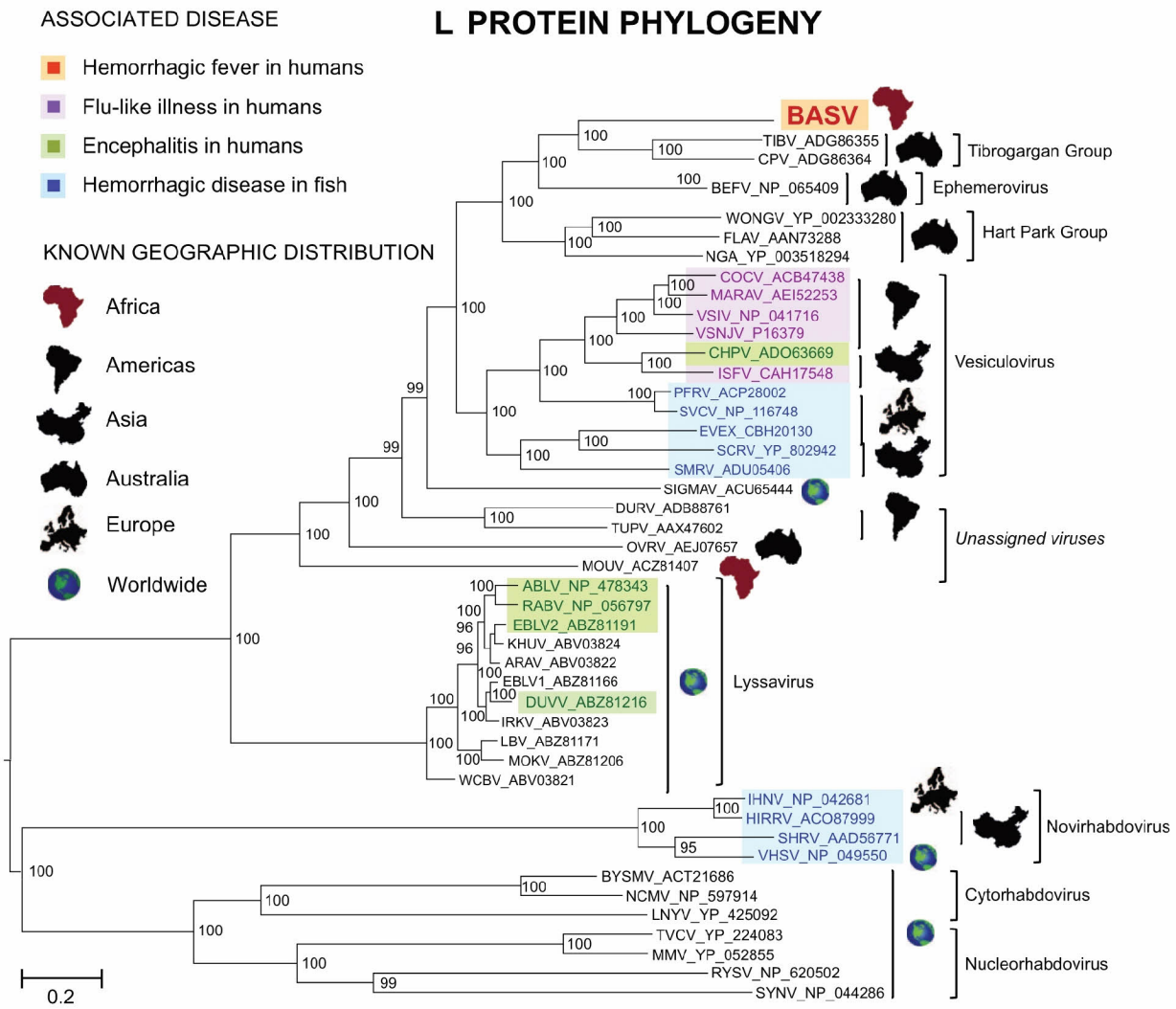


Figure 4. Phylogenetic analysis of the L proteins of BASV and other rhabdoviruses. The geographic distribution for each virus or group of viruses is indicated with a specific icon, while diseases associated with infection by certain rhabdoviruses are indicated by specific colors. Abbreviations and accession numbers used for the phylogenetic analysis are provided in Methods.
doi:10.1371/journal.ppat.1002924.g004

S1 and Fig. S2). Some enteric pathogens, such as E. coli O157:H7, Campylobacter, Shigella, and Salmonella, are diagnosed through fecal laboratory testing and not blood, and have been associated with hemorrhagic diarrhea [25]. However, these outbreaks are typically foodborne and associated with larger clusters and much greater numbers of clinical cases than reported here [26,27,28]. Furthermore, enteric diarrheal cases rarely present with systemic symptoms such as fever or generalized mucosal hemorrhage, with bleeding most often limited to the gastrointestinal tract, and overall mortality rates are generally low [26]. Thus, the clinical syndrome observed in 3 patients with hemorrhagic fever in the DRC, a region endemic for viral hemorrhagic fevers, is much more consistent with infection by a VHF disease agent. BASV is a plausible hemorrhagic fever candidate because it is a novel, highly divergent infectious virus, thus of unknown pathogenicity, and was detected at a titer of >1 million copies/mL in blood from an acutely ill individual. In addition, there is ample precedent for hemorrhagic disease from rhabdoviruses, as members of the genus *Novirhabdovirus* cause severe hemorrhagic septicemia in fresh and saltwater fish worldwide [29] (Fig. 4). The detection of BASV seropositivity in an asymptomatic close contact (Fig. 6) is not surprising given that up to 80% of patients infected with Lassa virus do not exhibit any hemorrhagic fever symptoms [30,31].

Prior to the BASV outbreak, no hemorrhagic disease cases had been reported in Boma Bungu Health Zone. BASV was also not detected in 43 serum samples from unknown, filovirus-negative cases or outbreaks of hemorrhagic fever from 2008 2010 spanning 9 of the 11 provinces in the DRC (Fig. 7A). In addition, a serosurvey of 50 random blood donors from Kasai-Oriental province in central DRC was negative for prior exposure to BASV (Fig. 7B). Taken together, these data suggest that the virus may have emerged recently and locally from Boma Bungu in Bas-Congo, DRC.

We were unable to isolate BASV despite culturing the RNA-positive serum in a number of cell cultures and inoculation into suckling mice. One explanation for these negative findings may be that the virus inoculation titers of <50 µL were insufficient, although this is surprising given the concentration of >1 million copies per mL of BASV in blood from the lone survivor. A more likely explanation is viral inactivation resulting from the lack of

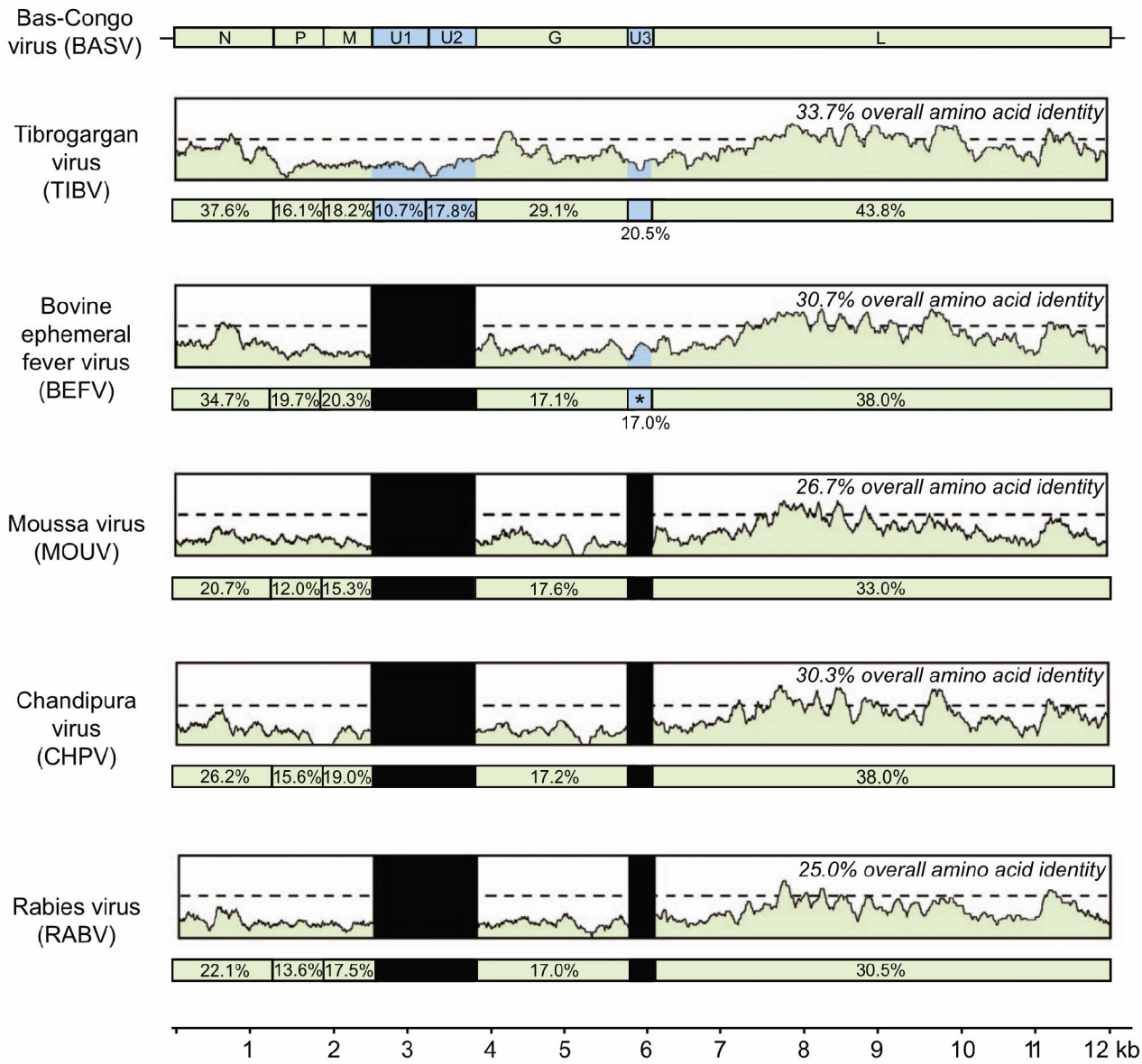


Figure 5. Schematic representation of the genome organization of BASV and its protein similarity plot compared to representative rhabdoviruses. The similarity plots are generated by aligning the concatenated rhabdovirus proteins and calculating scanning amino acid pairwise identities using a window size of 50 bp. The horizontal bar under each similarity plot shows the percent identity of the rhabdovirus protein relative to its corresponding protein in BASV. Genes coding for the 5 core rhabdovirus proteins are shown in green, while the accessory U1, U2, or U3 genes are shown in blue. Black bars correspond to accessory proteins which are not present in the genome. Note that BEFV contains 3 genes between G and L; only the alignment between the alpha-1 protein of BEFV and the U3 protein of BASV is shown (asterisk). The x-axis refers to the nucleotide position along the ~12 kb genome of BASV.
doi:10.1371/journal.ppat.1002924.g005

adequate cold chain facilities in remote Boma Bungu. Viral RNA can often still be detected by RT-PCR in sera that is culture-negative [32]. In support of this premise, we have observed that the BASV-G/VSVΔG-GFP pseudotyped virus efficiently infects and replicates in a variety of insect and mammalian (including human) cell lines (Steffen, *et al.*, manuscript in preparation). In the absence of a positive culture, a "reverse genetics" approach to produce recombinant BASV particles, if successful, would greatly facilitate further study of the virus, as established previously for other rhabdoviruses such as VSV [33].

Based on our findings, some speculations on the origin of and routes of transmission for BASV can be made. All 3 patients became ill with acute hemorrhagic fever over a 3-week period within the same 2500-m^2 area of Mangala village, suggesting that all 3 cases were infected with the same pathogen. Waterborne or airborne transmission would be expected to result in more numerous cases than the 3 reported. There were no reports of animal die-offs that would suggest potential exposures to infected wild animals or livestock. Taken together, these observations suggest that an unknown arthropod vector could be a plausible source of infection by BASV. This hypothesis is consistent with the phylogenetic and structural relationship of BASV to rhabdoviruses in the Tibrogargan group and *Ephemerovirus* genus, which are transmitted to cattle and buffalo by *Culicoides* biting midges [9]. In

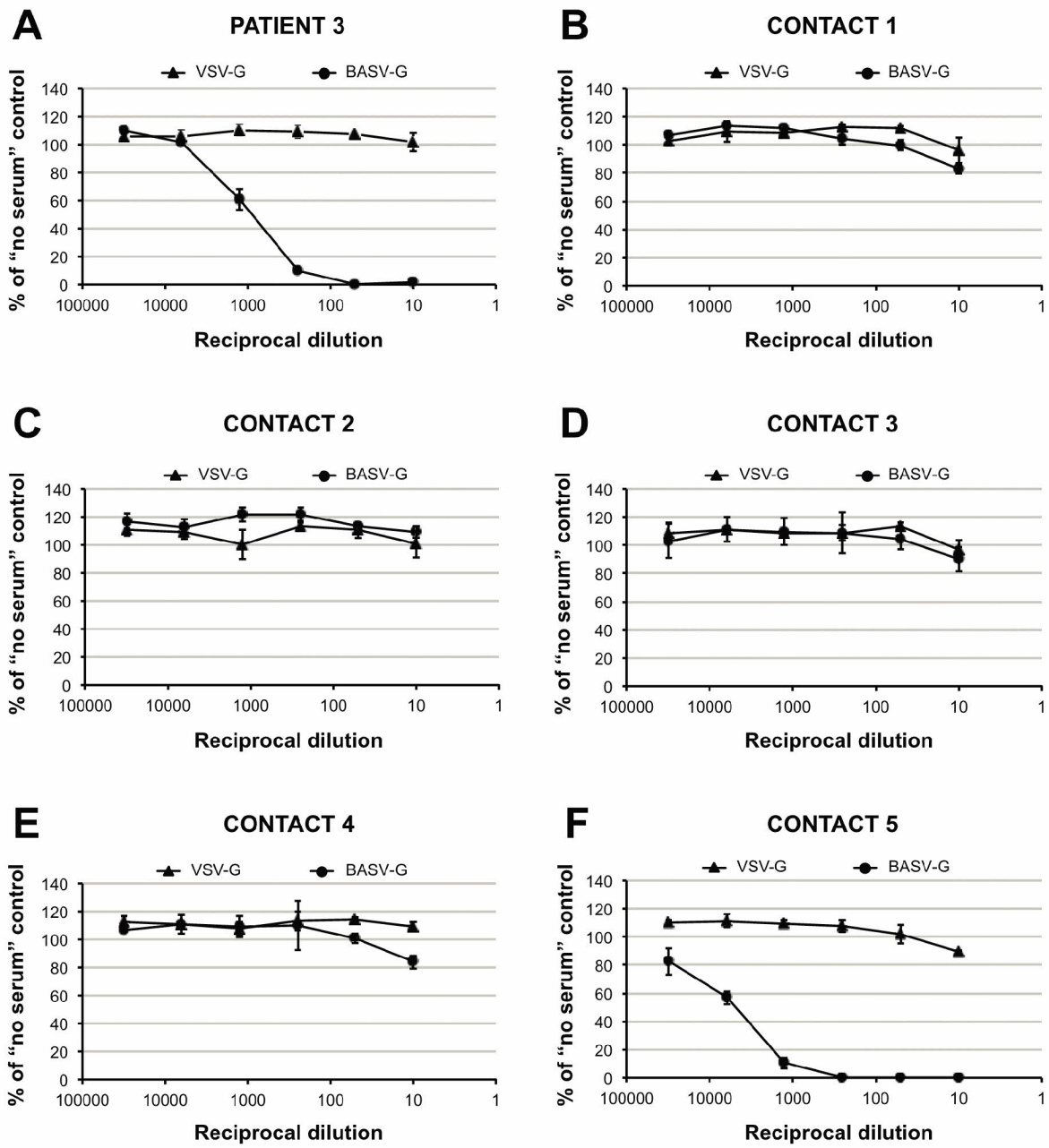
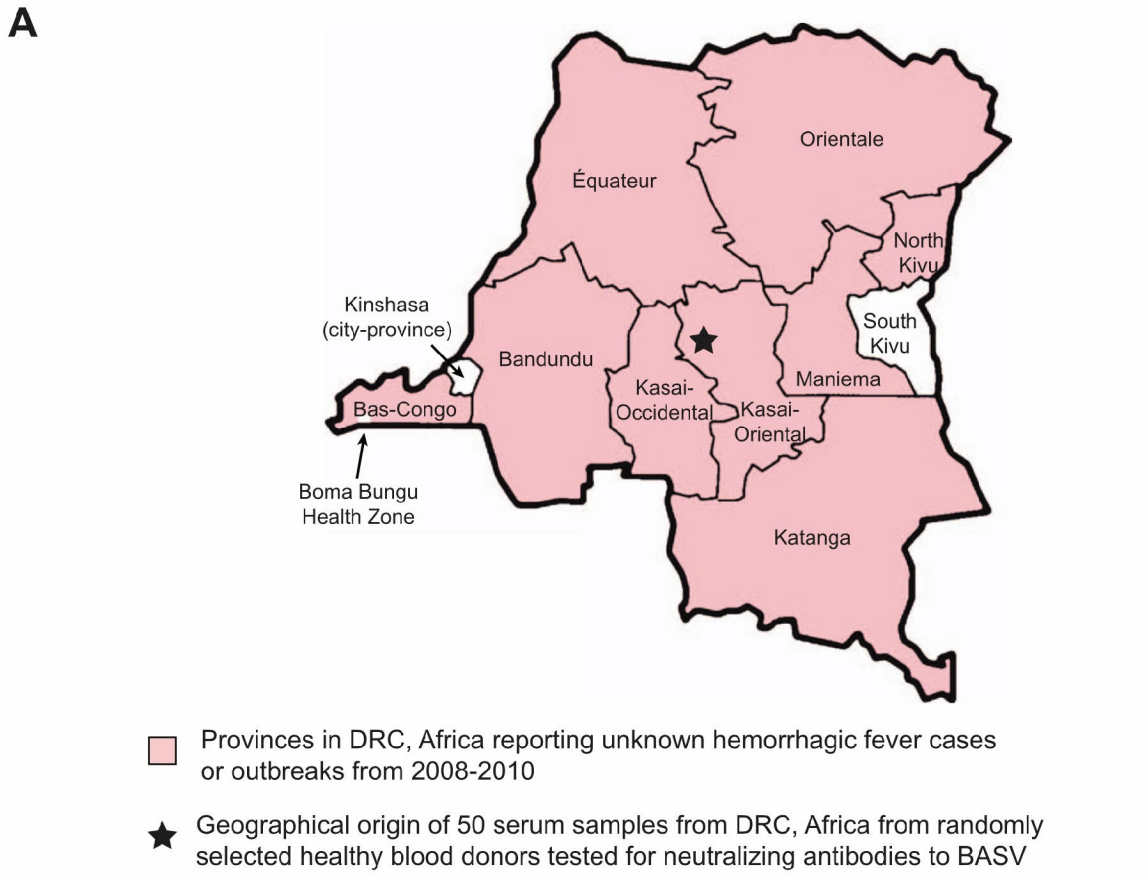


Figure 6. Detection of antibodies to BASV by serum neutralization of VSVΔG-GFP pseudotypes. Infectivities of VSVΔGFP pseudotypes bearing the glycoproteins of VSV or BASV, respectively, after incubation with 5-fold serial dilutions (1:10, 1:50, 1:250, 1:1,250, 1:6,250, 1:31,250) of sera from six individuals are depicted as percent of infectivity in the absence of serum. The six individuals tested include a patient with hemorrhagic fever (panel A, "Patient 3"), the nurse directly caring for him (panel F, "Contact 5"), and other health care workers in Mangala village (panels B–E). All data points represent the average of triplicate assays; error bars indicate standard deviations. Similar results were obtained in an independent experiment using murine leukemia virus (MLV)-based pseudotypes (data not shown).
doi:10.1371/journal.ppat.1002924.g006

addition, the recent discovery of Moussa virus (MOUV), isolated from *Culex* mosquitoes in Cote d'Ivoire, Africa [23], implies the presence of hitherto unknown arthropod vectors for rhabdoviruses on the continent. Nevertheless, at present, we cannot exclude the possibility of other zoonotic sources for the virus or even nosocomial bloodborne transmission (as Patients 1 and 2 have not clearly been established to be BASV cases by serology or direct detection), and the natural reservoir and precise mode of transmission for BASV remain unknown. A community-based serosurvey in Boma Bungu and an investigation to track down potential arthropod or mammalian (e.g. rodents and bats) sources for BASV are currently underway.

Although we cannot exclude the possibility of independent arthropod-borne transmission events, our epidemiologic and serologic data do suggest the potential for limited human-to-human transmission of BASV. Patient 3, a nurse, had directly taken care of Patients 1 and 2 at the health center, and another nurse (Contact 5), who had taken care of Patient 3 (but not



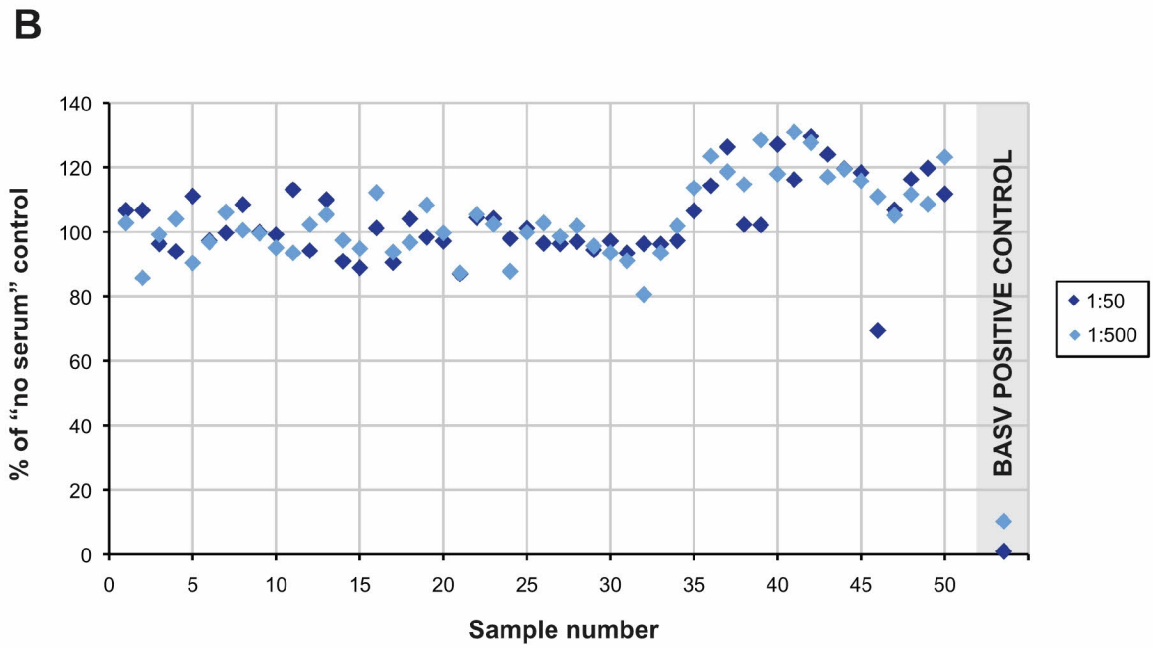


Figure 7. BASV Screening in DRC, Africa. (**A**) All 43 serum samples corresponding to unknown hemorrhagic fever cases or outbreaks in 2008–2010 from 9 provinces in DRC (pink) tested negative for BASV by PCR. (**B**) Sera from 50 donors in Kasai-Oriental province, DRC (Panel A, star) were tested for BASV-neutralizing antibodies. Sera at 1:50 (dark blue) or 1:500 dilution (light blue) were tested. Serum from the surviving Patient 3 was included as a positive control (grey shaded area). Data points represent an average of duplicate assays.
doi:10.1371/journal.ppat.1002924.g007

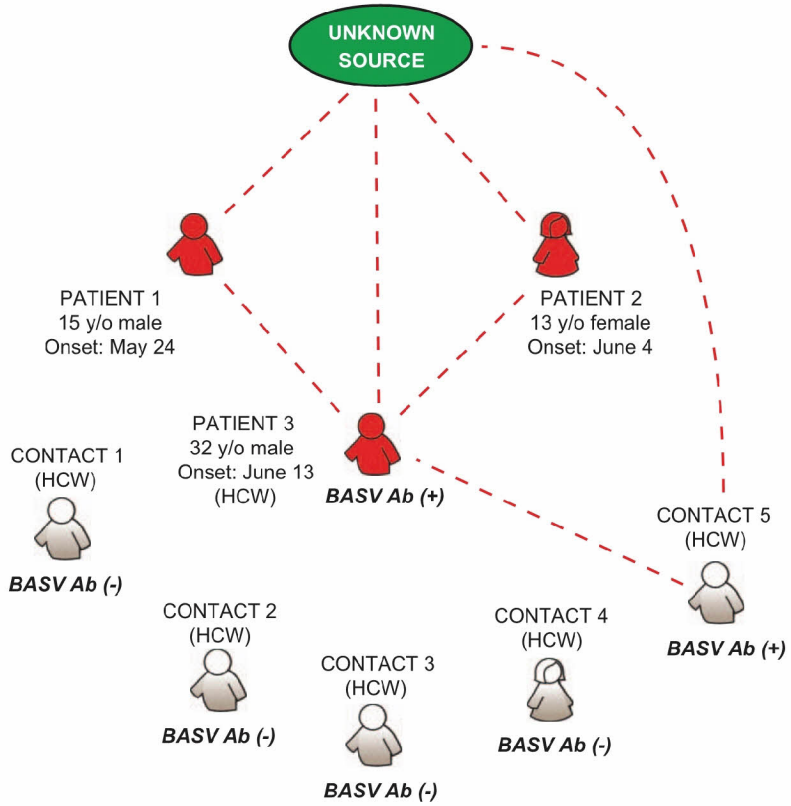


Figure 8. Proposed model for BASV transmission during the hemorrhagic fever outbreak in Mangala. Patients presenting with symptoms of acute hemorrhagic fever are depicted in red. Dashed red lines represent potential routes of BASV transmission. Contacts 1 through 5 are health care workers at the local health center in Mangala village. Abbreviations: HCW, health care worker; y/o, year-old; Ab, antibody.
doi:10.1371/journal.ppat.1002924.g008

Patients 1 or 2) had serologic evidence of asymptomatic BASV infection. We present a hypothetical model for BASV transmission during the hemorrhagic fever outbreak in which the initial infection of two children in Mangala (Patients 1 and 2) was followed by successive human-to-human transmission events involving two healthcare workers (Patient 3 and Contact 5) (Fig. 8). This pattern of transmission from the community to health care workers is also commonly seen in association with outbreaks of Ebola and Crimean-Congo hemorrhagic fever [6,34].

While rhabdoviruses are distributed worldwide, some authors have suggested that the *Rhabdoviridae* family probably originated from tropical regions of the Old or New World [9]. The discovery of BASV in Central Africa suggests that additional rhabdoviruses of clinical and public health importance likely await identification, especially in these poorly investigated geographic regions. Active epidemiological investigation and disease surveillance will be needed to fully ascertain the clinical and public health significance of BASV infection in humans, as well as to prepare for potentially larger human outbreaks from this newly discovered pathogen.

Methods

Ethics Statement

Written informed consent for publication of their case reports was obtained from the sole survivor of the hemorrhagic fever outbreak and the parents of the two deceased children. Written informed consent was obtained from the surviving patient and 5 of his close contacts for analysis of the serum samples reported in this study. Samples were analyzed under protocols approved by the institutional review boards of University of California, San Francisco, the University of Texas Medical Branch, and the National Institute of Biomedical Research (INRB) and CIRMF in Gabon, and the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Medical Branch.

Diagnostic Samples

No diagnostic samples were available from Patient 1 or Patient 2. Blood was collected in a red top serum tube from Patient 3 on June 16, during the acute phase, three days after hemorrhagic onset. The sample was transported at 4°C to the BSL-4 facility at CIRMF. Serum was obtained by centrifugation at 2300 rpm for 10 min. No other acute samples from Patient 3 were available. In January of 2012 (~2.5 years after the outbreak), convalescent sera were collected from Patient 3 and close contacts (other workers at the health center) for BASV neutralization testing. Forty-three serum samples from other unknown hemorrhagic fever cases or outbreaks representing 9 of 11 provinces in the DRC were available for BASV PCR testing (Fig. 7A). Fifty available plasma samples from random blood donors (median age 27.5 years; age range 1–76 years) in Kasai Oriental province, DRC, were also tested for antibodies to BASV (Fig. 7A and B; Table S2).

Nucleic Acid Extraction and Viral PCR Testing

RNA was extracted from 140 µl of serum using the QIAamp viral RNA mini kit (Qiagen). Taqman real-time reverse-transcription-PCR (RT-PCR) testing for known hemorrhagic fever viruses was performed using primers and probes specific for Marburg

virus (MARV), all four species of Ebola virus (Zaire, ZEBOV; Sudan, SEBOV; Côte d'Ivoire, CIEBOV, and Bundibugyo, BEBOV), Crimean-Congo hemorrhagic fever virus (CCHFV), Yellow fever virus (YFV), Dengue virus (DENV), Rift Valley fever virus (RVFV) and Chikungunya virus (CHIKV) (available upon request).

Discovery of the BASV Rhabdovirus by 454 Pyrosequencing

200 µL of serum sample were inactivated in 1 mL of TRIzol (Invitrogen), and nucleic acid extraction and purification were performed according to the manufacturer's instructions. Roche 454 pyrosequencing using randomly amplified cDNA libraries was performed as described previously [35]. Viral sequences were identified using BLASTn or BLASTx by comparison to the GenBank nonredundant nucleotide or protein database, respectively (E-score cutoff = 10^{-5}).

De novo Assembly of the BASV Genome by Illumina Sequencing

To recover additional BASV sequence, two sets of cDNA libraries were prepared from DNase-treated extracted RNA using a random PCR amplification method as described previously [36], or random hexamer priming according to the manufacturer's protocol (Illumina). The libraries were then pooled and sequenced on two lanes of an Illumina HiSeq 2000. Raw Illumina sequences consisting of 100 base pair (bp) paired-end reads were filtered to exclude low-complexity, homopolymeric, and low-quality sequences, and directly compared using BLASTn or BLASTx alignments to a library consisting of all rhabdovirus sequences in GenBank. The initial read obtained by 454 pyrosequencing as well as other reads aligning to rhabdoviruses were then inputted as "seeds" into the PRICE *de novo* assembler [37] (Fig. 2), with a criterion of at least 85% identity over 25-bp to merge two fragments. *De novo* assembly of the BASV genome was performed iteratively using PRICE and the Geneious software package (Biomatters) [38]. The near-complete whole genome sequence of the novel rhabdovirus (~98.2% based on protein homology to other rhabdoviruses) was determined to at least 3× redundancy by *de novo* assembly as well as PCR and Sanger sequencing of low-coverage regions. Sanger sequencing was also performed to verify the accuracy of the assembly and confirm the genomic organization of BASV (Fig. 2).

Deep Sequencing Analysis of the BASV Serum Sample for Other Pathogens

Rapid classification of the ~140 million 100-bp paired-end Illumina reads was performed using a modified cloud computing-based computational analysis pipeline [17] (Veeraraghavan, Sittler, and Chiu, manuscript in preparation). Briefly, reads corresponding to human sequences were taxonomically classified using SOAP and BLAT software [39,40]. Other reads were then identified using BLASTn or BLASTx by comparison to GenBank-derived reference databases (E-score cutoff = 10^{-5}).

PCR Quantitation of BASV Burden

To estimate the viral load in the patient's serum, we first designed a set of specific PCR primers for detection of BASV targeting the L protein, BASV-F (5′- CGCTGATGGTTTTTTGACATGGAAGTCC-3′)/BASV-R (5′-TAAACTTCCTCTCTCCTCTAG-3′), for use in a SYBR-Green real-time quantitative RT-PCR assay. A standard curve for the assay was constructed as described previously [36]. The viral load in the patient's serum was determined by comparison to the standard curve.

Structural Features and Phylogenetic Analysis

Predicted open reading frames (ORFs) in the BASV genome were identified with Geneious [38]. Multiple sequence (Figs. 3 and 4; Fig. S1) and pairwise (Fig. 5) alignments of BASV proteins relative to corresponding proteins from other rhabdoviruses were calculated using MAFFT (v6.0) with the E-INS-i option and at default settings [41]. To generate the phylogeny trees, all rhabdoviruses in GenBank were included as well as representative members of other families within the order *Mononegavirales*. Bayesian tree topologies were assessed with MrBayes V.32 software (20,000 sampled trees; 5,000 trees discarded as burn-in) [42]. Convergence was confirmed by the PSRF statistic in MrBayes, as well as by visual inspection of individual traces using TRACER from the BEAST software package [43]. Trees were visualized after midpoint rooting with FigTree V1.31 [43].

Virus Cultivation in Cell Cultures or Suckling Mice

Initial attempts were made to culture the virus using a total of 200 µL of BASV-positive serum inoculated onto confluent monolayers of Vero E6 and C6/36 (*Aedes albopictus* mosquito) cells in 6-well plastic tissue culture plates at 37°C and 28°C, respectively, in a 5% CO_2 environment as previously described [44]. From 20–50 µL of serum were used to inoculate the cells, which were examined daily for cytopathic effect (CPE) at days 5, 7, and 14. Supernatants were harvested and two additional blind passages were performed, each passage followed by 14 days of observation for CPE. Cell culture supernatants were also monitored for evidence of viral replication by quantitative RT-PCR.

Using the remaining 100 uL of BASV-positive serum, further attempts were made to culture the virus in 5 cell lines and in suckling mice. The serum sample was split in half and diluted 1:20 or 1:10 in phosphate-buffered saline with 20% fetal bovine serum (FBS) to allow sufficient volume to inoculate cell cultures or mice, respectively. The first diluted sample was inoculated intracerebrally into a litter (n = 12) of 1 day old mice. Pups were observed daily for 14 days for lethality or signs of clinical illness. The second diluted sample was inoculated into 12.5 cm^2 tissue culture flasks of Vero, BHK, LLC-MK_2 (rhesus monkey kidney), CCL-106 (rabbit kidney) and C6/36 cells. Vertebrate cells were held at 37°C for 14 days and observed for evidence of CPE. Mosquito cells were maintained at 28°C for 10 days. Since no CPE was observed in any of the cultures, cells were subsequently fixed for transmission electron microscopy to see if viral particles could be visualized [45].

Construction of VSVΔG-GFP Pseudotypes and BASV Serum Neutralization Testing

A pseudotype system based on a vesicular stomatitis virus (VSV) construct carrying a reporter gene for green fluorescent protein (VSVΔG-GFP) and bearing the predicted synthesized BASV glycoprotein (BASV-G) was used to generate a serum neutralization assay for BASV. Briefly, the predicted BASV glycoprotein (BASV-G) was synthesized (Genscript) and subcloned into the pCAGGS expression plasmid. Human embryonic kidney 293T cells were seeded (DMEM + 10% FBS + penicillin/streptomycin + Glutamax (Gibco) + non-essential amino acids (Gibco)) in 10 cm culture dishes 24 hours prior to transfection. Cells were transfected with 20 µg BASV-G, VSV-G, or empty pCAGGS DNA per dish following a calcium phosphate transfection protocol [46]. The culture medium was replaced 15 hours post-transfection and cells were stimulated with 6.2 mM valproic acid for 4 hours before the medium was replaced again. At 36 hours post-transfection the transfected cells were infected with VSVΔG-GFP/VSV-G pseu-

dotypes at a multiplicity of 0.1–0.3. The inoculum was removed after 4 hours and replaced by fresh culture medium. At 24 hours post-infection, infectious supernatants were harvested, filtered through 0.45 µm filters, and concentrated 10-fold by centrifugation through a 100-kDA filter (Millipore). Concentrated viruses were aliquoted and stored at −80°C.

For serum neutralization testing, human hepatoma Huh-7 cells were seeded (DMEM +10% FBS + penicillin/streptomycin + Glutamax (Gibco) + non-essential amino acids (Gibco)) in 48-well plates 24 hours prior to infection. Per well 10 µl of pseudovirus harboring either BASV-G or VSV-G (adjusted to obtain 25–50% infection of target cells) was mixed with 10 µl of the respective serum dilution and incubated for 45 minutes at 37°C. Subsequently, the mix was added to the target cells (performed in triplicate) and cells were incubated for 24 hours at 37°C. The infected cells were detached with trypsin and washed with PBS before fixing with 2% paraformaldehyde for 1 hour at room temperature. GFP expression in infected cells was quantified by flow cytometry using a LSR II (BD Biosciences) and the collected data was analyzed with FlowJo software (TreeStar).

Abbreviations and Nucleotide Sequence Accession Numbers

The annotated, nearly complete sequence of BASV has been submitted to GenBank (accession number JX297815). Deep sequencing reads have been submitted to the NCBI Sequence Read Archive (accession number SRA056894). Accession numbers used for the phylogenetic analyses in Figs 3, 4, and S1 are listed as follows, in alphabetical order: ABLV, Australian bat lyssavirus (NP_478343); ARAV, Aravan virus (ABV03822), BEFV, Bovine ephemeral fever virus (NP_065409); BYSMV, Barley yellow striate mosaic virus (BYSMV); CDV, Canine distemper virus (AAR32274); CHPV, Chandipura virus (ADO63669); CPV, Coastal Plains virus (ADG86364); COCV, Cocal virus (ACB47438); DURV, Durham virus (ADB88761); DUVV, Duvenhage virus (ABZ81216); EBLV1, European bat lyssavirus 1 (ABZ81166), EBLV2, European bat lyssavirus 2 (ABZ81191); EBOV, Ebola virus (AAG40171, AAA79970, BAB69010); EVEX, Eel virus European X virus (CBH20130); FDLV, Fer-de-lance virus (NP_899661); FLAV, Flanders virus (AAN73288); HeV, Hendra virus (NP_047113); HIRRV, Hirame rhabdovirus (ACO87999); HMPV, Human metapneumovirus (L_HMPVC); HPIV-1, Human parainfluenza virus type 1 (AA A69579); HPIV-2, Human parainfluenza virus type 2 (CAA 40788); HPIV-3, Human parainfluenza virus type 3 (AAA46854); HPIV-4, Human parainfluenza virus type 4 (BAJ11747); INHV, Infectious hematopoietic necrosis virus (NP_042681); IRKV, Irkut virus (ABV03823); ISFV, Isfahan virus (CAH17548); KHUV, Khujand virus (ABV03824); LBV, Lagos bat virus (ABZ81171); LNYV, Lettuce necrotic yellows virus (YP_425092); MARAV, Maraba virus (AEI52253); MARV, Marburg virus (YP_001531159); MeV, Measles virus (AF266288); MMV, Maize mosaic virus (YP_052855); MOKV, Mokala virus (ABZ81206); MOUV, Moussa virus (ACZ81407); MUV, Mumps virus (AF 201473); NCMV, Northern cereal mosaic virus (NP_597914); NDV, Newcastle disease virus (ADH10207); NGAV, Ngaingan virus (YP_003518294); NiV, Nipah virus (AAY43917); OVRV, Oak Vale rhabdovirus (AEJ07657); PFRV, Pike fry rhabdovirus (ACP28002); RABV, Rabies virus (NP_056797); RSV, Respiratory syncytial virus (NP_056866); RYSV, Rice yellow stunt rhabdovirus (NP_620502); SIGMAV, Sigma virus (ACU65444); SCRV, Siniperca chuatsi rhabdovirus (YP_802942); SHRV, Snakehead virus (AAD56771); SMRV, Scophthalmus maximus rhadovirus (ADU05406); SVCV, Spring viremia of carp virus (NP_116748); SYNV, Sonchus yellow net virus (NP_044286); TIBV, Tibrogargan virus (ADG86355); TUPV, Tupaia virus (AAX47602); TVCV, Tomato vein clearing virus (YP_224083); VHSV, Viral hemorrhagic septicemia virus (NP_049550); VSIV, Vesicular stomatitis virus, Indiana (NP_041716); VSNJV, Vesicular stomatitis virus, New Jersey (P16379); WCBV, West Caucasian bat virus (ABV03821); WONGV, Wongabel virus (YP_002333280).

Supporting Information

Figure S1 Phylogenetic analysis of the N, P, M, and G proteins of BASV and other rhabdoviruses. Each phylogenetic tree is rooted by using the corresponding protein from human parainfluenza virus type 1 (HPIV-1), a paramyxovirus, as an outgroup. Abbreviations and accession numbers used for the phylogenetic analysis are provided in Methods.
(TIF)

Figure S2 Confirmation of laboratory contamination by rotavirus and absence of rotavirus in BASV serum by specific PCR. An RT-PCR assay for detection of Group A rotaviruses was performed using primers NSP3F (5′-ACCATCTWCACRTRACCCTCTATGAG-3′) and NSP3R (5′-GGTCACATAACGCCCCTATAGC-3′), which generate an 87-bp amplicon (Freeman, et al., (2008) J Med Virol 80: 1489–1496). PCR conditions for the assay were 30 min at 50°C, 15 min at 95°C for the reverse transcription step followed by 40 cycles of 95°C, 30 s/55°C, 30 s/72°C, 30 s and 72°C/7 min for the final extension. PCR products are visualized by gel electrophoresis, using a 2% agarose gel and 1 kB ladder. Rotavirus is readily detected in extracted RNA from a stool sample taken from an ongoing study of viral diarrhea in the laboratory (lane 1), but not in two separate aliquots of extracted nucleic acid from the BASV serum sample (lanes 2 and 3).
(TIF)

Table S1 Viral reads in the deep sequencing data corresponding to the BASV-positive serum sample.
(DOCX)

Table S2 Demographics of 50 blood donors from Kasai-Oriental province, DRC, randomly selected for BASV antibody screening.
(DOCX)

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Author Contributions

Conceived and designed the experiments: GG JNF DL GS ED NDW CYC EML. Performed the experiments: GG DL ES IS RBT. Analyzed the

data: GG JNF DL J-JM NV MM PM GS ED NDW CYC EML. Contributed reagents/materials/analysis tools: TS JGR CW RBT JM AWR TT BSS GS ED NDW CYC EML. Wrote the paper: GG JNF DL IS RBT GS ED NDW CYC EML. Obtained consents from patients and their families: PM.

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Spillover and pandemic properties of zoonotic viruses with high host plasticity

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Christine Kreuder Johnson[1], Peta L. Hitchens[1], Tierra Smiley Evans[1], Tracey Goldstein[1], Kate Thomas[1], Andrew Clements[2], Damien O. Joly[3], Nathan D. Wolfe[3], Peter Daszak[4], William B. Karesh[4] & Jonna K. Mazet[1]

Most human infectious diseases, especially recently emerging pathogens, originate from animals, and ongoing disease transmission from animals to people presents a significant global health burden. Recognition of the epidemiologic circumstances involved in zoonotic spillover, amplification, and spread of diseases is essential for prioritizing surveillance and predicting future disease emergence risk. We examine the animal hosts and transmission mechanisms involved in spillover of zoonotic viruses to date, and discover that viruses with high host plasticity (i.e. taxonomically and ecologically diverse host range) were more likely to amplify viral spillover by secondary human-to-human transmission and have broader geographic spread. Viruses transmitted to humans during practices that facilitate mixing of diverse animal species had significantly higher host plasticity. Our findings suggest that animal-to-human spillover of new viruses that are capable of infecting diverse host species signal emerging disease events with higher pandemic potential in that these viruses are more likely to amplify by human-to-human transmission with spread on a global scale.

Emerging, re-emerging, and endemic zoonotic diseases continue to place a substantial burden on global health, particularly where dense human populations and pressures on environmental and economic resources are greatest. Over one billion cases of human zoonotic disease are estimated to occur annually, and novel emerging zoonoses have resulted in hundreds of billions of dollars in economic losses[1]. Given the rich diversity of animal life on our planet, it is not surprising that animals are the source of most human infectious diseases, with centuries of intimate contact with domesticated species facilitating the early transmission of the most adaptable pathogens to humans[2]. Recent recognition that the majority of emerging infectious disease events have wildlife origins[3] highlights the need for a deep understanding of the type of contact between wild animals and people that enables disease transmission. Opportunities for close contact between humans and wild animals are relatively rare compared to contact with domestic animals, yet recent emergence of many diseases, such as severe acute respiratory syndrome, Nipah virus encephalitis, and Ebola, highlight the threat that wildlife pathogens pose to global health security[4].

After centuries of documented outbreaks, we have now begun to unravel the mechanisms underlying disease transmission from animals to people. Here, we focus on zoonotic viruses, which are the most frequently emerging human pathogen, constituting less than 15% of all known species of human pathogens, but over 65% of pathogens discovered since 1980[5]. We seek to understand the mechanisms facilitating transmission of viruses from animals to people, with special attention to the human activities enabling direct and indirect contact with wild animal hosts resulting in recent human outbreaks. By evaluating data reported for all known zoonotic viruses, we test long-held assumptions regarding common traits among viruses that have spilled over from animals and activities facilitating their transmission. We use network analyses to evaluate sharing of viruses by animal hosts and high-risk transmission interfaces

[1]One Health Institute, School of Veterinary Medicine, University of California, Davis, CA USA. [2]USAID, Bureau for Global Health, Washington DC, USA. [3]Metabiota, San Francisco, CA USA. [4]EcoHealth Alliance, New York, NY USA. Correspondence and requests for materials should be addressed to C.K.J. (email: ckjohnson@ucdavis.edu)

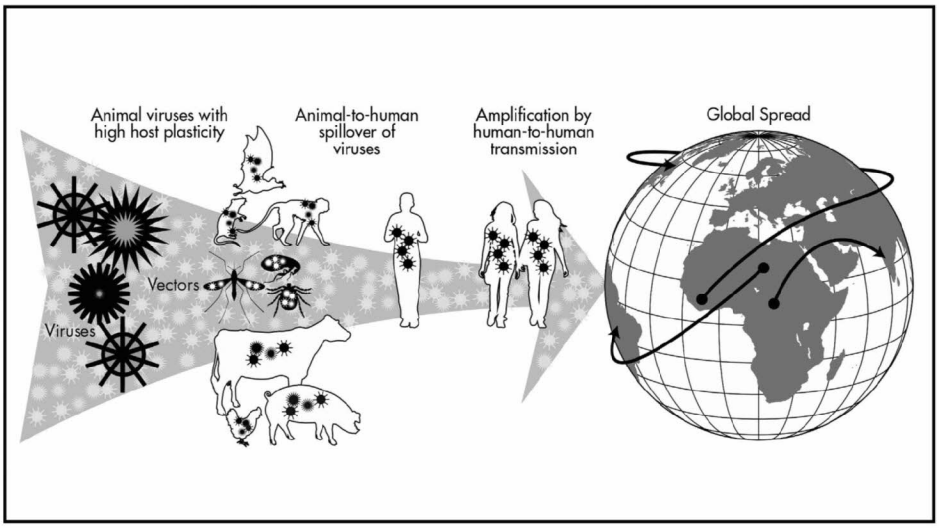

Figure 1. Pandemic properties of zoonotic viruses that spill over from animals to humans and spread by secondary transmission among humans. Key characteristics of pandemic potential that were evaluated for associations with viral traits and high-risk disease transmission interfaces include host plasticity, human-to-human transmissibility, and geographic distribution. Human practices that promote transmission of mutation-prone RNA viruses able to infect a wide range of taxonomically diverse hosts, including wild and domestic animals, act synergistically to facilitate viral emergence, particularly for viruses capable of human-to-human transmission and broad geographic spread (map and illustration created using Adobe Illustrator CS6).

involving wildlife, and we use regression modeling to identify human activities linked to key pandemic properties among viruses including viral sharing among taxonomically diverse hosts, amplification by human-to-human transmission, and international spread (Fig. 1). Our findings uncover key transmission mechanisms involved in zoonotic virus emergence to inform global disease surveillance and preventive measures needed to mitigate zoonotic threats.

Results

Through systematic evaluation of data reported in the scientific literature on zoonotic viruses, we identify several key virus characteristics and transmission mechanisms that are synergistic to zoonotic virus spillover, amplification by human-to-human transmission, and global spread. The majority (94%) of zoonotic viruses described to date ($n = 162$) are RNA viruses, which is 28 times higher (95% CI 13.9–62.5, exact $P < 0.001$) than the proportion of RNA viruses among all vertebrate viruses recognized, indicating that RNA viruses are far more likely to be zoonotic than DNA viruses, as has been reported among human pathogens[6]. Epidemiological circumstances involved in recent zoonotic transmission from animals to people are summarized here for 95 viruses with data on human activities enabling direct and indirect contact disease transmission and animal host taxa implicated in transmission. In general, wild animals were suggested as the source of zoonotic transmission for 91% (86/95) of zoonotic viruses compared to 34% (32/95) of viruses transmitted from domestic animals, and 25% (24/95) with transmission described from both wild and domestic animals (see Supplementary Table). Wild animals, which include a taxonomically diverse range of thousands of species, were significantly more likely to be a source for animal-to-human spillover of viruses than domesticated species (exact $P = 0.001$). Wild rodents were implicated as a source of spillover for 58% (55/95) of zoonotic viruses, particularly for zoonotic arenaviruses ($n = 8/8$, exact $P = 0.019$) and zoonotic bunyaviruses ($n = 20/24$, exact $P = 0.004$). Primates were implicated as a source of zoonotic retroviruses (exact $P = 0.017$), while bats were more implicated for zoonotic paramyxoviruses (exact $P = 0.011$) and most zoonotic rhabdoviruses (6/8, exact $P = 0.002$).

Emerging pathogens have been noted for their ability to infect a range of animal hosts[5,7–10]. We find that most (63%) zoonotic viruses infecting humans were reported in animal hosts from at least two different taxonomic orders, and 45% were reported in four or more orders, in addition to humans. The virus-host unipartite network illustrates high connectivity among host groups sharing zoonotic viruses and the central role domestic animals play in cross-species transmission (Fig. 2). In a Poisson model predicting host range and evaluating common hosts and high-risk transmission interfaces, viruses with domestic animal hosts occurred in twice as many host orders than other viruses (Table 1). Most domestic animal groups clustered in the middle of the host network with high centrality measures and a high number of shared viruses (Fig. 2), indicating that domestic animals play a key role in cross-species transmission of zoonotic viruses. Among viruses from wildlife, we found higher host plasticity (ie, hosts from a higher

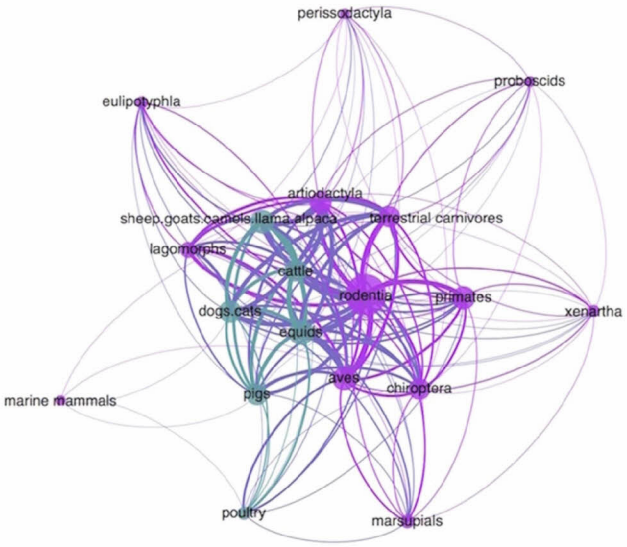

Figure 2. Host unipartite network map showing high host plasticity among zoonotic viruses with wild and domestic animal hosts connected by shared viruses. High connectivity between hosts by more shared viruses is evident for domestic animal hosts (green) and wild animal hosts (purple) that are most centrally located. Host node size is proportionate to the number of connections each host has to another host based on shared viruses. The width of each edge connecting hosts is relative to the number of viruses shared by the connection between hosts.

number of taxonomic orders) in viruses transmitted at high-risk interfaces involving wild animals kept as pets, maintained in sanctuaries or zoos, and sold at markets, which were collapsed into one category due to similar effect and significance in the final Poisson model. We also found that vector-borne viruses were reported in three times the number of host taxonomic groups than non-vector-borne viruses, indicating that vector-borne pathogens have significantly broader host range than non-vector-borne viruses.

Based on data published to date, transmission of zoonotic viruses to humans occurs by direct or indirect contact with wildlife in a diverse array of interconnected animal-to-human interfaces, with little overlap with viruses transmitted primarily by vectors (Fig. 3). Zoonotic virus spillover from wildlife was most frequent in and around human dwellings and in agricultural fields, as well as at interfaces with occupational exposure to animals (hunters, laboratory workers, veterinarians, researchers, wildlife management, zoo and sanctuary staff). Primate hosts were most frequently cited as the source of viruses transmitted by direct contact during hunting (exact $P = 0.051$) and in laboratories (exact $P = 0.009$), while rodent hosts were more likely to be implicated in transmission by indirect contact in and around human dwellings (exact $P < 0.001$) and in agricultural fields (exact $P = 0.001$). Approximately 40% of zoonotic viruses involving wild animals required arthropod vectors for transmission to humans, with vectors providing an effective bridge for transmission of diseases from wild animals that do not normally contact humans. Zoonotic viruses with wild avian hosts were most likely to involve vectors (exact $P < 0.001$). Network analysis of disease transmission from wild animals illustrates that vector-borne viruses were the least connected to other transmission interfaces (Fig. 3), consistent with effective control of vector-borne diseases by elimination of vectors or contact with vectors. In contrast, 22% of viruses transmitted from domestic animals to humans were by vector only, with close proximity interactions with domestic animals enabling direct pathogen transmission to humans.

Once animal viruses have spilled over into humans, human-to-human transmission of zoonoses facilitates sustained spread of disease with a rapidity and reach infeasible for zoonotic viruses requiring contact with animal hosts for each transmission opportunity. Human-to-human transmissibility was described for 20% of zoonotic viruses investigated here (Supplementary Table). We find virus host plasticity to be positively correlated with capability for human-to-human transmission (Table 1). In a logistic regression model predicting virus capability for human-to-human transmission, we find viruses were significantly more likely to be human-to-human transmissible with each increase in virus host plasticity (count of host orders and ecological groups). Furthermore, we find viruses in the arenaviridae and filoviridae families to be more likely to possess human-to-human transmissibility, along with viruses transmitted by direct contact with hunted and consumed wildlife (Table 1). Hunting poses special risk for cross-species disease transmission of blood-borne zoonotic viruses[11,12] as evidenced by re-emerging threats, including ebolaviruses[13] and primate retroviruses[14–16]. Our findings therefore support speculation that hunting of high-risk host species carries an increased probability of spillover of zoonotic viruses that can be further spread by human-to-human transmission[13].

Poisson regression predicting virus host plasticity (number of host groups)[a]			
	Incidence Rate Ratio	**P value**	**(95% CI)**
Transmission from domestic animals to humans	1.97	<0.001	(1.56–2.49)
Transmission by direct contact with wildlife at markets	2.00	0.040	(1.03–3.88)
Transmission by direct contact with wild animals kept as pet or in zoos or sanctuaries	1.55	0.039	(1.02–2.34)
Transmission by vector	3.01	<0.001	(2.32–3.91)
Logistic regression predicting human-to-human transmissibility[b]			
	Odds Ratio	**P value**	**(95% CI)**
Host plasticity (number of host groups)	1.20	0.039	(1.01–1.44)
Transmission by direct contact with wild animals hunted or consumed*	10.43	0.004	(2.10–51.80)
Ordered logistic regression predicting geographic spread[c]			
	Odds Ratio	**P value**	**(95% CI)**
Host plasticity (number of host groups)	1.22	0.001	(1.08–1.37)
Transmission by direct contact with wild animals in trade or laboratories	6.14	0.014	(1.45–26.10)

Table 1. Host and epidemiologic correlates of zoonotic virus emergence. Multivariable regression models with viral traits and transmission interfaces significantly associated with zoonotic virus host plasticity, human-to-human transmissibility, and geographic spread. [a]Viral family was included as a main effect in the model. Viral families significantly related to number of host orders were reovirus (IRR = 2.07 (1.21–3.55), P = 0.008), rhabdovirus (IRR = 1.59 (1.13–2.24), P = 0.008), and a collapsed virus family group with bornavirus and hepatitis E virus (IRR = 4.48 (2.77–7.25), P < 0.001). [b]Viral family was included as a main effect in the model. Viral families with a significantly higher probability of human-to-human transmission were arenavirus (OR = 62.6 (8.09–485), P < 0.001) and filovirus (OR = 52.92 (3.90–719), P = 0.003). [c]Virus family was included as a random effect using robust standard error estimation clustered on virus family. *High-risk disease transmission interface categories 'hunting' and 'consumed' were similar in their association with virus capability for human-to human transmission so these categories were collapsed for this model.

We further characterized zoonotic virus capacity for spread by categorizing viruses according to geographic range in a single country (16%), >1 country in 1–3 World Health Organization-defined (WHO) regions (55%), or ≥4 WHO regions (29%), and used ordinal logistic regression to evaluate characteristics of viruses in broader range categories. We find viruses were more likely to be in broader geographic range categories with increasing host plasticity (Table 1). Among all high risk interfaces and hosts, only viruses transmitted to humans by contact with wild animals in the wildlife trade and in laboratories, such as lymphocytic choriomeningitis virus[17], monkeypox virus[18], herpes B virus[19], and Marburg[20], were more likely to have broader geographic reach.

Discussion

Wild animals were implicated as a source of disease spillover to humans for the vast majority of zoonotic viruses, further substantiating the concept that the diversity of wildlife on our planet has provided a rich pool of viruses, a fraction of which have successfully adapted to infect humans. Our findings indicate that high viral host plasticity is an important trait that is predictive of pandemic potential of viruses in the zoonotic pool, not only because wide host range was common among viruses that have spilled over from animals to humans, but also because this trait was associated with increased human-to-human transmission and spread on a global scale. Reporting bias must be considered in the interpretation of any association based on data reported in the literature, and the relationship between human-to-human transmissibility and host plasticity could be biased by increased research effort for viruses that have been shown to be transmissible among humans. However our analyses identified a strong linear relationship between host plasticity and likelihood of human-to-human transmissibility, and we estimate zoonotic viruses found in 10 host orders are 12 times more likely to be human-to-human transmissible than zoonotic viruses found in only one animal host order. Human-to-human transmission of viruses with high host plasticity is consistent with the hypothesis that evolutionary selection for viruses with greater ability to adapt rapidly to new hosts co-selects for viruses capable of effective intraspecies transmission in the new host. Evolutionary selection of viruses capable of infecting a wide range of hosts has been a key hypothesis underpinning disease emergence theory[7,21], and we provide evidence for the importance of viral host plasticity as a synergistic trait aiding mechanisms of disease transmission, particularly at the high-risk human-animal interfaces reported here.

Human practices facilitating heightened contact between taxonomically diverse animal hosts has likely facilitated selection of viruses with high host plasticity and sharing of zoonotic diseases. Zoonotic

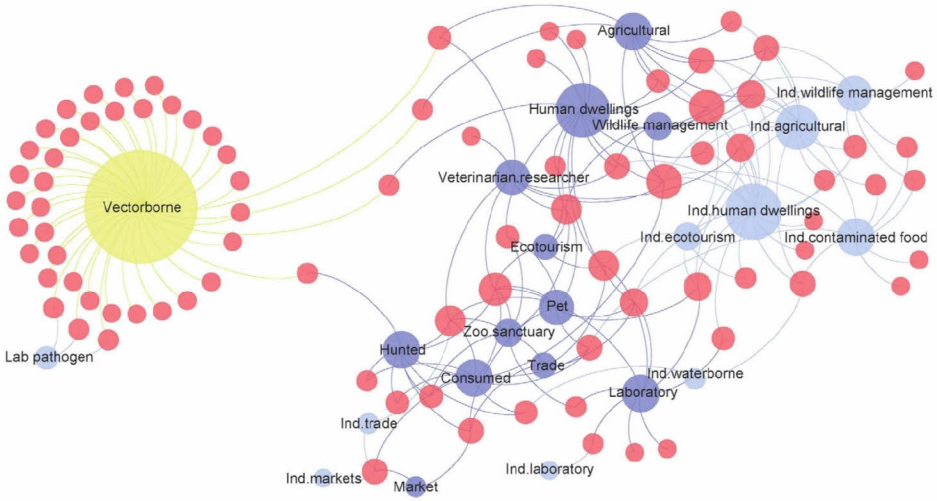

Figure 3. Epidemiologic bipartite network map showing high-risk disease transmission interfaces shared by zoonotic viruses transmitted from wildlife to humans. High-risk interfaces are shown with node size proportionate to the number of viruses reported for each transmission interface, categorized according to (1) direct contact with wildlife (dark blue), (2) indirect contact with wildlife (light blue), and (3) transmission by vector (yellow). Virus node size (red, n = 86 viruses) reflects the number of connections to different transmission interfaces and ecological plasticity of viruses through use of multiple transmission opportunities. Highly connected and more central interfaces facilitated transmission of more viruses, providing an epidemiologic picture of circumstances likely to promote future disease emergence, and important targets for disease surveillance and preventive measures.

viruses reported in domestic animals had a significantly wider host range than viruses not shared by domesticate species. Increased research effort targeting diseases in domesticated species could bias data towards this finding, but we also detected increased host range among viruses transmitted by wildlife kept in similarly confined circumstances. Increased host plasticity among viruses shared by domestic animals supports the concept that the breeding and keeping of taxonomically diverse domesticated species in regular close contact with people for centuries has enabled evolutionary adaptive selection for mutation-prone RNA viruses capable of cross-species transmission[2]. For the many viruses shared by wildlife and domestic animals, domesticated species play a critical role in facilitating direct contact with people, as well as amplification of disease transmission in intensive animal production facilities.

Our finding of significantly higher host plasticity among viruses transmitted by direct contact with wildlife kept as pets or in zoos and sanctuaries provides additional evidence to support the premise that confining taxonomically diverse species in close proximity selects for transmission of adaptable viruses with high host plasticity, even among wildlife. Diverse species of wild animals that are confined in zoos, sanctuaries, kept as pets, and sold at markets are also subject to circumstances that facilitate cross-species virus transmission via intimate contact, particularly for zoonotic viruses already adapted to transmission among domesticated animals. Vectorborne transmission similarly enables opportunities for effective contact across diverse animal hosts, which is consistent with our finding of higher host plasticity among vectorborne viruses. Through this mechanism, vector-borne transmission has facilitated emergence of animal diseases in humans, particularly those from wildlife, and, for viruses with generalist vectors, this transmission route is an effective method for interspecific dispersal[6].

Here we provide an epidemiologic picture of the animal-human transmission networks likely to perpetuate future disease emergence, and our findings add to previous efforts to guide global health research geographically[3]. In addition to an emphasis on vector control, the myriad of other high-risk interfaces with human activities that have facilitated animal-to-human viral spillover should be a focus for education and interventions directed at disease prevention. More in depth investigation of the epidemiology of zoonoses at high risk human-animal interfaces is needed to assess risk of viral disease emergence and direct global, as well as local, disease prevention and control. Risk for a new human pandemic is likely highest at the high-risk interfaces facilitating disease threats in the past. Unfortunately, wild animal hosts and high-risk interfaces facilitating spillover of zoonotic viruses, particularly beyond their first emergence, remains vastly under-reported. Adequate data on circumstances at the point of disease spillover are lacking for many viruses because animal involvement in zoonotic disease exposure is very difficult to ascertain and this information is often not linked to diagnoses in published reports. Global animal disease data are largely incomplete due to inadequate livestock and wildlife health surveillance worldwide. Resulting ascertainment biases are especially problematic for spillover events that do not involve professions likely to self-report, as is likely the case for veterinarians, researchers, and scientists working

at laboratory facilities. Detailed patient histories that elucidate activities precipitating animal exposure will greatly assist in completing the epidemiologic picture underlying the emergence of many zoonotic viruses. This, together with heightened surveillance to gather data on human practices enabling contact with animals in settings with diverse host assemblages, particularly at high-risk interfaces under-reported to date, will direct us towards critical control points for disease control and behavior change interventions aimed at prevention.

Methods

Zoonotic Virus Datasets. Peer-reviewed scientific literature was searched for reports on zoonotic viruses transmitted from animals to humans using the Web of Science electronic library database for published reports through 2010. An initial list of zoonotic pathogens was established with database searches for topic keywords (zoonotic, zoonoses, and infectious animal disease, emerging wildlife disease) and cross checked with World Health Organization (WHO), Food and Agricultural Organization (FAO), Centers for Disease Control and Prevention (CDC), and the World Organization for Animal Health (OIE) web-based reports, and previously published compilations of human infectious diseases and human emerging infectious disease events[3,22]. Individual pathogen-specific searches using the Web of Science database were then made using pathogen common and scientific names to identify general transmission properties and specific circumstances involved in disease transmission from animals to humans reported in the peer-reviewed literature. Among 162 zoonotic viruses, data on animal hosts and human activities associated with naturally occurring animal-to-human transmission from 1990–2010 were collated and summarized for each virus. Viral family categories and virus genome characteristic (RNA vs DNA) was compiled using the National Center for Biotechnology Information[23].

Zoonotic viruses were included in analyses of interfaces and hosts if data were available on the circumstances surrounding virus transmission from animals to humans from 1990–2010 in scientific reports searched as described above (n = 95 viruses, Supplementary Table). Viral transmission from animals to humans was determined as documented infection or seroconversion, without regard to disease severity. General transmission categories were used to summarize disease transmission by i) direct or indirect contact with wild animals, ii) transmission from direct or indirect contact with domestic animals, iii) transmission by vector involving a wildlife host, domestic animal host, or both. Each virus was also categorized as human-to-human transmissible if horizontal human-to-human transmission was reported, as for transmission from animals to humans (by documented infection or seroconversion, without regard to disease severity) based on search of all reports for each virus in the scientific literature. For this study, human-to-human transmission excluded transmission between humans by vectors.

For all viruses transmitted from wildlife, data on circumstances of transmission were collated from all reports for each virus to identify interfaces that best described the human activities suspected or confirmed to enable effective contact and natural (ie non-experimental) transmission of zoonotic viruses to people. Transmission interfaces involving wildlife were stratified by direct and indirect contact transmission and summarized in categories describing human contact as follows i) wild animals in and around human dwellings, ii) wild animals hunted, iii) wild animals consumed, iv) wild animals kept as pets, v) wild animals housed in laboratories, vi) wild animals sold in markets, vii) wild animals kept in zoos and sanctuaries, viii) wild animal exposure during agricultural activities, ix) wild animal exposure during ecotourism activities, x) wild animal exposure during wildlife management activities in protected areas, xi) virus exposure in laboratory settings (lab pathogen), and xii) virus exposure via contaminated water.

For all viruses included in analyses, an extended search was conducted to identify confirmed or suspected hosts serving as a source of spillover as reported in the peer-reviewed literature based on virus detection by molecular assay, serological assay, or virus isolation. Animal species included were implicated in the scientific reports as hosts suspected in animal-to-human transmission of a given virus, either through direct contact, indirect contact or vector-borne transmission. Host species were then classified a priori according to ecological circumstances for human contact (domesticated species, wild terrestrial species, and wild marine mammal species), which we expected to modify any potential host-pathogen phylogenetic relationships based on purely taxonomic classification. Stratification of animal host categories according to general circumstances of human contact was also important so that analyses could inform on risk interfaces and intervention strategies. Wild terrestrial host species were then categorized further by taxonomic order, except for orders within the superorder xenarthra, which were collapsed into one category (n = 6 zoonotic viruses). Marine mammal orders were also combined due to sparse data, as marine mammals were implicated in spillover of only 3 zoonotic viruses. Due to the large number of viruses reported in domesticated animals, domestic species were grouped according to taxonomy and stratified by similarity in circumstances for human use of animals and their products; i) cattle, ii) equids, iii) goats, sheep, llamas, alpaca, camels, iv) pigs, v) poultry (chickens, ducks, geese), and vi) dogs and cats. Virus host range (host plasticity) was calculated as the total count of animal taxonomic orders and ecological groups recognized as hosts involved in animal-to-human spillover for each virus.

A literature search was similarly conducted to identify geographic range reported for each virus in humans and animals. Geographic distribution in animals and people encompassed importation to another country by infected persons or animals if secondary amplification by animal-to-human or human-to-human transmission occurred. Zoonotic viruses were further classified according to 3 categories of international spread based on published reports as to whether viruses had been reported within

1) a single country only, 2) more than one country but only 1–3 WHO regions, or 3) more than one country and ≥4 WHO regions[24] (Supplementary Table).

While search effort was standardized for all viruses in our approach to the literature review, viruses varied in the number of scientific reports available describing their traits, hosts, and geographic range. All virus, host species, and interface data were summarized as binomial variables for each individual virus, in order to account for a variable number of reports and documented spillover events per virus, and adjust for likely increased research effort for viruses that infect humans and domesticated species. Each virus was designated as the unit of analysis for which we compared viral traits, animal hosts involved in spillover, and human activities noted at the point of spillover.

Statistics. Virus genome category (RNA vs DNA) was compared between zoonotic viruses (n = 162) and all viruses reported to infect humans and other vertebrates minus the zoonotic viruses (n = 956) using Fisher's exact test. Bipartite affiliation (two-mode) networks were generated for virus-host and virus-interface matrix data to evaluate connectedness between host orders and between high-risk disease transmission interfaces involving wildlife. Betweenness centrality was measured for all viruses to indicate the number of connections with wild and domestic animal hosts in the virus-host network, along with the centrality of each virus within the host network, relative to all other zoonotic viruses. Betweenness centrality for each virus was calculated as the number of geodesic paths that pass through a node, standardized by the total number of virus nodes in the network, multiplied by 100. A unipartite (one-mode) network was generated to illustrate host taxonomic orders and groups connected by shared viruses. Network analyses were conducted in the network analysis platform Gephi, using the force-directed algorithm ForceAtlas2 to generate a virus-interface network display[25]. Centrality indices were normalised for two-mode data[26] using specialized software for social network analysis (UCINET 6 for Windows).

Unadjusted bivariate relationships between viral family, interface categories, and host taxa were examined using exact statistics. Viral traits and transmission circumstances were further evaluated for multivariable associations with virus host plasticity using Poisson regression to evaluate the influence of putative viral traits and high-risk interfaces on the count of host taxonomic orders and ecological groups reported for each virus. Factors evaluated for their relationship with host range included viral family, general transmission category involving domestic animals, wild animals, or vectors, and specific direct and indirect contact wildlife transmission interfaces. Incidence rate (indicating count of host orders) ratios were estimated for all significant independent factors associated with virus host range in the Poisson model ($P < 0.05$). Viral traits, general transmission categories, wildlife transmission interfaces, and virus host plasticity measures were similarly evaluated for associations with virus capability for human-to-human transmission. Virus host plasticity, general transmission categories, and wildlife transmission interfaces were also evaluated for associations with international spread using ordered logistic regression adjusting for clustering of random effects within virus family.

For all multivariable models, putative risk factors with $P < 0.20$ in univariable analyses were entered forward stepwise and retained in models if $P < 0.05$. Correlated variables were not included in the same model but deviance measures were used to evaluate changes in model fit to the data with each parameter independently. In all models, variables with <3 categories were evaluated for difference in magnitude, direction, and significance of effect between categories using the likelihood ratio statistic and similar categories were collapsed. Overall model fit was evaluated using Hosmer-Lemeshow goodness-of-fit test and measures of information criteria. Incidence rate and odds ratios were estimated with 95% confidence intervals. Univariable and multivariable statistical analyses were conducted using STATA 13.1 SE (College Station, TX, USA).

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Author Contributions

C.K.J., P.L.H., T.G., A.C., D.O.J., N.D.W., P.D., W.B.K. and J.K.M. designed the research, C.K.J., P.L.H., T.S.E. and K.T. conducted the literature search, C.K.J. and P.L.H. developed analytical models and analyzed data, C.K.J. wrote the manuscript with contributions and review by all authors.

Additional Information

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Rift Valley fever virus: Unanswered questions

Brian H. Bird [a, b, *], Anita K. McElroy [a, c]

[a] Viral Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA
[b] One Health Institute, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
[c] Pediatric Infectious Disease, Emory University Atlanta, GA 30322, USA

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ABSTRACT

This mosquito-borne pathogen of humans and animals respects no international or geographic boundaries. It is currently found in parts of Africa, Madagascar, and the Arabian Peninsula where periodic outbreaks of severe and fatal disease occur, and threatens to spread into other geographic regions. In recent years, modern molecular techniques have led to many breakthroughs deepening our understanding of the mechanisms of RVFV virulence, phylogenetics, and the creation of several next-generation vaccine candidates. Despite tremendous progress in these areas, other challenges remain in RVF disease pathogenesis, the virus life-cycle, and outbreak response preparedness that deserve our attention. Here we discuss and highlight ten key knowledge gaps and challenges in RVFV research. Answers to these key questions may lead to the development of new effective therapeutics and enhanced control strategies for this serious human and veterinary health threat.

1. Introduction

Rift Valley fever virus (RVFV; family *Bunyaviridae*, genus *Phlebovirus*) is a mosquito-borne human and veterinary pathogen associated with large outbreaks of severe disease in parts of Africa and the Arabian Peninsula (Nanyingi et al., 2015; Pepin et al., 2010). The virus is a complex and classic example of the multi-faceted intersection of human and animal health and vector ecology that characterize the "One-Health" aspects of many zoonotic arboviruses (Fig. 1) (Bird et al., 2009; Linthicum et al., 1988; Swanepoel and Coetzer, 1994). The hallmark of RVFV outbreaks is widespread abortion and lethality among livestock animals. Human cases result primarily from transmission from infected mosquitoes or contact with virus contaminated livestock tissues, fluids, or aborted materials. The majority of human cases are self-limiting, but a small percentage can progress to more serious sequelae including hepatitis, retinitis, delayed onset encephalitis, and most severely a hemorrhagic syndrome with high case fatality.

The rapid and sudden development of thousands to tens of thousands of human cases and the widespread agricultural impact of RVFV on potentially millions of livestock adds an enhanced layer of complexity to control strategies. RVFV is the only significant hemorrhagic fever virus of humans that also causes high-level mortality and morbidity in livestock, thus threatening both human and animal health and our food supply. RVF control programs must balance the needs of multiple stakeholders ranging from individually affected human patients, local livestock herdsmen, medical and veterinary practitioners, national level authorities working in public health, agriculture, and food-safety, and importantly a general public who is increasingly concerned about animal welfare issues and the impacts of emerging viruses (Bird and Nichol, 2012; Kortekaas, 2014).

Even though RVFV has been studied for more than 80 years, many aspects of its maintenance and ecology in endemic areas, mechanisms of expansion to other regions, and basic questions related to disease pathogenesis remain unanswered. The development of modern molecular techniques, such as full genome sequencing and reverse genetics systems, have dramatically increased our understanding of the evolutionary history and genomics of the virus and some aspects of its virulence mechanisms, yet as this molecular-level understanding has increased substantially over the past 25 years, other areas have lagged behind. Herein, we highlight ten key unanswered questions ranging from the host factors underlying RVF disease pathogenesis, to virus ecology, to control and eradication plans that we feel warrant additional study and research priority.

2. RVFV pathogenesis

2.1. What genetic factors and co-morbidities contribute to severe disease outcomes in humans?

The majority of human cases are relatively mild and never progress to severe or life-threatening disease. However, in the 1–2% of cases that do become severe, what underlying host factors are responsible? Identifying human genetic factors such as HLA type or single nucleotide polymorphisms that influence disease progression is an important next step. In fact, recent cross-sectional studies in Kenya identified for the first time genetic polymorphisms related to disease severity in several innate immune response genes (Hise et al.,

* Corresponding author. University of California, Davis School of Veterinary Medicine One Health Institute, USA.
Email address: bhbird@ucdavis.edu (B.H. Bird)

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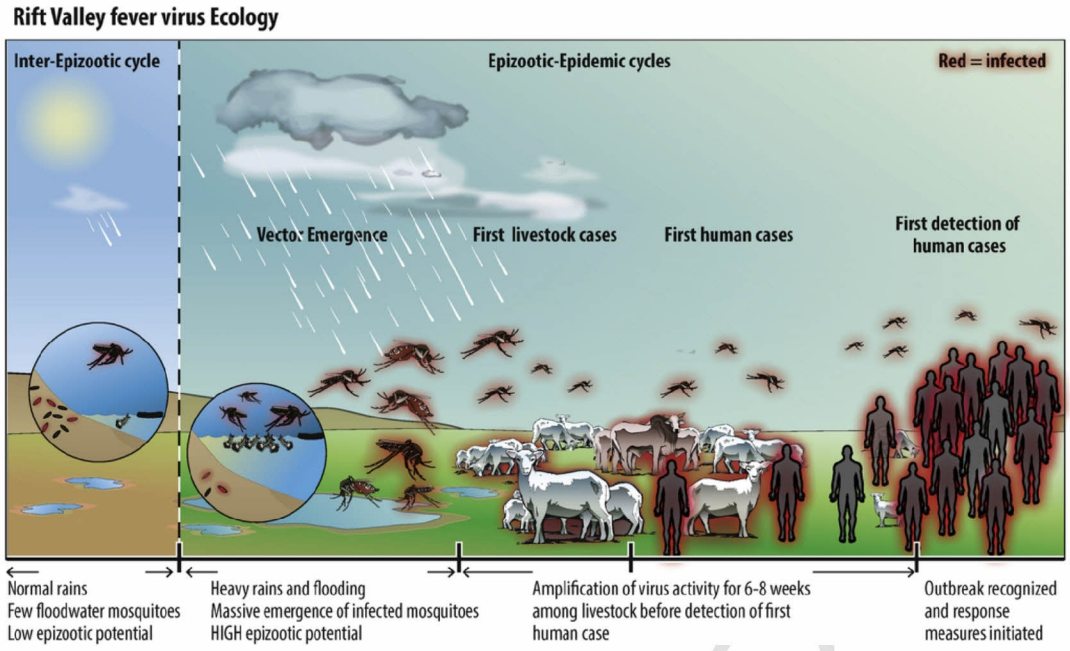

Fig. 1. .

2015; LaBeaud et al., 2015). These studies identified mutations in several important virus sensing and response signaling pathways (TLR2, TLR7, TLR8, MyD88, TRIF, MAVS, and RIG-I) that were associated with more severe RVF disease outcomes. However, they did not evaluate any aspect of the adaptive immune response, nor did they evaluate genes involved in other cellular processes that could be important in modifying RVF disease severity. These studies are a great and needed first step towards understanding the influence of the host response on RVF severity, but additional large-scale investigations in multiple human populations are needed.

Patient comorbidities may also contribute prominently to RVF outcome. For example, during one outbreak in Tanzania, patients who were HIV positive developed RVFV encephalitis at a higher rate and had a higher case fatality ratio compared to those who here HIV negative (Mohamed et al., 2010). It is possible that other viruses (e.g., Hepatitis B or C virus), or other diseases (diabetes, obesity, parasitism etc.) play a determinant role in RVFV infection outcomes. In order to address these possibilities, long-term prospective cohort studies in endemic areas that span the inter-epidemic period and an active epidemic period are needed. This will allow for the follow-up of enrolled participants through the entire course of their clinical disease from initial onset to eventual recovery. However, given the highly episodic nature of large-scale RVFV outbreaks this might require longitudinal research programs spanning 10 years, or more in enzootic regions. A viable alternative could be to target efforts in areas where climate conditions are conducive to low-level but continuous RVFV transmission rather than the explosive, but rare, transmission cycles observed classically in East Africa.

2.2. Why is human maternal to fetal transmission of RVFV so rare?

RVFV epizootics are notorious for widespread and devastating abortion storms in ruminant livestock, yet case reports of human congenital disease or fetal losses during outbreaks or in retrospective studies examining this are rare (Abdel-Aziz et al., 1980; Adam et al., 2010; Adam and Karsany, 2008; Laughlin et al., 1979; Niklasson et al., 1987). In approximately 70–90% of affected livestock animals, the virus easily crosses placental barriers and can cause a variety of fetal malformations or death and resorption/abortion depending on the stage of gestation (Bird et al., 2009). Typically, aborted fetuses and gestational fluids contain exceptionally high titers often exceeding 1.0×10^7 plaque forming units (PFU)/g and pose a great risk for human exposure and infection (Swanepoel and Coetzer, 1994). Is the lack of similar findings among humans simply attributable to epidemiologic surveillance bias or does this represent true differences in either placental or fetal immunology between species? It is clear from two published case reports that human maternal to fetal transmission can occur (Adam and Karsany, 2008; Arishi et al., 2006) but what is unclear is why this appears to be such a rare event. Are human fetuses and the placenta only susceptible to infection during a very narrow time-window during gestation, does the human placenta contain cell populations that prevent virus translocation, or does it produce some anti-viral factor that is restrictive to virus replication, unlike what is observed in animals? The striking differences between the histologic structure and anatomical arrangement of human (hemochorial, discoid) and ruminant (epitheliochorial, cotyledonary) placentation likely influence the susceptibility of the fetus to trans-placental virus infection, reviewed in (Furukawa et al., 2014; Gundling and Wildman, 2015). However, to date no detailed cross-species in vitro or ex-vivo experimental studies have been reported. The recent discovery of the highly neurotropic nature of a different arbovirus, Zika virus, in human fetuses (Martines et al., 2016) further highlights the importance of this intriguing area of comparative anatomy, immunology, and virology.

2.3. What underlies the pathogenesis of RVF delayed-onset encephalitis and retinitis?

The main target organ for high-level virus replication and tissue injury in all species is the liver. However, in a small subset of human patients even those without significant hepatic involvement, retinitis/visual impairment can develop 5–20 days after the initial febrile pe-

riod, and/or a delayed onset encephalitis which typically occurs 14–28 days post-onset of initial clinical symptoms can develop (Al-Hazmi et al., 2003; Alrajhi et al., 2004; Newman-Gerhardt et al., 2013; Siam and Meegan, 1980; Siam et al., 1980). These could lead to long-term debilitating sequelae ranging from visual impairment and blindness to cognitive and neurologic defects. The mechanism of virus invasion into the CNS or the eye and the possibility that the disease process might be, at least in part, immune-mediated remain unaddressed areas of research. The possible routes of virus entry are numerous, including direct neuronal invasion via the cranial or peripheral nerves, hematogenous spread due to high viremia, or infection of antigen presenting cells (monocytes, dendritic cells) and their use as Trojan horses to introduce virus into the CNS or eye. Recent work in mice using an attenuated RVFV strain lacking the NSs gene (ΔNSs) demonstrated that $CD4^+$ T cells are required for the prevention of RVFV encephalitis, however activated T-cell infiltration in to the CNS may cause deleterious effects to the host as the cells attempt to control CNS infection (Dodd et al., 2013, 2014). These two possible conflicting roles of host T cells, in both preventing and possibly exacerbating encephalitis requires further clarification, ideally in a model that makes use of WT rather than attenuated viruses.

The unique immune-privileged environment of the eye and mechanisms of ocular RVFV disease have not been examined in any detail experimentally. However, preliminary human data suggests that ocular disease may be immune mediated (Newman-Gerhardt et al., 2013). The reassessment of the gerbil or rat encephalitis models (Anderson et al., 1988; Bird et al., 2007; Bucci et al., 1981) which demonstrated delayed onset encephalitis and low level virus replication in retinal tissues, for the development of more robust animal models of ocular disease could be useful. The recent recognition of ocular damage as part of the long-term sequelae of another hemorrhagic fever virus, Ebola virus, in human survivors from West Africa 2014–2016 epidemic (Tiffany et al., 2016; Varkey et al., 2015) serves to strengthen the importance of developing these experimental models to further understand the pathogenesis of these debilitating disease outcomes.

2.4. Can targeted antiviral therapeutics that can cross the blood-brain-barrier be effective in treating RVF neurologic disease?

The lack of effective antivirals capable of crossing the blood-brain barrier (BBB) is a problem not only restricted to RVFV, but is relevant to a variety of neurotropic viruses (Laksitorini et al., 2014; Ludlow et al., 2016). Penetration across the BBB is an important issue in RVF treatment. Evidence from animal models demonstrates that antiviral therapeutics can be effective in treating the primary hepatic manifestations of RVF disease and increase survival (Scharton et al., 2014). The benefits of antiviral therapeutics in treating RVFV infection are without question. However, medical professionals should be aware that because of potentially poor penetration across the BBB, systemically delivered antivirals may not prevent all cases of delayed-onset neurologic RVF (Scharton et al., 2014; Smith et al., 2010). The potential for these delayed neurological disease outcomes should be considered in the design of any prospective human therapeutic trial to ensure the close monitoring and medical management of study participants should neurologic symptoms appear. Further research into novel compounds capable of penetrating the BBB or trans-BBB delivery systems in animal model systems is needed (Reed et al., 2013).

2.5. What is the importance of cell-mediated immunity against RVFV?

It is well established that the key early determinant of immune clearance of many viral infections including RVFV is robust intracellular innate pathway signaling followed closely by the development of high titer neutralizing IgG antibodies, reviewed in (Lihoradova and Ikegami, 2014). These are clear correlates of early and late RVFV vaccine induced protection in animals and humans. Significantly less is known regarding the contributions and function of $CD4^+$ and $CD8^+$ T lymphocytes, natural killer cells, and other immune effector cells in promoting and maintaining appropriate immune control of RVFV infection (Dodd et al., 2014; Roberts et al., 2015; Xu et al., 2013). Does the virus have any mechanism to abrogate these higher-order cell mediated responses and subvert this arm of the immune system? Does cell mediated immunity play a role in modulation of RVFV disease? Using an attenuated strain of RVFV that would not normally cause disease in mice, it was recently demonstrated that this virus was able to gain entry into the CNS and cause encephalitis in $CD4^+$ T cell depleted mice when inoculated into the footpad (Dodd et al., 2013). This finding did not appear to be related the role of $CD4^+$T cells in providing help for the production of antibodies because mice unable to generate any antibodies (muMT mice) did not develop encephalitis in this model, suggesting another role of the $CD4^+$ T cell in the prevention of encephalitis. This mouse data, combined with the finding of increased encephalitis in humans with HIV (whose viral target is the $CD4^+$ T cell), suggests that $CD4^+$ T cells play a role in the prevention of RVFV encephalitis. Therefore, cell mediated immune mechanisms could be an important, and as yet undefined, aspect of RVFV immune control.

3. Wildlife and risk factors

3.1. What is the importance (if any) of cryptic small mammal hosts or other wildlife in "ancestral homelands" and endemic areas?

Transovarial transmission in *Aedes* spp. flood-water mosquitoes is undoubtedly an important mechanism for the persistence of RVFV in endemic areas (Linthicum et al., 1985). These mosquitoes, perpetuate virus maintenance by depositing virus-infected eggs at the edges of water-filled depressions (dambos) following flooding (Linthicum et al., 1988). At the end of flooding periods, these dambos dry and the eggs may remain viable for long-time periods waiting for the next cycle of inundating rains. In East Africa, when abnormally heavy rains return (approximately every 10 years) the resulting flooding leads to abundant emergence of RVFV infected mosquitoes and initiates the amplification of transmission into large-scale epizootics/epidemics (Fig. 1). However, it is not known if transovarial transmission alone is sufficient to maintain RVFV endemicity over longer time periods or if there is a requirement for continuous low-level virus amplification and vector-host interactions in-between these large-scale outbreaks. What role (if any) small mammals or other wildlife may play in virus maintenance at low levels during the inter-epidemic periods is unclear (Gora et al., 2000; Manore and Beechler, 2015). Experimental animal model data demonstrates that generally RVFV is highly virulent in most types of common laboratory rodents and is unlikely to be maintained in these species (Ross et al., 2012). Data from serosurveys of wild caught rodents native to endemic areas and experimental inoculation studies of a limited number of species suggest that other cryptic transmission cycles are also unlikely, but possible (Olive et al., 2013; Swanepoel et al., 1978). and reviewed in

(Olive et al., 2012). Understanding if these cryptic virus life cycles exist will be key to understanding what would be required to eradicate or substantially control RVFV in endemic areas.

3.2. Are common European and North American wildlife and non-traditional livestock susceptible to infection and disease and can mosquito vectors in these areas transovarially transmit RVFV?

Many wild ungulates and other wildlife species have been shown to be seropositive in endemic areas suggesting a role for these animals in the virus life-cycle (Britch et al., 2013; Evans et al., 2008). To date, no experimental infections of any large European or North American wildlife have been reported. This is a critical gap in our understanding of what may occur after RVFV introduction into these areas. In the United States alone, the estimated population of white tail and other related deer (*Odocoileus* spp.) exceeds 20 million (Taber, 1997), yet we know nothing about the effects of RVFV replication in these animals or in other less numerous wild ruminants such as big-horn sheep (*Ovis canadensis*), elk (*Cervus canadensis*), and other deer and antelope species. Recently, a step forward was made by an investigation of cell lines derived from North American large mammal wildlife (deer, antelope, coyote) that demonstrated the in vitro susceptibility of these cell lines to RVFV infection (Gaudreault et al., 2015). It remains to be seen however, if this susceptibility will be confirmed by in vivo animal challenge studies.

A further example of an unexpected impact of RVFV on agriculture was the surprising susceptibility of New World camelids (llamas and alpacas) to RVFV infection and severe disease. In South Africa these animals have been imported for the fiber industry (alpacas and llamas), and during the 2009–2010 outbreak in that country many were infected and died; Dr. Sophette Gers, personal communication and (Pienaar and Thompson, 2013). While the United States and European herds of these animals is relatively small they are common in parts of South America and should be considered when designing public health communication strategies regarding RVFV infection risks and included in potential animal vaccination or culling interventions in the event of introduction of RVFV.

Many mosquito species present in North America are capable of serving as biological vectors of RVFV reviewed in (Linthicum et al., 2016). However, despite the importance of mosquito transovarial transmission (TOT) in Africa (Linthicum et al., 1985), little is known regarding North American species' capacity to perpetuate the RVFV life-cycle by this mechanism. If transovarial transmission can occur, it may complicate the eventual control and eradication of the virus if it were introduced onto the continent. Experimental data exploring the potential for transovarial mosquito transmission, and the role of wildlife and other non-traditional livestock in the Americas or Europe will be essential to aid the development of comprehensive RVFV biodefense strategies.

3.3. What is the virus load in raw milk and meat products from infected animals?

RVFV outbreaks can greatly disrupt the public's trust in the safety of animal products and the food-supply (Hartley et al., 2011). A recurring question during previous outbreaks relates to the potential infectivity and risk of raw milk products or meat obtained from infected animals. Anecdotal reports and epidemiologic evidence has suggested that consumption of unpasteurized milk is associated with infection (Anyangu et al., 2010; LaBeaud et al., 2011; Woods et al., 2002), but there has been little direct evidence that this may be a relevant high-risk driver of human virus transmission. Likely due to practical difficulties, only one study examining the shedding of live infectious virus in the milk of experimentally infected lactating animals has been reported. This study conducted in 1951 suggests that virus can be found in acutely infected cattle milk for 3–5 days (Alexander, 1951; Easterday, 1965). The lack of experimental data is striking, and studies in major milk producing species such as cattle, sheep, goats, and camels are clearly needed to accurately communicate the potential food-borne risk of RVFV in raw milk products.

Likewise, the risks of RVFV transmission from infected meat is well described in epidemiological studies (Anyangu et al., 2010), however there is a lack of clear experimental data regarding the actual persistence of residual infectious virus in infected meat products. Once infected, how long does meat harbor infectious virus and what factors influence the degradation of infectivity (i.e., holding temperatures ranging from warm ambient temperatures of outdoor markets (>30 C) to refrigerated coolers (~4 C), processing techniques, pH, humidity etc.)? This knowledge will be helpful for clear public communications to describe the potential risks of RVFV within the food-supply in endemic areas and following the introduction of the virus to previous unaffected regions.

4. Control and eradication strategies

4.1. Can one-health vaccination approaches for livestock actually contain virus spread and reduce human infections?

While widespread livestock vaccination could potentially save millions of animal lives and protect the economic livelihoods of thousands of herdsmen, it has yet to be demonstrated that a one-health vaccination approach would effectively reduce human RVF cases or provide a buffer for a ring-vaccination strategy to control RVFV in non-endemic areas. Ideally, field trials in endemic areas should be completed to demonstrate the feasibility of large-scale animal vaccination campaigns and their ability to end the transmission cycle to humans. In the United States, only one vaccine (MP-12, a modified live attenuated vaccine strain) has received conditional licensure. Several recently developed recombinant vaccines with enhanced safety profiles over historical vaccines have been developed, reviewed in (Bird and Nichol, 2012; Indran and Ikegami, 2012; Kortekaas, 2014). Further trials and commercial development of these types of vaccines are needed so that the groundwork for potential emergency use authorization by relevant agricultural and human health authorities is in place prior to the next large RVF outbreak. Rapid deployment of safe and efficacious vaccines delivered via "needle-free" technologies to prevent animal-to-animal spread of wild-type RVFV or other pathogens on contaminated needles will likely be essential for eventual control of RVFV as a significant health threat.

4.2. Can we develop integrated comprehensive eradication plans for successful control of RVFV following introduction into non-endemic areas?

Thus far RVFV has been able to establish itself endemically in many areas of sub-Saharan Africa, and is likely now endemic or is being repeatedly re-introduced into areas such as the western provinces of Saudi Arabia, Madagascar, and the Comoros Islands. During the large 1976–1979 Egyptian outbreak, massive efforts including mosquito abatement and animal vaccination were undertaken to prevent the spread of the virus across the Sinai and into the greater Middle East region (Hoogstraal et al., 1979; Meegan, 1979; Shimshony and Barzilai, 1983). However, it is unclear if these human

efforts or other naturally occurring factors (climate, long distances across desert areas etc.) were ultimately responsible in preventing further incursion into other naïve territories. Since that time, sporadic RVFV activity continues along the Nile River in Egypt but has not been detected in the greater Middle East region (Ahmed Kamal, 2011; Hanafi et al., 2011). Similarly, in Saudi Arabia since 2000 there are occasional reports of RVFV activity, but not beyond the originally involved outbreak areas in the western Jizan region (Memish et al., 2015). Taken together, the incursion of the virus into these two previously unaffected areas suggests that once the virus is introduced it may be able to establish itself endemically.

In Europe and the Americas numerous competent mosquito vectors are present and at least initially, RVFV transmission could possibly be maintained for some time if suitable mammalian hosts are present (Turell et al., 2008, 2013). This coupled with the lack of large scale rapidly deployable vaccine stockpiles, no knowledge of the role of wildlife and other non-traditional livestock, and the potential for mosquito transovarial transmission in maintaining RVFV transmission make it difficult to predict what would happen following an incursion of the virus into Europe or the Americas. The time is now to generate integrated cross-disciplinary surveillance, eradication and control plans, before the virus appears in novel areas. The appearance of novel arboviruses in the Americas such as West Nile virus, Heartland virus, and more recently Zika virus serve as obvious warnings that greater understanding and preparedness for RVFV (and arboviruses in general) are warranted before they arrive unwelcomed.

5. Summary

It has now been almost 5 years since the end of the last Southern African RVFV outbreak (2009–2010), and almost a decade since the last large East African outbreak (2006–2007) (Bird et al., 2008; LaBeaud et al., 2015; Pienaar and Thompson, 2013). Although there were strong indications from climate models during late 2015, that the potential for another large scale outbreak on the plains of East Africa might occur in early 2016, at the time of this writing, it appears that the risk for this is decreasing. Despite the difficulties in predicting when and where RVFV outbreaks may occur, the fact that they will reoccur is a certainty. Perhaps before the next large epizootic, answers to some of the key questions raised here will be found thus aiding our collective efforts to control and eventually eliminate RVFV as a significant human and veterinary health threat.

Ethics statement and funding sources

Dr. Bird is co-inventor of a RVFV vaccine platform that is under commercial development. Dr. McElroy has no financial interests pertaining to this manuscript to declare. This manuscript was funded by core funds from the Centers for Disease Control and Prevention.

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EMERGING DISEASE INSIGHTS
Research from the PREDICT Modeling & Analytics team

Distribution and Seasonality of Potential Ebola Bat Reservoirs

January 8, 2016

For details on methods or analysis contact: PREDICTmodeling@ecohealthalliance.org

Identification of the natural Ebola virus (EBOV) reservoir has remained elusive. Thirty-five mammalian species in Africa and Asia, including wild primates, rodents, carnivores, and ungulates, have tested positive via PCR or serology for at least one of the five different viral strains of Ebola virus (Bundibugyo, Cote d'Ivoire/Tai Forest, Reston, Sudan, Zaire)[1]. Bats likely play a key role in EBOV ecology, with 23 species found positive or seropositive. Ten of these species occur in Africa (Table 1), where all human EBOV cases have originated.

To better understand spatial risk of EBOV spillover, the PREDICT-2 Modeling & Analytics team used ecological niche models to predict the spatial occurrence of these ten African bat species. In addition, to examine seasonal changes in spillover risk, we conducted a thorough literature review for these species to better understand the role of life history traits (Table 1) and reproductive seasonality (Table 2) in Ebola disease dynamics.

Table 1: Life-history traits of the ten potential African Ebola bat hosts.

group	species	diet	birth periods	strain	source
Megachiroptera	*Eidolon helvum*	fruits	one	Reston, Sudan, Zaire	2,3
	Epomops franqueti	fruits	two	Zaire	4,5
	Epomorphus gambianus	fruits	two	Reston, Zaire	6
	Hypsignathus monstrosus	fruits	two	Zaire	4,5
	Micropteropus pusillus	fruits	two	Zaire	4
	Myonycteris torquata	fruits	two	Zaire	4,5
	Rousettus aegyptiacus	fruits	two	Zaire	4
	Nanonycteris veldkampii	fruits	two	Reston/Zaire	6
Microchiroptera	*Mops condylurus*	insects	two	Zaire	4
	Hipposideros gigas	insects	one	Zaire	4

GEOGRAPHY OF EBOV SPILLOVER RISK

An aggregate ecological niche model (ENM) for the ten potential bat EBOV reservoir species is shown in Figure 1. Pigott et al. (2014) modeled the zoonotic niche of EBOV using occurrence

data of three EBOV reservoirs: *E. franqueti*, *H. monstrosus*, and *M. torquata* as one component. We expanded this to include all known African EBOV-positive bat species and used an ensemble approach to minimize model uncertainty.

Gatherer (2014) proposed that the ranges of *H. monstrosus* and *M. torquata* overlapped Meliandou village in the Guéckédou Region of Guinea (location of the index for the 2014 Ebola outbreak)[8]. Thirteen species of bats have been captured in southeastern Guinea near this village, including four known EBOV hosts*: E. helvum*, *N. veldkampii*, *M. condylurus*, and *M. torquata*[9]. Our ENMs confirm the presence of three of these species, *E. helvum*, *N. veldkampii*, *M. torquata,* and suggested that *H. gigas* likely occurs there (Fig. 2).

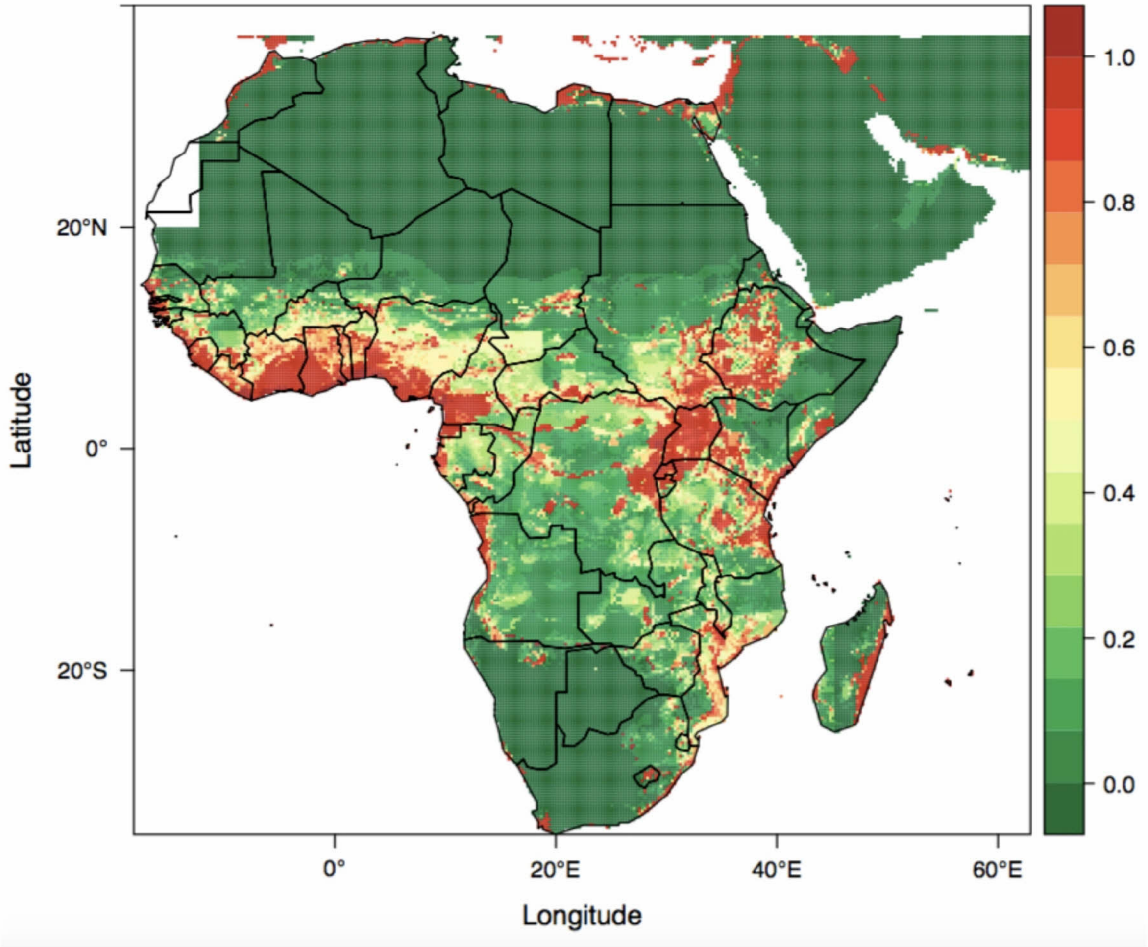

Figure 1. Stacked ecological niche models for the ten African bat species that potentially harbor the Ebola virus.

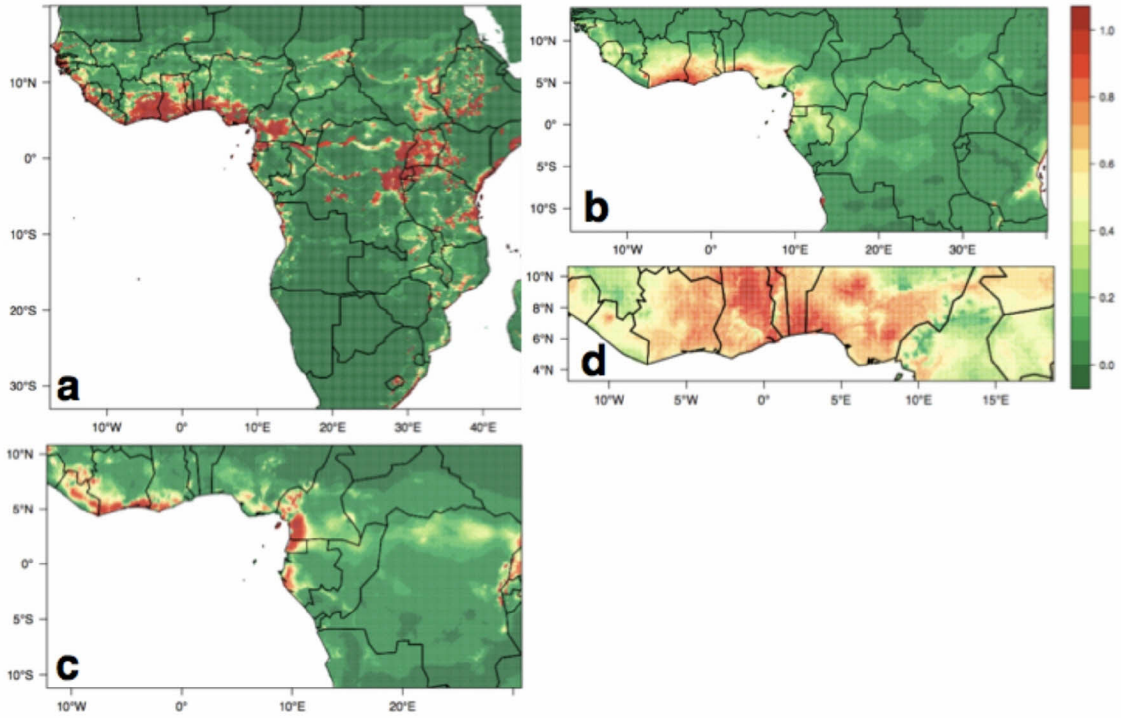

Figure 2. Ecological niche models for the four bats (a. E. helvum, b. H. gigas, c. M. torquata, d. N. veldkampii) whose suitable range include Meliandou village in Guinea, the index case of the 2014 EBOV outbreak.

POTENTIAL FOR SEASONALITY OF EBOV SPILLOVER RISK

Previous work has shown seasonal pulses of human Marburg virus cases, and of viral prevalence within the bat *R. aegyptiacus*. It is therefore logical that EBOV may also exhibit seasonal pulses within its bat reservoir hosts, tied to their life history traits. Bats have highly synchronous mating strategies, with the most energetically costly periods (late pregnancy and early lactation) occurring during the wet season, when food sources are most abundant[10]. This provides two potential drivers for EBOV spillover: 1) population pulses of recently emerged susceptible juveniles may increase risk of viral transmission[11]; and 2) abundant fruit in the wet season may increase the potential interface between humans and bats. Analysis of the literature for all likely EBOV bat reservoirs show that the reproductive cycles of West African bats exhibit birth periods from February-to-April and August-to-October, at the onset of the wet seasons (Table 2). Weaning and first flight of juvenile bats is most often during peak rainfall of May – June. These patterns suggest that there is a reasonable likelihood that seasonal patterns of EBOV spillover risk occur, perhaps with two peaks per year, at the time when maternal antibodies wane in juvenile bats, a few weeks after birth. PREDICT surveillance plans will need to include multiple field visits each year to analyze the change in viral spillover risk over time and identify peak seasonal risk.

CONCLUSIONS

1. Ensemble Ecological Niche Modeling of all 10 likely EBOV bat reservoirs suggests widespread risk of future EBOV spillover across West and Central Africa, and provides fine scale risk maps to target surveillance.

2. Analysis of life history traits for all likely EBOV bat reservoirs reveals evidence of seasonality that could drive seasonal spikes of EBOV spillover risk, perhaps with two peaks each year. Surveillance of EBOV in bats will therefore need to be planned to examine these seasonal fluctuations.

Table 2: Reproductive cycles of the West African bats that have demonstrated evidence of EBOV exposure. *Key*: G, gestation; P, parturition; L, lactation; W, weaning. Blue fill shows the wet seasons for West Africa.

Species	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
E. helvum[12]	G	G	GP	PL	L	W			G	G	G	G
E. gambianus[13,14]	G	G	GL	PL	PL	GW	GW	G	G	PL	G	G
H. gigas[15]	L	L	L	L	LW	W	G	G	G	GP	L	L
H. monstrosus[16]	G	GP	PL	L	W	G	G	GP	PL	L	W	G
M. pusillus[13]	G	P	PL	L	GL	GW	G	P	PL	L	GL	G
M. torquata[17]	G	P	PL	L	GW	GW	G	P	PL	L	GW	GW
N. veldkampii[14,18]	GL	G	G	G	P	PL	L	G	G	G	GP	PL

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PLoS MEDICINE

Health in Action

A "One Health" Approach to Address Emerging Zoonoses: The HALI Project in Tanzania

Jonna A. K. Mazet[1]*, Deana L. Clifford[1], Peter B. Coppolillo[2], Anil B. Deolalikar[3], Jon D. Erickson[4], Rudovick R. Kazwala[5]

1 Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, California, United States of America, **2** Wildlife Conservation Society, Bozeman, Montana, United States of America, **3** Department of Economics, University of California, Riverside, California, United States of America, **4** Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, Vermont, United States of America, **5** Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, Tanzania

Need for Integrated Health Approaches

Every day thousands of children and adults die from underdiagnosed diseases that have arisen at the human–animal–environment interface, especially diarrheal and respiratory diseases in developing countries [1,2]. Explosive human population growth and environmental changes have resulted in increased numbers of people living in close contact with wild and domestic animals. Unfortunately, this increased contact together with changes in land use, including livestock grazing and crop production, have altered the inherent ecological balance between pathogens and their human and animal hosts. In fact, zoonotic pathogens, such as influenza and SARS (severe acute respiratory syndrome), account for the majority of emerging infectious diseases in people [3], and more than three-quarters of emerging zoonoses are the result of wildlife-origin pathogens [4]. While zoonoses represent a significant emerging threat to public health, many of these diseases, such as diarrheal diseases arising from poor water sanitation, are neglected by funding agencies [5].

Role of Water and Natural Resource Limitation

Nowhere in the world are these health impacts more important than in developing countries, where daily workloads are highly dependent on the availability of natural resources [6,7]. Water resources are perhaps most crucial, as humans and animals depend on safe water for health and survival, and sources of clean water are dwindling due to demands from agriculture and global climate change. As water becomes more scarce, animals and people are squeezed into smaller and smaller workable areas. Contact among infected animals and people then increases, facilitating disease transmission. Water scarcity also means that people and animals use the same water sources for drinking and bathing, which results in serious contamination of drinking water and increased risk of zoonotic diseases. In addition, poor sanitation and animal management can result in fecal contamination of both animal and human food. When this situation is complicated by high HIV/AIDS prevalence, the impacts of otherwise minimally virulent or difficult-to-transmit pathogens can be catastrophic to families and entire communities, and ultimately to the environment through impacts on human capacity, natural resource management, and land use [8].

The conditions of land-use change, water scarcity, and overlapping human, livestock, and wildlife populations are particularly prevalent in rural Africa and near remaining wildlands. Human population in sub-Saharan Africa doubled between 1975 and 2001 [9], and the African Population and Health Research Center predicts another doubling from 2008 levels to 1.9 billion by 2050. Such rapid population growth and consequent demands for natural resources are making African wildlands increasingly vulnerable to conversion to other land uses, such as logging, agriculture, and pasturage. A recent analysis by Wittemyer et al. [10] found that average annual population growth rates were higher in buffers to protected areas than in rural areas in Africa and Latin America. Protected areas provide some of the last supplies of ecosystem goods and services for expanding human populations, including firewood, bush meat, clean water, medicinal plants, and areas of safety during civil strife. Their porous edges also provide refuge for the vectors of zoonotic disease transmission.

The One Health Approach

The interconnectedness of human, animal, and environmental health is at the heart of One Health, an increasingly important prism through which governments, NGOs (nongovernmental organizations), and practitioners view human health [11]. An important implication of the One Health approach is that integrated policy

The Health in Action section is a forum for individuals or organizations to highlight their innovative approaches to a particular health problem.

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Abbreviations: BTB, bovine tuberculosis; EPTB, extrapulmonary tuberculosis; HALI, Health for Animals and Livelihood Improvement; NGO, nongovernmental organization

* E-mail: jkmazet@ucdavis.edu

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interventions that simultaneously and holistically address multiple and interacting causes of poor human health—unsafe and scarce water, lack of sanitation, food insecurity, and close proximity between animals and humans—will yield significantly larger health benefits than policies that target each of these factors individually and in isolation. By its very nature, the One Health approach is transdisciplinary, since it is predicated on agricultural scientists, anthropologists, economists, educators, engineers, entomologists, epidemiologists, hydrologists, microbiologists, nutritionists, physicians, public health professionals, sociologists, and veterinarians working collaboratively to improve and promote both human and animal health. Figure 1 depicts the relationship among health, safe water, and food supply and their dependence upon plants, animals, and the environment, as well as the influences that interact to affect human health. This complexity necessitates a collaborative approach among professionals from multiple disciplines for the design of effective interventions.

Applying the One Health Paradigm

The HALI Project

Assessing and reducing the impacts of zoonotic diseases and resource limitation on health and livelihoods requires governments, NGOs, and academic institutions to work with citizens to develop interventions that are cost effective, sustainable, and conservation minded. In 2006, the Health for Animals and Livelihood Improvement (HALI; http://haliproject.wordpress.com/) project was initiated to test the feasibility of the One Health approach in rural Tanzania and to find creative solutions to these problems by investigating the impact of zoonotic disease on the health and livelihoods of rural Tanzanians living in the water-limited Ruaha ecosystem. HALI, from the Swahili word for state of health, addresses these complex disease and natural resources issues on a platform that recognizes that the health of domestic animals, wildlife, and people is inextricably linked to the ecosystem and natural resources on which all depend [12].

The Ruaha landscape is one of Tanzania's largest wild areas, covering a region larger than Denmark (>45,000 km^2). This sprawling ecosystem is of extraordinary conservation significance and supports approximately 30,000 elephants (*Loxodonta africana*) and the continent's third largest population of critically endangered African wild dogs (*Lycaon pictus*) [13]. The socioeconomic importance of the Ruaha region rivals its biological significance, as virtually all communities depend entirely on the natural resource base, and agriculture accounts for about 80% of these livelihoods [14]. This importance is immediately apparent at the village level, where livestock are widespread, abundant, and central to traditional natural resource management. Unfortunately, livestock-dependent households are among the poorest in the nation [15]. This local poverty fuels the demand for illegal wildlife hunting for meat, another known driver for disease emergence [16].

Zoonotic diseases known to be of public health importance, such as rabies and Rift

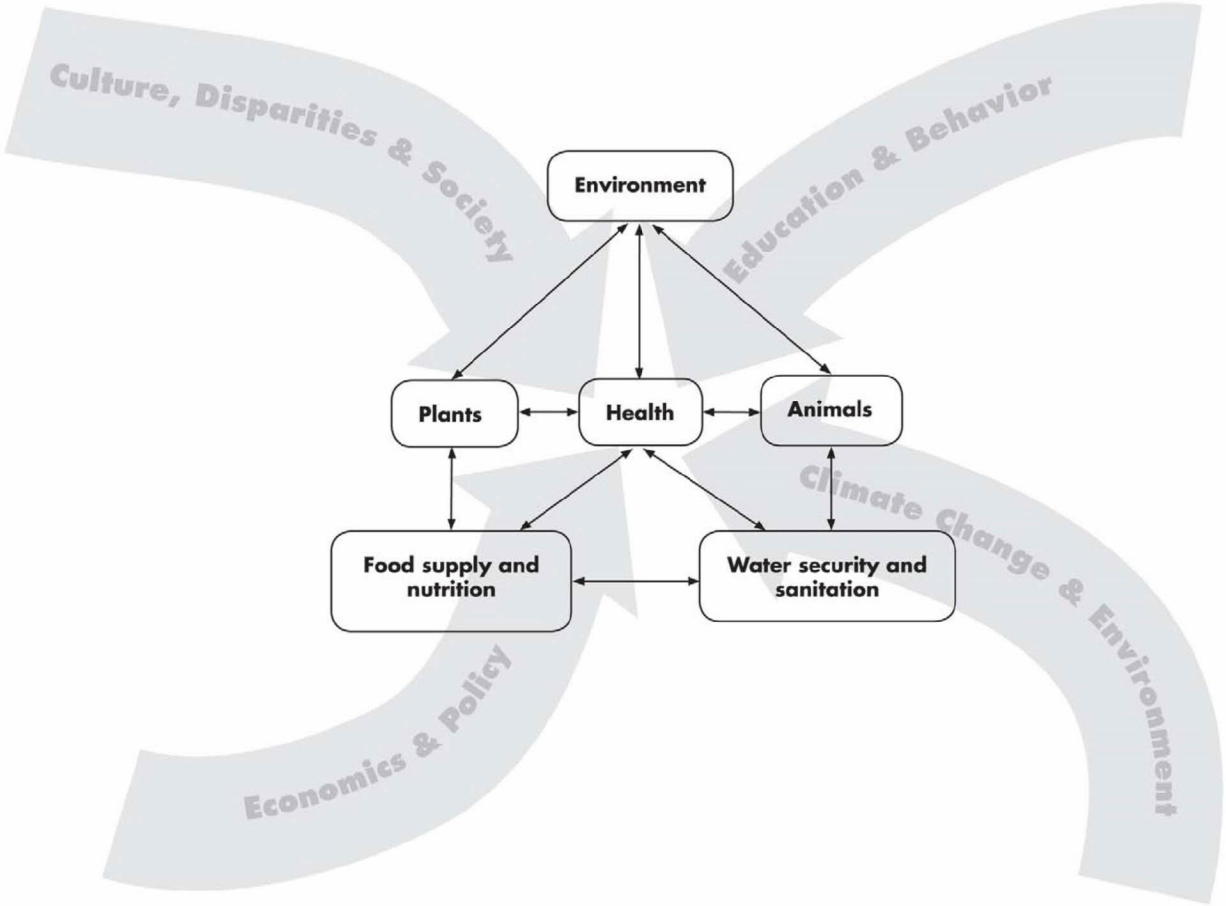

Figure 1. The local and global influences impacting human health, including the interdependence of people, animals, plants, and the environment, and the associated food and water availability, safety, and security. (Graphic artist credit: A. Kent).
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Table 1. The HALI Project's multilevel approach to assessing the impact of the interactions between water and disease in the Ruaha ecosystem by simultaneously investigating the medical, ecological, socioeconomic, and policy issues driving the system.

System Drivers	Objective	Activities
Medical	Assess wildlife, livestock, and their water sources for zoonotic pathogens and disease including bovine tuberculosis, brucellosis, *Salmonella*, *Cryptosporidium*, *Giardia*, *E. coli*, and *Campylobacter*.	• 70 wildlife samples tested
		• 1,368 live cattle from 102 pastoralist households tested
		• 228 livestock carcasses tested
		• Ten water sources sampled monthly for 2 years
	Evaluate pastoralists' perceptions about disease impacts and risk of transmission from animals and water.	• 159 household surveys estimating disease impacts and examining transmission risk factors (subset resampled seasonally)
	Introduce new diagnostic techniques for disease detection.	• Transfer of five technologies between University of California, Davis (US) and Sokoine University of Agriculture (Tanzania)
	Train Tanzanians of ALL education levels about zoonotic disease.	• Community outreach to over 950 local people
		• Training for 24 game scouts and technicians
		• Four honors bachelor and extern projects
		• Two masters theses
Ecological	Environmental monitoring of water quality and availability	• Ten water sources sampled monthly for 2 years
	Assess wildlife population health and demography	• Surveys in association with Wildlife Conservation Society, Tanzania National Parks, and the local community managing wildlife
	Examine landscape-level risk factors for disease	• Integration of spatial data on wildlife and livestock density, regions of water scarcity, and land use regimes
Socioeconomic	Evaluate livestock and human disease impacts on livelihoods of pastoralist households	• 159 household surveys examining economic risk factors (subset resampled seasonally)
	Examine land and water use impacts on daily workloads and village economies	• 18 detailed household diaries, including gender differences (Figure 3)
		• 20 village and district leader interviews
		• Village stakeholder workshops
	Advanced degree training for African national	• Rwandan PhD, Ecological Economics (University of Vermont)
Policy	Develop new health and environmental policy interventions to mitigate the impacts of zoonotic diseases	• Strong partnerships with local governments, health and environment ministries, and policy and education NGOs
		• USAID policy briefs
		• Integrative modeling
	Raise awareness about the links among health, livelihoods, and natural resources	• Active participation in stakeholder meetings, international conferences, and ministry presentations
		• HALI Project blog
		• Public outreach through movie nights, radio programs, and zoonotic disease calendar

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Valley fever, are present in wildlife, domestic animals, and people in Tanzania [17]; however, the role of underdiagnosed diseases, such as bovine tuberculosis (BTB), has only just begun to be characterized [18,19]. Nearly 40,000 new cases of tuberculosis (human, bovine, or atypical strain) are diagnosed per year in Tanzania [20], with anywhere from 21% to 77% of Tanzanian tuberculosis patients also infected with HIV [21]. The extrapulmonary form of tuberculosis (EPTB) in people, often associated with BTB infection from animals, accounts for 20% of the reported cases in Tanzania [20]. Therefore, bovine tuberculosis became a focal disease for the HALI project due to its high livestock prevalence [22], wildlife data paucity, and the large, susceptible HIV-infected human population living in close association with livestock and wildlife. Additional priorities for HALI were determined through gender-balanced interviews with affected communities, including village chair people; leaders of agricultural, water, and women's cooperatives; and heads and members of pastoralist households. An overwhelming consensus emerged from follow-up stakeholder meetings of diverse communities, including multiple levels of government (including public hospital physicians), nonprofit organizations, academic institutions, and citizens:

> *A significant proportion of the rural population in the Ruaha landscape is affected by diseases impacted by water supply, and these diseases are affecting health, agricultural productivity, food security, and biodiversity in the region.*

HALI's Multilevel Approach

Accordingly, the HALI project is assessing the impact of the interactions between water and disease in the Ruaha ecosystem by simultaneously investigating the medical, ecological, socioeconomic, and policy issues driving the system (Table 1). The map in Figure 2 illustrates our multilevel approach, which includes: testing of wildlife, livestock, and their water sources for zoonotic pathogens and disease; environmental monitoring of water quality, availability, and use; assessing wildlife population health and demography; evaluating livestock and human disease impacts on

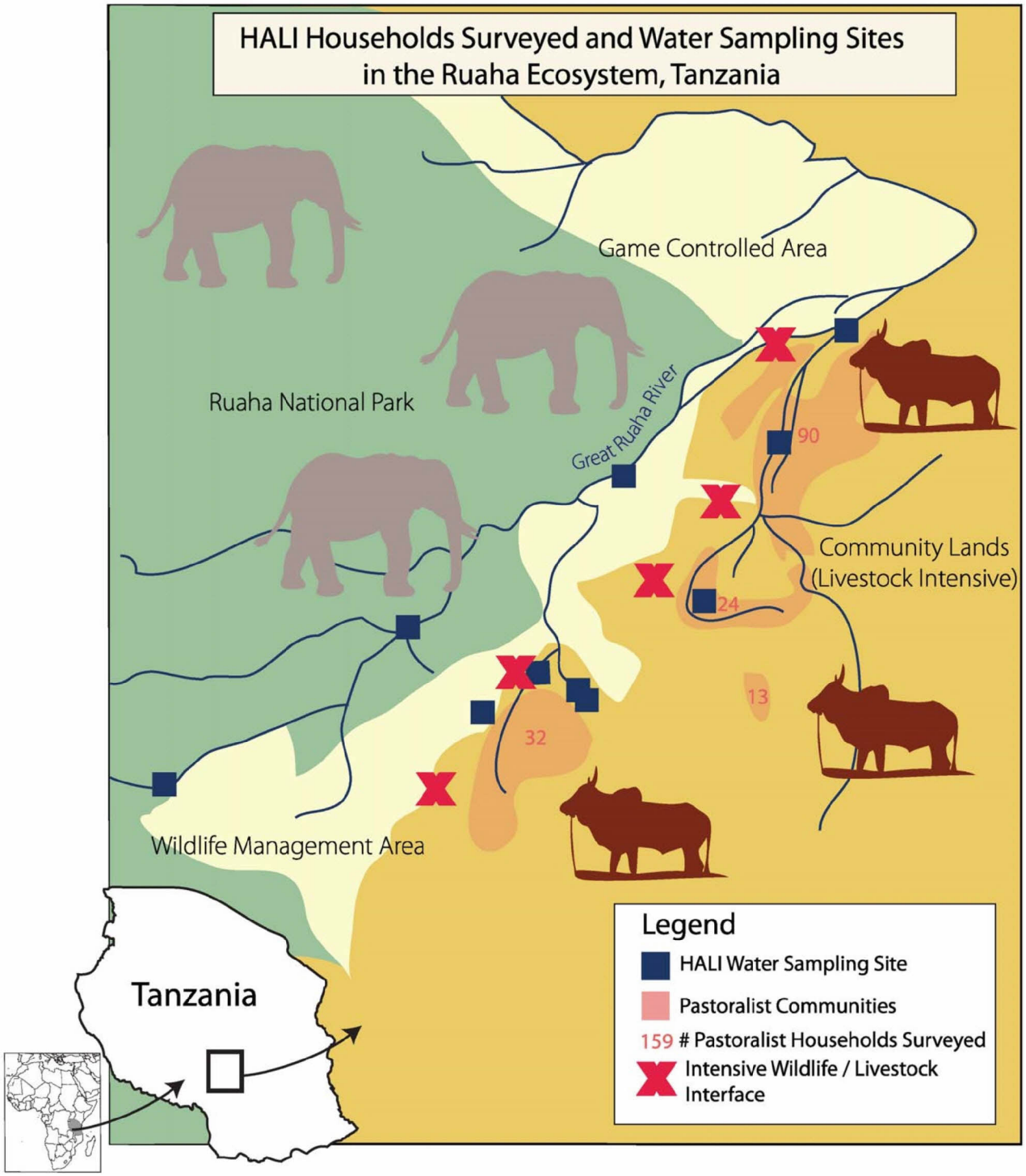

Figure 2. Map of the HALI Project study site in the Ruaha ecosystem, Tanzania. (Graphic artist credit: A. Kent).
doi:10.1371/journal.pmed.1000190.g002

livelihoods of pastoralist households; examining land and water use impacts on daily workloads and village economies; introducing new diagnostic techniques for disease detection; training Tanzanians of all education levels about zoonotic diseases; and developing new health and environmental policy interventions to mitigate the impacts of zoonotic diseases. Perhaps most importantly, the HALI project is examining these issues in a common framework with specific emphasis on the interactions between them, instead of attempting to isolate a single issue.

The HALI project has identified bovine tuberculosis and brucellosis in livestock and wildlife in the Ruaha ecosystem and is using this information to identify geographic areas with varying water availability where risk of transmission among wildlife, livestock, and people may be high.

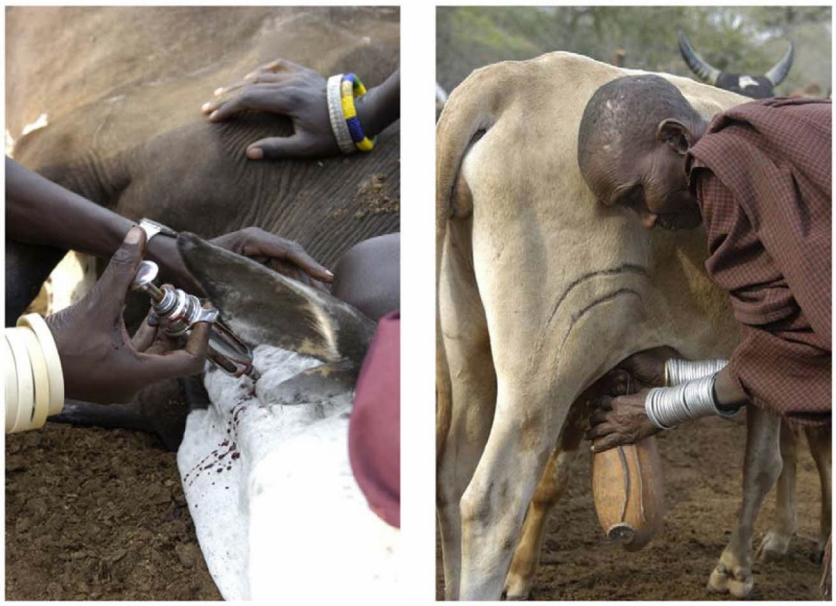

Figure 3. Men's and women's disease risks from livestock likely differ: men have occasional, but intense contact with sick animals (left), while women have regular, close contact with animals, particularly poultry and lactating cows and goats (right). (Photo credit: M. Kock-Wildlife Conservation Society).
doi:10.1371/journal.pmed.1000190.g003

In addition, *Salmonella*, *Escherichia coli*, *Cryptosporidium*, and *Giardia* spp. that can cause disease in humans and animals have been isolated from multiple water sources used by people and frequented by livestock and wildlife. These data are now being used to examine spatial and temporal associations between landscape factors and disease and to identify risk factors and health impacts that may be mitigated through policy changes and outreach. Preliminary findings also indicate that more than two-thirds of participating pastoral households do not believe that illness in their families can be contracted from livestock, and nearly half believe the same of wildlife. Furthermore, when the HALI project began working in this region, 75% of households did not consider sharing water sources with livestock or wildlife a health risk, illustrating the need for effective community education.

Lessons Learned for Planning One Health Projects and Interventions

The HALI platform has reinforced the importance of the One Health concept and provided lessons for the development of a new approach to global health.

First, it is crucial to recognize that zoonotic pathogens are present and emerging in rural communities and that their emergence is spatially and temporally variable within these communities. Most people living in high risk areas are not aware of the danger or what can be done to reduce it. In addition, transmission can be exacerbated by common animal husbandry and food and water handling practices (Figure 3) [23]. Therefore, data collection strategies should include the evaluation of spatial, temporal, and demographic patterns of pathogen prevalence and disease in human, domestic animal, and wildlife populations in likely hotspots for disease emergence. The underlying water- and land-use determinants of disease and the social, economic, and cultural barriers to control and prevention must be explored [24,25]. While local stakeholders and international institutions actively involved in animal health, conservation, and livelihood assessment and improvement were quick to engage in HALI, physicians and public health experts (local and international) have been slower, likely due to competing demands on time and resources already dedicated to addressing malaria and tuberculosis of human origin [5]. Concerns over the financial escalation of projects directly measuring pathogens in humans was also an obstacle to engaging medical professionals for these neglected diseases.

Second, the role of water in disease transmission and zoonosis emergence should be further explored. Water scarcity increases work stress, especially in women and children, and brings animals and people together more frequently, increasing the likelihood of water contamination and transmission of infectious diseases. Likewise, the manner in which water is used for agricultural and animal production affects worker health, food safety, and the health of those who drink and bathe in it. Improving water safety and security, including sanitation, in ecologically appropriate ways that reduce disease risk will require a transdisciplinary approach in which economists, ecologists, epidemiologists, and engineers play important roles with public and animal health practitioners.

Finally, the determinants and consequences of zoonotic diseases, as well as the interventions to mitigate their deleterious effects, are all cross-sectoral. Effective surveillance, assessments, and interventions are possible only by bridging the organizational gaps among institutions studying and managing wildlife, livestock, water, and public health. It is clear that education in global health, especially emerging zoonotic diseases, is urgently needed at all levels from research institutions to pastoralist communities. Collecting detailed data regarding land use and agricultural practices, food consumption and water use habits, illness in animals and people, and access to health care will help appropriately tailor education efforts for priority diseases and pandemic prevention. The donor community should be encouraged to transcend disciplinary conventions and invest in holistic health projects that have the best chance of affecting change.

Author Contributions

ICMJE criteria for authorship read and met: JAKM DLC PBC ABD JDE RRK. Wrote the first draft of the paper: JAKM. Contributed to the writing of the paper: DLC PBC ABD JDE RRK. Coordinated and supervised project activities in Tanzania: DLC. Coordinated laboratory analyses in Tanzania: RRK.

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PLOS | NEGLECTED TROPICAL DISEASES

Inter-epidemic Acquisition of Rift Valley Fever Virus in Humans in Tanzania

Robert David Sumaye , Emmanuel Nji Abatih, Etienne Thiry, Mbaraka Amuri, Dirk Berkvens, Eveline Geubbels

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Abstract

Background

In East Africa, epidemics of Rift Valley fever (RVF) occur in cycles of 5–15 years following unusually high rainfall. RVF transmission during inter-epidemic periods (IEP) generally passes undetected in absence of surveillance in mammalian hosts and vectors. We studied IEP transmission of RVF and evaluated the demographic, behavioural, occupational and spatial determinants of past RVF infection.

Methodology

Between March and August 2012 we collected blood samples, and administered a risk factor questionnaire among 606 inhabitants of 6 villages in the seasonally inundated Kilombero Valley, Tanzania. ELISA tests were used to detect RVFV IgM and IgG antibodies in serum samples. Risk factors were examined by mixed effects logistic regression.

Findings

RVF virus IgM antibodies, indicating recent RVFV acquisition, were detected in 16 participants, representing 2.6% overall and in 22.5% of inhibition ELISA positives (n = 71). Four of 16 (25.0%) IgM positives and 11/71 (15.5%) of individuals with inhibition ELISA sero-positivity reported they had had no previous contact with host animals. Sero-positivity on inhibition ELISA was 11.7% (95% CI 9.2–14.5) and risk was elevated with age (odds ratio (OR) 1.03 per year; 95% CI 1.01–1.04), among milkers (OR 2.19; 95% CI 1.23–3.91), and individuals eating raw meat (OR 4.17; 95% CI 1.18–14.66). Households keeping livestock had a higher probability of having members with evidence of past infection (OR = 3.04, 95% CI = 1.42–6.48) than those that do not keep livestock.

Conclusion

There is inter-epidemic acquisition of RVFV in Kilombero Valley inhabitants. In the wake of declining malaria incidence, these findings underscore the need for clinicians to consider RVF in the differential diagnosis for febrile illnesses. Several types of direct contact with livestock are important risk factors for past infection with RVFV in this study's population. However, at least part of RVFV transmission appears to have occurred through bites of infected mosquitoes.

Author Summary

Rift Valley fever (RVF) is a disease of animals and people that is caused by the RVF virus. During epidemics, humans get RVF through direct contact with animals or through mosquito bites. In East Africa, epidemics occur every 5–15 years following unusually high rainfall. In between epidemics, the transmission of RVF might occur at low level. In an epidemic-free period, we measured whether people in the Kilombero Valley in Tanzania had evidence of past and recent RVF infection in their blood sample, and studied risk factors. Three per cent of people had been infected recently, and 12% had evidence of past infection, with increased risk with age, among milkers and among people eating raw meat. Some people with past or recent infection reported they had not had contact with animals. Households keeping livestock had more members with evidence of past infection. The findings show that people get infected with RVF in between epidemics, and that various types of contact with livestock are important risk factors. There is also evidence that some people get infected with RVFV by mosquitoes in the epidemic free period. Clinicians in the Kilombero Valley should consider RVF in the differential diagnosis of patients with fever.

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Introduction

Rift Valley fever (RVF) is one of the major viral zoonoses in Africa. The disease is caused by the Rift Valley fever virus (RVFV) of the genus *Phlebovirus* in the family *Bunyaviridae* [1], and it is transmitted to animals through infectious mosquito bites and other arthropod vectors [2]. People become infected either from mosquito bites or by direct or indirect contact with infectious material when exposed to blood, body fluids or tissues of viraemic animals when handling sick or dead animals as well as through aerosol transmission, consumption of raw milk, meat or blood [3–5].

The disease was first described in the Rift Valley of Kenya in the early 1900s and the etiological agent demonstrated in the early 1930s [6]. RVF epidemics occur in cycles of 5–15 years in the Eastern Africa region as a result of abnormally high precipitation, for example during the warm phase of the El Niño/Southern Oscillation (ENSO) phenomenon [7]. In other regions the disease has been driven by floods caused by other sources including construction of hydroelectric dams [8]. During the outbreaks the disease causes devastation in livestock populations and economies of livestock keepers as a result of morbidity, mortality in new-borns and abortions (irrespective of gestation period) with direct negative consequences in the next crop of new-borns [9].

Public health consequences during epidemics involve a wide range of clinical manifestation in people including mild illnesses characterized by fever, muscle pain, joint pain, and headache, which can cause RVF to be confused clinically with other febrile illnesses such as malaria. In mild cases, symptoms persist for about a week and subside without specific treatment. A small percentage (0.5–2%) of patients may develop severe forms of the disease characterized by either ocular disease, meningo-encephalitis or haemorrhagic fever which last for 1–4 weeks after onset of symptoms [10, 11]. People most at risk include those in close contact with infected animals and infectious materials [4], but also those unprotected from infectious bites of infected mosquitoes. Apart from general supportive therapy, there is no established treatment for people, and a commercial vaccine for humans is not available either. The control of RVF therefore relies mainly on vaccination of livestock and preventive measures by humans (including protection from mosquito bites and avoidance of contact with infected animals and infectious material during epidemics). [11].

Inter-epidemic transmission has increasingly been reported in recent years, including in our study area, but its consequences are not fully understood and its incidence not explored enough for future epidemic preparedness [8, 12–16]. Relatively little is known regarding the natural history of RVF as the epidemics occur in remote areas inaccessible during heavy rains; on the other hand, inter-epidemic RVF transmission presents an opportunity for studying its natural history as it normally occurs when affected areas are accessible.

In Tanzania, RVF with human involvement has been reported in the past [17, 18], with few studies demonstrating inter-epidemic transmission in livestock and people [12, 19]. During the 2006/07 RVF epidemic in Tanzania, livestock and people in the Kilombero Valley were affected [20], and a sero-survey in livestock indicated presence of inter-epidemic period transmission of RVF [12]. The Kilombero Valley is a seasonally inundated floodplain between the densely forested escarpment of the Udzungwa mountains to the northwest and the grass covered Mahenge mountains to the southeast. The annual floods in the valley mimic flooding that may occur elsewhere during ENSO years. In the Kilombero Valley, there has been intense malaria transmission due to abundance of the *Anopheles gambiae* complex, but other mosquito species including vectors of RVF virus (e.g. *Culex* spp., *Aedes* spp. and

Mansonia spp.) are present [21]. The current study therefore aimed to 1) determine whether people do acquire RVF during the inter-epidemic period in the Kilombero Valley and 2) evaluate the demographic, behavioural, occupational, and spatial determinants of recent and longstanding RVF sero-positivity in people.

Methodology

Study population and area

We conducted the study in rural areas of the Kilombero River Valley, located in the Kilombero and Ulanga districts in south-eastern Tanzania [22]. The Kilombero Valley is characterized by seasonal flooding which supports reproduction of large numbers of mosquitoes including arbovirus vectors such as *Aedes* spp [21]. The inhabitants of the two districts engage mainly in smallholder farming, fishing, and livestock keeping. A serological survey was carried out from March to August 2012 in six villages, three from each study district, with a total population of 14,517 in 3716 households. About a quarter of households keep livestock [23]. We selected the villages from hotspots of RVF transmission in the livestock populations in the Kilombero Valley [12]. This aimed at maximizing the probability of detecting inter-epidemic virus activity in the human population, since the hotspots indicated presence of ecological features that promote RVF transmission.

Data and sampling

The sample size calculation took into account the fact that sampling was done in households (clusters), with an average cluster size of 5 individuals per household considered appropriate for the valley [22] so a design effect of 3 was applied. The design effect adjusted sample size was further adjusted for the expected number of covariates we hoped to evaluate, which overall gave a sample size of 726 in 145 clusters. To ensure equal representation, we selected livestock keepers' and farmers' households independently as sampling units, because the two sub-populations are exposed in different ways to RVF risk factors [24]. In the four villages that were within the health and demographic surveillance system (IHDSS) of the Ifakara Health Institute, we randomly selected farmers' households from the master list of IHDSS [23]. For farmers' households in the other two villages and for livestock keepers' households in all villages, we obtained the lists of households from the village office and manually picked every n^{th} household from the list.

We took blood samples from all members of the household who provided written consent to participate in the study. For children under 18 years the written consent was provided by parents or guardians. We collected blood samples into vacutainer tubes containing clot activator and after clotting, eluted the sera into cryovial tubes and kept these in a car fridge until transferred to the laboratory. We collected demographic characteristics and individuals' exposure to risk factors to RVF through a structured questionnaire.

Serological analyses

We analysed the serum samples for presence of RVFV antibodies by two commercial enzyme-linked immunosorbent assay (ELISA) kits, an inhibition ELISA and a capture ELISA. The inhibition ELISA simultaneously detects immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against RVFV in humans, domestic and wildlife ruminants (Biological Diagnostic Supplies Limited, Dreghorn, United Kingdom) [25]. We converted the net optical density (OD) reading for each sample to a percentage inhibition (PI) value using the equation: [(100 –(net OD of test sample / mean net OD of negative control) x 100]. Test results producing PI values ≥38.6 are considered positive (following the manufacturer's recommendations) whereas below that threshold is negative, with sensitivity and specificity being 99.5% and 99.7% respectively [25]. To determine recent infection, we then tested the positive samples for the presence of IgM using the capture IgM ELISA (Biological Diagnostic Supplies Limited, Dreghorn, United Kingdom) [26, 27]. For this test, we used the two intermediate net OD values of positive controls (C+) for the calculation of the net mean OD value of C+. We then used this value in subsequent calculations of percentage positivity (PP) of C+, C- and test sera as follows: [PP = (net OD serum/net mean OD C+) x 100]. The cut off for positive samples' PP values was ≥7.1, with sensitivity and specificity being 96.4% and 99.6% respectively [27].

Data analyses

We analysed the data in STATA version 13 (Stata Corp., College Station, Texas, USA). Samples that were positive by inhibition ELISA were considered to give evidence of past infection in the individual, as IgG antibodies last long in persons infected in the past [26]. Samples that were positive by IgM ELISA were considered to indicate recent infection in the individual, as IgM antibodies are short lived following infection by RVF virus [26, 28]. To examine risk factors of RVF virus infection and help identify households at higher risk for targeted public health interventions, we developed three separate mixed effect logistic models. We built two models for individual level risk factors for recent and past infection as outcome variables respectively and treated households as a random effect variable. We built a third model for household level factors with household sero-positivity as outcome variable and villages as random effect variable. For each model, we first determined the univariable association of individual factors with the outcome by fitting a logistic regression model. Variables with p-value <0.25 were selected as potential covariates in the multivariable analysis, where a p-value ≤ 0.05 was considered statistically significant. We performed manual forward model-building with subsequent models evaluated against sparser models by means of the Akaike Information Criterion (AIC). We also tested two-way interactions between variables included in the model. Lastly, all factors that were dropped in the process of model building were later tested for any confounding effect. We considered factors to be a confounder if they led to a change of ≥25% in the coefficient estimates. We calculated the population attributable fraction (PAF), a fraction of all cases in the study population due to exposure to a certain risk factor, as follows: PAF = (Px*(RR-1))/(1+(Px*(RR-1))), where Px = estimated population exposure and RR = risk ratio.

Ethics statement

We obtained ethical approval from both the Institutional Review Board of the Ifakara Health Institute (IHI-IRB) and Medical Research Coordination Committee of the Tanzania's National Institute for Medical Research for this study, permit number NIMR/HQ/R.8a/Vol.IX/1101. Prior to study procedures, participants were explained the study purpose and procedures and upon agreeing to participate, individual adult participants provided a written informed consent whereas parents or guardians provided written consent for the under-age participants.

Results

The analyses were based on data from 606 participants in 141 households with complete questionnaire and laboratory results. We could not attain the a priori calculated sample size because of consenting issues among household members and because family size was smaller than expected. We do not anticipate this has introduced underrepresentation of participants with certain characteristics given the number of clusters involved. Out of 606 participants, 55.6% were females with age ranging between 2 and 90 years. Fifty four per cent and 46% of the participants originated from Kilombero and Ulanga districts respectively.

The inhibition ELISA results indicated an overall RVF sero-prevalence of 11.7% (95% CI = 9.2–14.5). There was a linear increase in sero-prevalence in the 10 year cohorts (Fig. 1). Evidence of recent infection by RVFV was found in 16 participants representing 2.6% overall (n = 606) and 22.5% of inhibition ELISA positive individuals (n = 71). Four of 16 (25.0%) IgM positives and 11/71 (15.5%) of individuals with inhibition ELISA sero-positivity reported they had had no previous animal contact, suggesting that at least part of the transmission in the area occurred through infected mosquito bites.

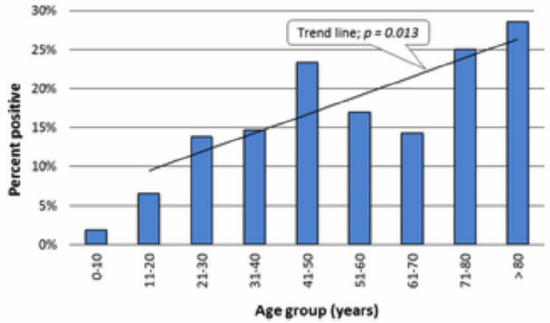
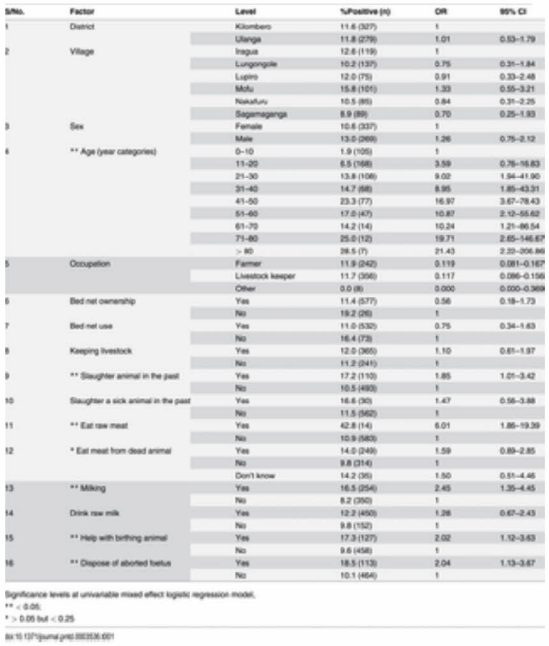


Fig 1. Prevalence of Rift Valley fever by age groups.
The trend line indicates gradual increase of sero-positivity with age.
http://dx.doi.org/10.1371/journal.pntd.0003536.g001

In the univariable analyses, factors associated with past RVF infection were history of participating in slaughter of animals (odds ratio [OR] 1.85; 95% CI 1.01–3.42), assisting birthing animals (OR 2.02; 95% CI 1.12–3.63), milking animals (OR 2.45; 95% CI 1.35–4.45), eating raw meat/blood (OR 6.01; 95% CI 1.86–19.39), disposing aborted foetus (OR 2.04; 95% CI 1.13–3.67) and being older (OR 1.03 per year; 95% CI 1.02–1.04) (Table 1). In the multivariable model, age (OR 1.03; 95% CI 1.01–1.04), milking animals (OR 2.19; 95% CI 1.23–3.91) and eating raw meat/blood (OR 4.17; 95% CI 1.18–14.66) remained significantly associated with past infection (Table 2). The PAFs of milking animals and eating raw meat in the past were 29% and 6% respectively. None of the risk factors studied were associated with recent infection (results not shown).



S/No.	Factor	Level	%Positive (n)	OR	95% CI
1	District	Kilombero	11.6 (327)	1	
		Ulanga	11.8 (279)	1.01	0.53–1.79
2	Village	Iragua	12.6 (119)	1	
		Lungongole	10.2 (137)	0.75	0.31–1.84
		Lupiro	12.0 (75)	0.91	0.33–2.48
		Mofu	15.8 (101)	1.33	0.55–3.21
		Nakafuru	10.5 (85)	0.84	0.31–2.25
		Sagamaganga	8.9 (89)	0.70	0.25–1.93
3	Sex	Female	10.6 (337)	1	
		Male	13.0 (269)	1.26	0.75–2.12
4	** Age (year categories)	0–10	1.9 (105)	1	
		11–20	6.5 (168)	3.59	0.76–16.83
		21–30	13.8 (108)	9.02	1.94–41.90
		31–40	14.7 (68)	8.95	1.85–43.31
		41–50	23.3 (77)	16.97	3.67–78.43
		51–60	17.0 (47)	10.87	2.12–55.62
		61–70	14.2 (14)	10.24	1.21–86.54
		71–80	25.0 (12)	19.71	2.65–146.67
		> 80	28.5 (7)	21.43	2.22–206.86
5	Occupation	Farmer	11.9 (242)	0.119	0.061–0.167
		Livestock keeper	11.7 (356)	0.117	0.086–0.156
		Other	0.0 (8)	0.000	0.000–0.369
6	Bed net ownership	Yes	11.4 (577)	0.56	0.18–1.73
		No	19.2 (26)	1	
7	Bed net use	Yes	11.0 (532)	0.75	0.34–1.63
		No	16.4 (73)	1	
8	Keeping livestock	Yes	12.0 (365)	1.10	0.61–1.97
		No	11.2 (241)	1	
9	** Slaughter animal in the past	Yes	17.2 (110)	1.85	1.01–3.42
		No	10.5 (493)	1	
10	Slaughter a sick animal in the past	Yes	16.6 (30)	1.47	0.58–3.88
		No	11.5 (562)	1	
11	** Eat raw meat	Yes	42.8 (14)	6.01	1.86–19.39
		No	10.9 (583)	1	
12	* Eat meat from dead animal	Yes	14.0 (249)	1.59	0.89–2.85
		No	9.8 (314)	1	
		Don't know	14.2 (35)	1.50	0.51–4.46
13	** Milking	Yes	16.5 (254)	2.45	1.35–4.45
		No	8.2 (350)	1	
14	Drink raw milk	Yes	12.2 (450)	1.28	0.67–2.43
		No	9.8 (152)	1	
15	** Help with birthing animal	Yes	17.3 (127)	2.02	1.12–3.63
		No	9.6 (458)	1	
16	** Dispose of aborted foetus	Yes	18.5 (113)	2.04	1.13–3.67
		No	10.1 (464)	1	

Significance levels at univariable mixed effect logistic regression model,
** < 0.05;
* > 0.05 but < 0.25

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Table 1. Prevalence of RVF inhibition ELISA sero-positivity and association of individual-level variables with sero-positivity.
http://dx.doi.org/10.1371/journal.pntd.0003536.t001

No.	Factor	Level	OR	95% CI
1	Help with birthing animal	No	1	
		Yes	0.83	0.36–1.90
2	Age (years)	n/a*	1.03	1.01–1.04
3	Milking	No	1	
		Yes	2.19	1.23–3.91
4	Eat raw meat	No	1	
		Yes	4.17	1.18–14.66
5	Dispose of aborted foetus	No	1	
		Yes	1.35	0.59–3.09

* Age was included as a continuous variable,
OR = odds ratio,
CI = confidence interval

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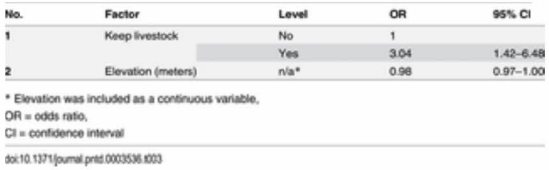
Table 2. Multivariable analysis of correlates of RVF antibody sero-positivity.
http://dx.doi.org/10.1371/journal.pntd.0003536.t002

Though keeping livestock was not associated with individuals' sero-positivity, households keeping livestock had a higher chance of having at least one member with past infection (OR = 3.04, 95% CI = 1.42–6.48) than households that do not keep livestock (table 3). Participant's gender, eating meat from dead animals, drinking raw milk, bed net use, proximity to the main flood area, elevation and district were not associated with inhibition ELISA sero-positivity.



No.	Factor	Level	OR	95% CI
1	Keep livestock	No	1	
		Yes	3.04	1.42–6.48
2	Elevation (meters)	n/a*	0.98	0.97–1.00

* Elevation was included as a continuous variable,
OR = odds ratio,
CI = confidence interval

doi:10.1371/journal.pntd.0003536.t003

Table 3. Household-level factors for RVF sero-positivity.
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Discussion

We report here presence of IgM antibodies against RVFV among inhabitants of Kilombero Valley. This confirms recent infection and thus transmission of RVF which is not linked to the previous epidemic which happened five years prior in the study area [20]. This finding affirms our previous report, which highlighted IEP transmission of RVF in livestock [12]. Inter-epidemic sero-positivity to RVF in people has also been previously documented in other parts of Tanzania and Africa [13, 14, 16, 29], with IgM antibodies detected in Nigeria and Chad [14, 29]. The observed sero-prevalence by inhibition ELISA (11.7%) in this study is high compared to studies from other parts of Tanzania during inter-epidemic period with sero-prevalence of 5.2% and 4% in Mbeya and Tanga regions respectively [13, 19], possibly as a result of our sampling of participants from hotspots of RVF circulation in animals. In Gabon, a country with no RVF epidemic history, a sero-prevalence of 3.3% has been reported [30], in Kenya, an epidemic prone country, a mixed picture for inter-epidemic sero-positivity has been recorded in people in different geographical locality and time [16, 31].

In this study, participants who milked animals were more likely to have evidence of past RVF infection. This points to a potential public health consequence of RVFV shedding in milk which occurs during the viraemic phase of the disease. The traditional milking practices create a lot of aerosols, and if one is milking a viraemic animal the RVFV containing milk particles could result into infection to milkers through inhalation of the infectious aerosols [32]. Also skin abrasions on hands of milkers could form an easy route of infection when people have broken skin. However, drinking raw milk was not associated with longstanding sero-positivity. Although raw milk consumption is considered an important risk factor during epidemics [18, 33], the infection through oral route comes across barriers including acidic environment in the stomach [34]. The findings in our study might also be explained by the practice of consuming fermented milk by the livestock keepers in which case the virus would die when exposed to acidic environment of sour milk [34].

People who ate raw meat (including blood and internal organs such as kidneys and liver) were more likely to have evidence of past RVF infection. The animal products (meat and blood) from infected animals could have a high concentration of RVFV which has the ability to persist at neutral pH in carcasses. When meat is consumed raw before the pH drops with rigor mortis this could lead to infection in people. Eating meat from animals that died before slaughter was not associated with sero-positivity which might be because individuals who reported eating meat from dead animals also reported cooking the meat before consumption, which would have destroyed the virus.

The high PAF values for milking and for eating raw meat as risk factors present important educational intervention targets for risk reduction even during epidemic free periods. The increased sero-prevalence in older individuals suggests stable rates of on-going transmission in the population. The increased sero-prevalence was also evident when participants were categorized into ten-year cohorts, with drops in the 51–60 and 61–70 groups. Older individuals might have either been infected in one or more previous epidemics or through clinically undetected low-level virus circulation in the study area.

Although there was no significant risk difference between individual livestock keepers and farmers, households keeping livestock had a higher probability of having at least one member with past RVF infection. This might imply presence of either higher risk through animal contact as compared to mosquito bites or higher exposure to infectious mosquito bites among livestock keepers, as mosquitoes living in close proximity to livestock can pick up infection from amplifying infected hosts and transmit to livestock keepers even in circumstances of low-level virus circulation in the general vector populations.

Helping with birthing animals and disposal of aborted foetuses are high risk activities when dealing with infected animals or infectious materials especially when not wearing proper protective attire. In this study both were not statistically significant in the final model. People who reported participating in slaughtering animals in the past (including skinning and butchering) were more likely to be sero-positive but the sero-positivity was not associated with slaughtering sick animals suffering from other unknown conditions. Slaughtering animals sick from RVF exposes individuals through direct contact with infectious materials such as aerosols from oozing blood and other organs during skinning and butchering [3].

Although sero-prevalence in male individuals was slightly higher, sex was not associated with sero-positivity. The sex difference in RVF prevalence has been reported in some studies [16, 30] but was not apparent in others [13, 19, 35] and where it existed, it has been mostly attributed to gender-biased distribution of animal handling in affected populations. The lack of association between gender and sero-positivity in this study indicates that either the specific risk-increasing animal handling activities are equally distributed between genders or that direct mosquito bites as source of infection to people in the valley was equally important. The latter possibility is supported in our study area because men were more involved with animal handling duties.

Bed net ownership and use were not associated with sero-positivity. This is possibly because in the study area there is high bed net coverage [36], but also because the main RVF vector *Aedes* mosquitoes are day biting mosquitoes.

Conclusion

These findings, coupled with our previous report in livestock [12], indicate persistent IEP transmission of RVFV in both livestock and human populations in the Kilombero Valley. The animal contact risk factors, especially milking and eating raw meat are important and present educational intervention targets for risk reduction. In the wake of declining malaria incidence [37] these findings

underscore the need for clinicians to consider RVF in the differential diagnosis for febrile illnesses among Kilombero Valley inhabitants. This is relevant regardless of the person's occupation, because part of the transmission likely happens through infectious mosquito bites. The findings also suggest the opportunity and need to further investigate the circulating RVFV strain as well as the main vectors responsible for IEP transmission.

Supporting Information

S1 Checklist. STROBE Checklist.

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(DOC)

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Author Contributions

Conceived and designed the experiments: RDS DB EG. Performed the experiments: RDS MA EG. Analyzed the data: RDS ENA ET DB. Wrote the paper: RDS ENA ET DB EG.

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SUPPLEMENT ARTICLE

Emergence of Divergent Zaire Ebola Virus Strains in Democratic Republic of the Congo in 2007 and 2008

Gilda Grard,[1] Roman Biek,[2] Jean-Jacques Muyembe Tamfum,[3,4] Joseph Fair,[4] Nathan Wolfe,[4] Pierre Formenty,[5] Janusz Paweska,[6] and Eric Leroy[1,7]

[1]Centre International de Recherches Médicales de Franceville, Gabon; [2]College of Medical, Veterinary and Life Sciences and Medical Research Council–University of Glasgow Centre for Virus Research, University of Glasgow, United Kingdom; [3]Institut National de Recherche Biomédicale, Kinshasa-Gombe, Democratic Republic of the Congo, [4]Global Viral Forecasting, San Francisco, California; [5]Department of Epidemic and Pandemic Alert and Response, World Health Organization, Geneva, Switzerland; [6]Special Pathogens Unit, National Institute for Communicable Diseases, Sandringham, South Africa; and [7]UMR MIVEGEC (IRD 224-CNRS 5290-UM1), Montpellier, France

Background. *Zaire ebolavirus* was responsible for 2 outbreaks in Democratic Republic of the Congo (DRC), in 1976 and 1995. The virus reemerged in DRC 12 years later, causing 2 successive outbreaks in the Luebo region, Kasai Occidental province, in 2007 and 2008.

Methods. Viruses of each outbreak were isolated and the full-length genomes were characterized. Phylogenetic analysis was then undertaken to characterize the relationships with previously described viruses.

Results. The 2 Luebo viruses are nearly identical but are not related to lineage A viruses known in DRC or to descendants of the lineage B viruses encountered in the Gabon–Republic of the Congo area, with which they do, however, share a common ancestor.

Conclusions. Our findings strongly suggest that the Luebo 2007 outbreak did not result from viral spread from previously identified foci but from an independent viral emergence. The previously identified epidemiological link with migratory bat species known to carry *Zaire ebolavirus* RNA support the hypothesis of viral spillover from this widely dispersed reservoir. The high level of similarity between the Luebo2007 and Luebo2008 viruses suggests that local wildlife populations (most likely bats) became infected and allowed local viral persistence and reemergence from year to year.

The genus *Ebolavirus* comprises 4 species: *Reston ebolavirus*, *Côte d'Ivoire ebolavirus* (CIEBOV), *Sudan ebolavirus* (SEBOV), and *Zaire ebolavirus* (ZEBOV) [1] as well as the recently discovered Bundibugyo Ebola virus (BEBOV) that may be classified as a new species [2]. Genetic diversity among species ranges from 25% to 35%, and *Zaire ebolavirus* is the most virulent, accounting for 1390 human cases in 13 recorded outbreaks over the preceding 35 years, with a case-fatality rate of ≤90%.

The first recognized ZEBOV epidemic occurred at Yambuku in the Democratic Republic of the Congo (DRC) in 1976 [3]. An isolated case was registered in Tandala, DRC, in 1977 [4], succeeded by a 17-year epidemically silent period before the Mekouka outbreak in Gabon in 1994. Subsequently, Gabon experienced 2 epidemics in 1996 at Mayibout and Booué [5, 6] whereas DRC saw a reappearance of ZEBOV in 1995, at Kikwit [7, 8]. Between 2001 and 2005, the cross-border area between northeast Gabon and northwest Republic of Congo (Gabon-RC) was hit by 5 ZEBOV outbreaks [9, 10]: Mekambo 2001–2002 (Gabon), Mbomo–Kellé 2001–2002 (RC), Kellé 2003 (RC), Mbandza–Mbomo 2003 (RC), and Etoumbi 2005 (RC). Like the 1994–1996 Gabonese epidemics, these cross-border outbreaks were marked by large wildlife epizootics [11–13], inducing

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Correspondence: Gilda Grard, PhD, Centre International de Recherches Médicales de Franceville, BP 769, Franceville, Gabon (gildagrard@gmail.com).

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mortality rates of ≤80% in great apes [14–16]. Chimpanzees, gorillas, and duikers were susceptible hosts responsible for viral introduction into human populations [11, 12].

The sequential appearance of ZEBOV outbreaks from 1976 to 2003 led to a hypothesis of wavelike viral dispersion into new territories to explain the explosive emergence of ZEBOV in the Gabon-RC area [17]. Indeed, the spatial and temporal pattern of ZEBOV outbreaks correlated with the genetic distance among strains, having apparently evolved continuously in a single lineage, westward from Yambuku (DRC) in 1976 to Mvoula (RC) in 2003. However, subsequent characterization of new *GP* and *NP* sequences demonstrated the existence of a second genetic lineage [10]. The previously recognized lineage A included ZEBOV strains from the 1976–1996 outbreaks (DRC and Gabon), whereas the newly described lineage B included animal-derived sequences since 2001 and the human strains from the Mbandza_Mbomo 2003 and Etoumbi 2005 outbreaks. The strains responsible for the previous 3 outbreaks were phylogenetically linked to lineage A in the *GP* gene, whereas they grouped with lineage B in the *NP* gene, strongly suggesting a recent recombination event [10]. Although the spatial and temporal pattern of outbreaks since 2001 still suggested progressive spread of the virus, the identification of 2 genetically divergent lineages did not fit the expected scenario in which all outbreaks during this period would be linked by ongoing transmission. Instead, the existence of the 2 lineages implied independent introductions into human populations following multiple viral spillovers from a reservoir host, a scenario referred to as the *multi-emergence hypothesis* [10, 11]. The data available from outbreaks up to 2005 thus indicated that the history of ZEBOV could not be explained by a single spreading wave of infections across multiple countries. In addition, ZEBOV sequence data collected from outbreaks up to that point revealed another puzzling aspect of ZEBOV biology, in that observed levels of sequence divergence were low (<5%), especially compared with other filoviruses, such as Marburg virus [18]. Molecular clock-based analyses suggested that these low levels of viral diversity might be due to a recent genetic bottleneck and that the estimated most-recent common ancestor of all viruses in the ZEBOV clade dated back only to the early 1970s [10, 17], just prior to the first outbreak at Yambuku in 1976.

In stark contrast to the temporal and geographical clustering of the Gabon-RC outbreaks, ZEBOV unexpectedly reemerged in DRC in 2007 in the Luebo area (Kasaï Occidental province), nearly 1000 km southeast of the last foci registered only 2 years before in RC. The Luebo 2007 outbreak occurred from June to November, affecting 264 persons and causing 187 deaths (70% case-fatality rate [19]). ZEBOV reappeared in the same area 1 year later, from November 2008 to February 2009, in the Luebo 2008 outbreak. No epidemiological survey was performed and official estimates were 32 human cases and 15 deaths (47% case-fatality rate [20]).

Although the first recorded ZEBOV outbreak took place in DRC in 1976, the virus reemerged in this country only once (Kikwit 1995) before the 2 Luebo outbreaks, whereas the neighboring countries were repeatedly affected during this time frame. In addition to the spatial discontinuity with the Gabon-RC outbreaks network, the Luebo outbreaks were in temporal discontinuity with the Kikwit epidemic that took place only 290 km eastward but 12 years before. Making the issue more complex, epidemiological and ecological investigations suggested that susceptible hosts, such as great apes and duikers, sustaining ZEBOV epizootics in Gabon and RC [11], were not involved in the Luebo epidemics [21]. Instead, the source of the Luebo 2007 outbreak appeared to be the hunting of fruit bats. These hunted bats, including *Hypsignatus monstrosus* and *Epomops franqueti,* undergo large annual migrations and are suspected ZEBOV reservoirs [21, 22].

Here, we report virological and phylogenetical analyses to determine whether the Luebo viruses (i) are related to viruses previously seen in DRC; (ii) are descendants of the recent Gabon-RC viruses, or (iii) represent newly emerged variants. We also investigated if the 2 Luebo outbreaks in 2007 and 2008 were caused by the same or different viruses and if the new sequence data collected would change the estimated age of the ZEBOV clade. Our phylogenetic analyses show that the Luebo viruses are not direct descendants of viruses characterized so far but share a common ancestor with lineage B viruses, despite the fact that the 2 groups are found nearly 1000 km apart. The Luebo 2007 and Luebo 2008 strains were nearly identical, despite being sampled 1 year apart. Given the limited divergence from other ZEBOV viruses, the estimated age of the ZEBOV clade overall is unaffected by these new sequence data.

MATERIALS AND METHODS

Ethical Considerations

Blood samples were collected from patients during the acute phase of illness by healthcare workers from the Health Ministries of DRC, Médecins sans Frontières (MSF), and the World Health Organization (WHO), all of whom were participating in the international response. Blood samples were collected at the patient's home or in hospital isolation wards with verbal consent, and either were sent to international laboratory partners for diagnostic evaluation or were immediately processed for ZEBOV detection in the field laboratories.

Outbreaks

The Luebo 2007 outbreak occurred between May and November 2007 in the Kasaï Occidental province of DRC (Figure 1) as previously described [21]. A diagnosis of ZEBOV hemorrhagic fever was made on September 2007, both by Centre International de Recherches Médicales de Franceville, Gabon (CIRMF) and Centers for Disease Control and Prevention

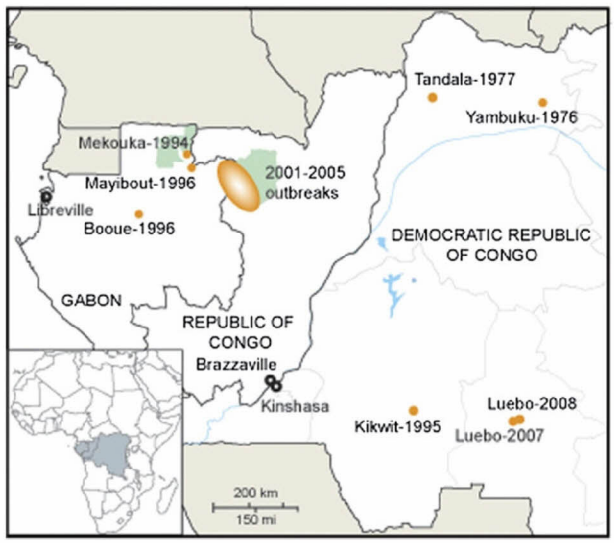

Figure 1. *Zaire ebolavirus* (ZEBOV) outbreak locations since 1976. The Minkebe National Park (Gabon) and Odzala National Park (Republic of the Congo) are in green.

(CDC), Atlanta, Georgia. Outbreak control and investigations were supported by (i) an international WHO team including DRC Ministry of Health (MoH); Institut National de Recherche Biomédicale (INRB), Kinshasa, DRC; MSF; CIRMF; and National Microbiology Laboratory (NML), Winnipeg, Canada and (ii) a CDC team. Field laboratories and an isolation ward were set up for case monitoring. Because of its remote location, the outbreak's initial cases could not be observed and the international response was considerably delayed. Most investigations were done retrospectively and were impaired by the concomitant occurrence of *Salmonella typhi* and *Shigella* outbreaks. The Luebo 2008 outbreak occurred from November 2008 to February 2009. The first identified case was an 18-year-old woman who died from hemorrhagic complications during premature delivery on 27 November. Subsequently, 13 contacts became ill and 9 died. MSF set up an isolation ward, and on 23 December, 3 suspect samples were taken to CIRMF and National Institute for Communicable Disease (NICD), South Africa. On 24 December, laboratory confirmation of 2 ZEBOV infections was obtained, and an alert was declared on 25 December 2008. Further diagnostics were performed at INRB, with laboratory and training support provided by CIRMF and NICD teams involved in the WHO international response. No epidemiological investigation was undertaken, so the source of the outbreak is unknown and the number of cases and deaths may be underestimated.

Diagnostic. Samples received at CIRMF were chemically or heat-inactivated in biosafety level-4 (BSL-4) facilities before reverse-transcriptase polymerase chain reaction (RT-PCR) and antigen-capture assays. RNA was extracted from 140 µL of patients' sera with the QIAamp viral RNA extraction kit (Qiagen) according to the manufacturer's instructions. Partial L-gene sequences were amplified with degenerate primer pairs PanFilo-L1/Panfilo-L2 (5′-ATMGRAAYTTTTCYTTYTCWYT-3′/5′-TGWG–GHGGRYTATAAWARTCACTDACAT-3′) and PanFilo-L3/Panfilo-L4 (5′-GCNAARGCMTTYCCHAGYAAYATGATGG-3′/5′-ATAAWARTCACTDACATGCATRTARCA-3′).

A sandwich enzyme-linked immunosorbent assay (ELISA) for ZEBOV antigen detection was applied to 4-fold dilutions (1:4 to 1:256) of heat-inactivated sera (56°C, 30 minutes), as previously described [23]. Investigators coated 96-well plates with a cocktail of monoclonal antibodies against ZEBOV and coated control wells with normal mouse ascetic fluid. After sera incubation, ZEBOV antigens were detected by adding hyperimmune rabbit polyvalent Ebola antiserum [24] followed by peroxidase-conjugated goat antibodies. Reaction development was performed with the tetramethylbenzidine (TMB) detection system (Dynex Technologies).

Virus Isolation. Tissue culture was performed in a biosafety level 4 laboratory. Vero-cell monolayers in 25 cm^2 flasks were incubated for 1 hour at 37°C with 200 µL of serum at 1:10 dilution in Dulbecco's modified eagle medium (DMEM). Medium containing 2.5% fetal bovine serum and antibiotics was added and the cells were incubated at 37°C with 5% CO2 for 6 days. Supernatants were harvested and stored at −80°C until their use for genomic characterization.

Genomic Characterization. RNA was extracted from Vero-cell supernatants with the QIAamp viral RNA extraction kit (Qiagen). A set of 20 overlapping primer pairs (available on request) was designed from ZEBOV sequence alignments for RT-PCR (SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity, Invitrogen). Polymerase chain reaction product sizes averaged 1500 nucleotides and melting temperatures ranged from 55°C to 65°C. Contiguous sequences were assembled using ChromasPro 1.5 software (Technelysium Pty Ltd).

Phylogenetic Analysis

The new sequence data were aligned with existing full-length *GP* and *NP* sequences using the MUSCLE algorithm [25] implemented in the Geneious program [26]. Phylogenetic trees without a molecular-clock assumption were estimated for the *GP* and *NP* data set using MrBayes version 3.1.2 [27] in 2 independent runs of 2 million states with a sampling frequency of 1000 generations and a burn-in of 1 million samples. Phylogenies with a molecular-clock assumption and specific divergence dates were estimated using BEAST version 1.5 [28]. Analyses were calibrated using the year and month of sampling and applied a relaxed clock model with uncorrelated log–normally distributed rates [29] and a constant population size prior (alternative priors had a negligible effect on node height estimates). 2 independent runs of 10 million states were performed with a sampling frequency of 10 000 and a burn-in period of 1 million states. Both Bayesian inference methods applied a SDR06 model

of molecular evolution [30] and used sequences from CIEBOV and BEBOV as outgroups for rooting the ZEBOV clade. Runs were examined visually in program Tracer version 1.4.1 [31] to confirm convergence among runs and that chains had reached a stationary phase. Genbank accession numbers for the nucleotide sequences used in this study are available online (see Supplemental content S1).

RESULTS

Genome Description and Genetic Distances

The final sequence contigs cover 99% of the whole genomic sequences, with 18 808 and 18 775 nucleotides for the Luebo 2007 and Luebo 2008 strains, respectively, with partial leader and trailer sequences. The 2 Luebo strains are almost identical, differing at 11 nucleotide positions, of which 3 lie in noncoding region (NCRs; including intergenic regions and partial leader and trailer sequences), 5 are silent mutations (*NP, VP40, GP, VP24,* and *L* proteins), and 3 are nonsynonymous mutations, 1 in *VP35* and 2 in the *L* protein. This resulted in a p-distance of <0.1%, maximum divergences being observed in *VP35, VP40,* and *VP24* coding sequences (CDS) and not in the *NP* and *GP* variable genes. Gene sizes, transcriptional signals, *GP* editing site, cleavage sites, and immunosuppressive motifs were identical to those of other available ZEBOV sequences. The ectopic stop codon identified in the group R (recombinant) viruses [10] was absent from the *GP* Luebo sequences.

Compared with the fully characterized ZEBOV strains (Figure 2), the Luebo viruses appeared slightly more similar to the Yambuku 1976 strain (1.9% mean p-distance) than to the more recent Kikwit 1995 strain (2.15% mean p-distance). The maximum p-distances were recorded in the NCRs (1.9%–3.5%), *NP* (1.3%–2.1 %) CDS, and *VP40* (1.4%–2.1%) CDS, whereas the smallest distances were observed in *VP30* (<0.7%) CDS. Based on the inclusion of partially characterized strains, *NP* sequences from group B and R viruses are the sequences most closely related to the Luebo strains (p-distance < 2.1%), whereas higher p-distances are observed with lineage A viruses, namely the Booué 1996, Mayibout 1996, and Mekouka 1994 Gabonese strains (2.4%–2.8% p-distance). Conversely, in *GP* CDS, group A and B viruses appeared equally distant from the Luebo strains (~2.4%), whereas group R viruses were slightly more divergent, with 2.9%–3.1% p-distance.

Phylogenetic Analysis

Phylogenetic trees were derived from *NP* (1448 nucleotides, Figure 3) and *GP* (2031 nucleotides, Figure 4) CDS. In addition to the Bayesian method, molecular clock–based analysis was used to take into account the expectation that viruses sampled >30 years apart have evolved, and to estimate the lineage divergence dates (Figures 3*B* and 4*B*). The CIEBOV and BEBOV sequences were included as outgroups, because these are the viruses most closely related to ZEBOV.

The 4 phylogenetic trees display 2 genetic lineages, with higher support values observed with molecular-clock constraints. Lineage A included ZEBOV strains from 1994–1996 whereas lineage B included animal-derived sequences from 2001 and human isolates from Mbandza 2003 and Etoumbi 2005 outbreaks. With both methods and with high support values, the group R viruses display a phylogenetic incongruence, being associated with lineage B in the *NP* gene (Figure 3) and with lineage A in the *GP* gene (Figure 4). Except for the Bayesian

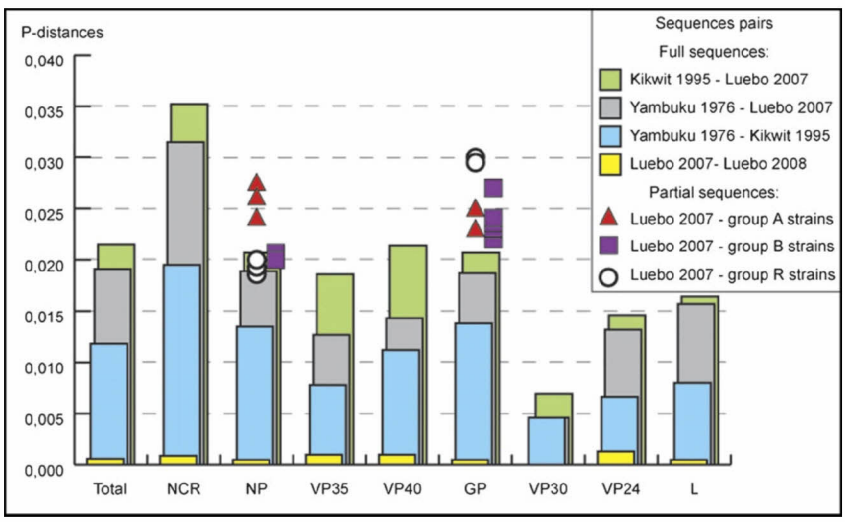

Figure 2. *Zaire ebolavirus* (ZEBOV) p-distances with respect to Luebo 2007 and Luebo 2008 sequences. Available complete sequences were first aligned for comparison with Luebo viruses. The p-distances between Yambuku 1976 and Kikwit 1995 are reported for reference (in blue). Complete *GP* coding sequences and partial *NP* coding sequences were then added to compare distances between Luebo viruses and other group A, B, and R partially characterized strains. NCR:, noncoding regions.

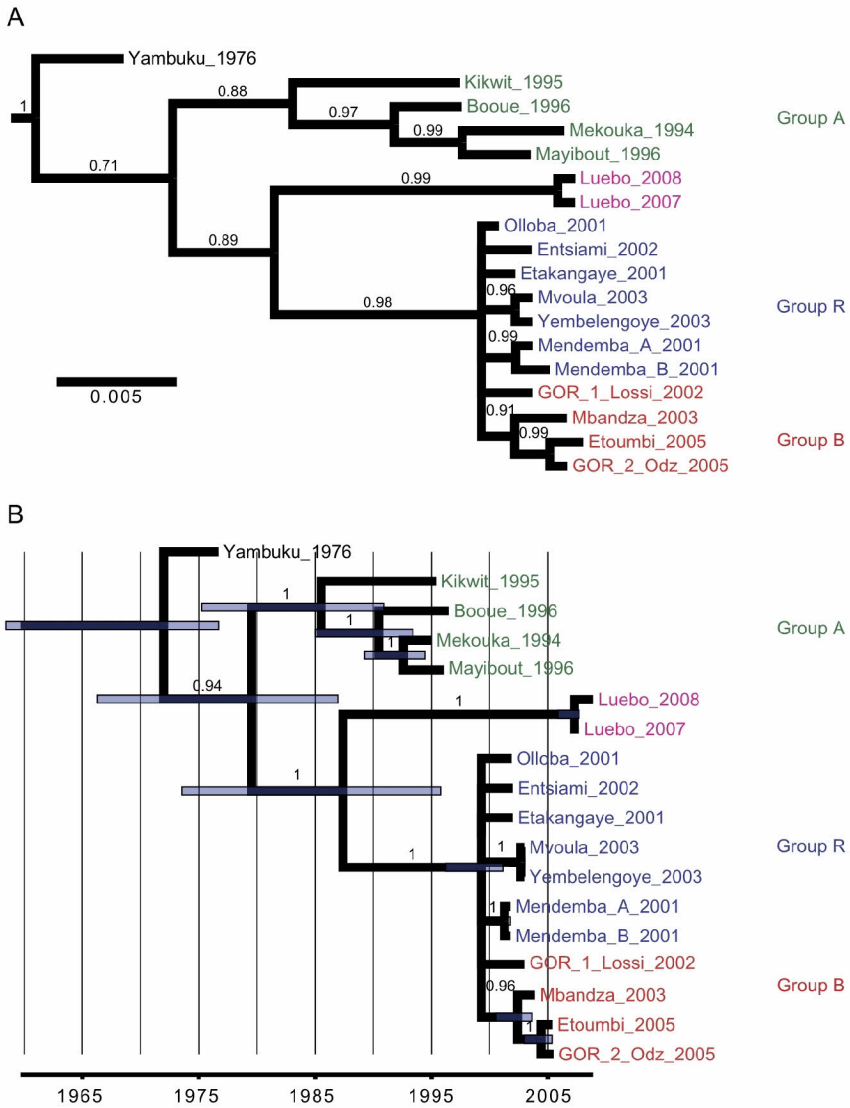

Figure 3. Bayesian phylogenies inferred from *NP* sequences, representing a consensus tree without molecular clock assumption (*A*) and a maximum clade credibility tree inferred under a 'dated tips' molecular clock model (*B*). *Côte d'Ivoire ebolavirus* (CIEBOV) and Bundibugyo Ebola virus (BEBOV) sequences were included as outgroups. Green: group A strains; red: group B strains; blue: group R strains. Branch support in form of Bayesian posterior probabilities is indicated above branches. In panel B, purple bars indicate the upper and lower limits of the 95% highest posterior density interval for estimated node ages.

analysis of *GP* sequences without the molecular-clock assumption, the Yambuku 1976 strain fell at the most basal position of the ZEBOV tree, just before the split into lineages A and B.

The Luebo 2007 and Luebo 2008 viruses grouped together tightly in all 4 trees. In most cases, they formed a strongly supported sister group to lineage B viruses, with the exception of the 1 *GP* analysis, which placed the Luebo viruses at the most basal position of the tree (Figure 4*A*).

According to the relaxed molecular-clock model applied to the *NP* (Figure 3*B*) and *GP* data (Figure 4*B*), the most-recent common ancestor (MRCA) of all known ZEBOV isolates was estimated to date back to 1972 and 1975, respectively. Lineage A and B would have split off sometime between 1979 and 1985, whereas the Luebo viruses would have separated from group B viruses between 1987 and 1996. The group R viruses shared an MRCA between 1998 and 2000, respectively.

DISCUSSION

The last 2 ZEBOV outbreaks occurred in DRC, within the same area, 1 year apart, in spatial and temporal discontinuities with the locations and time of occurrence of the previous sequential outbreaks. Following viral isolation, we characterized the full coding sequences of the Luebo 2007 and Luebo 2008 strains and performed phylogenetic analyses to investigate their relationships with previously characterized viruses.

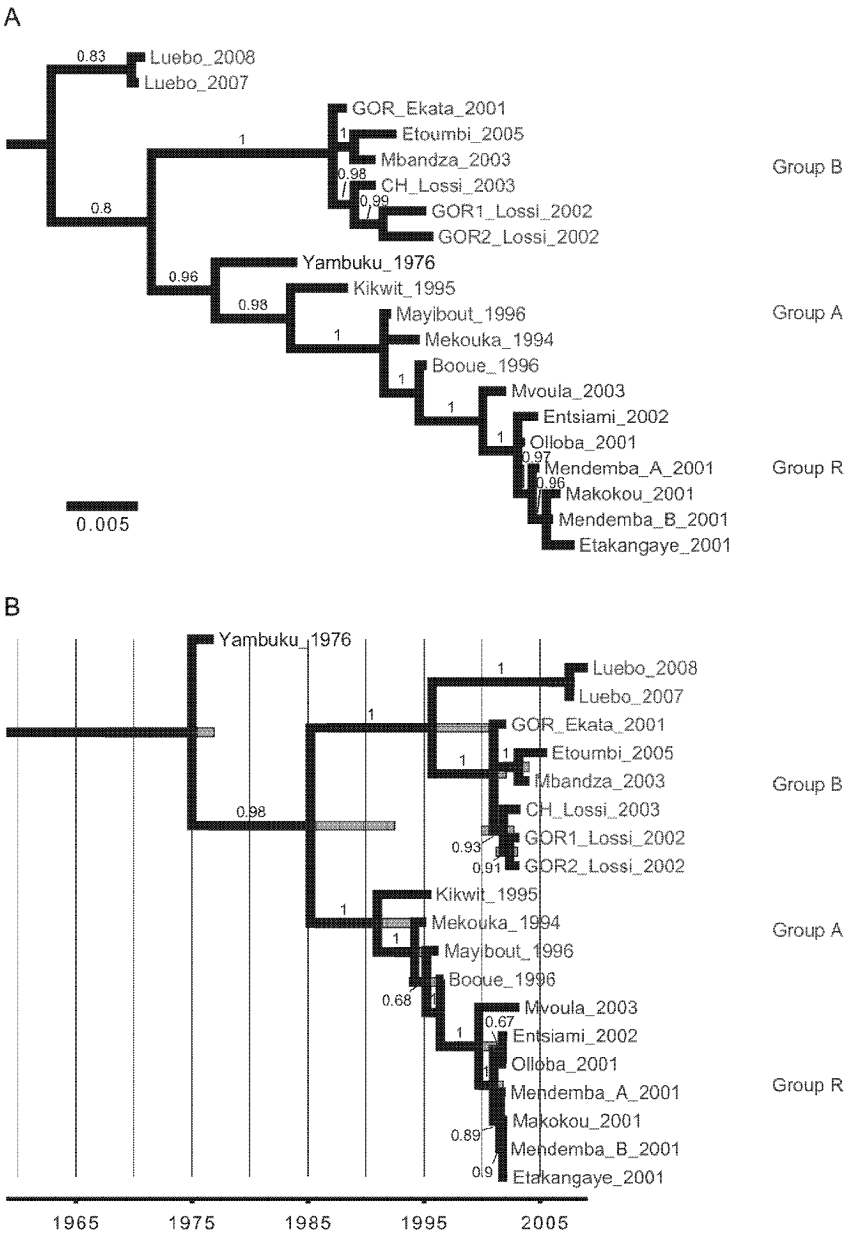

Figure 4. Bayesian phylogenenies inferred from *GP* sequences, representing a consensus tree without molecular-clock assumption (*A*) and a maximum clade credibility tree inferred under a 'dated tips' molecular-clock model (*B*). *Côte d'Ivoire ebolavirus* (CIEBOV) and Bundibugyo Ebola virus (BEBOV) sequences were included as outgroups. Green: group A strains; red: group B strains; blue: group R strains. Bayesian posterior probabilities are indicated above branches. In panel *B*, purple bars indicate the upper and lower limits of the 95% highest posterior density interval for estimated node ages.

The phylogenetic trees inferred from the *GP* and *NP* data sets, with and without a molecular-clock constraint, confirmed previous findings [10] including (i) the split of the ZEBOV clade into distinct lineages, (ii) the clustering of animal-derived sequences in the recently discovered B lineage, and (iii) the phylogenetic incongruence between the *GP* and *NP* phylogenies for the group R viruses. As in previous studies [10, 17], some uncertainty remained regarding the inferred position of the ZEBOV tree root. Most of our current analyses support a root-near position of the Yambuku 1976 virus and placed this virus outside groups A and B. The only exception to this was the *GP* analysis with no molecular clock assumption, which characterized the newly identified viruses from Luebo as a sister clade to all remaining ZEBOV isolates. However, the deeper tree nodes were not well resolved. More-definitive resolution of the early evolutionary history of the ZEBOV clade will likely not be possible until more full-length genomes of both lineage A and B viruses are available.

Surprisingly, from the p-distances observed all along the genome, the Luebo viruses shared greater homology with the Yambuku 1976 strain than with the more recent and

geographically closer Kikwit 1995 strain. Consistent with this, our phylogenetic analysis did not identify the Luebo viruses as belonging to the same genetic lineage A as Kikwit 1995. Instead, the Luebo viruses formed a well-supported sister group to the lineage B viruses, previously encountered only in Gabon-RC. The analysis clearly demonstrates that the Luebo viruses are not direct descendants of viruses sampled during previous outbreaks, which strongly suggests that the Luebo outbreaks did not arise through viral spread from any of the recently recognized foci. By contrast, the Luebo viruses are phylogenetically distinct from the strains characterized so far, suggesting that these outbreaks were epidemiologically independent of the 1994–2005 network. The most plausible scenario for such independent emergences is multiple viral spillover from a reservoir host widely dispersed throughout Central Africa.

Despite representing a ZEBOV lineage that is clearly divergent from any viruses previously described, the new DRC sequence data, like those collected during the 2001–2005 outbreaks [10], did not increase the estimated age of diversification of the ZEBOV clade as a whole. Rather, our date estimates confirm earlier results, placing the most recent common ancestor of the entire group at a time just prior to the virus' first emergence at Yambuku in 1976. Although this recent origin of the ZEBOV clade could result from a recent bottleneck and subsequent dispersion of ZEBOV across much of central Africa, the nature of the event that gave rise to this pattern is unknown.

Whereas the Luebo 2007 outbreak appeared to be linked to migratory fruit bats [21], the source of the 2008 outbreak could not be identified. The scenario of a second spillover from the migratory bat population appears unlikely, given that the 2 viruses were genetically almost identical. Such similarity would seem improbable for a virus originating from a large reservoir population that would be expected to simultaneously maintain multiple, and thus genetically diverse, viral lineages. Interestingly, the latter pattern was observed for Marburg viruses sampled from cavernous bats and humans in Uganda in 2007–2008 [32] and during the Durba outbreak (DRC, 1999–2000) [33], with different introductions into the human population, usually involving genetically divergent viruses. Despite the limited viral sampling, the high similarity between the 2 Luebo viruses is consistent with a direct epidemiological link between the 2 epidemics. Based on our evolutionary rate estimates for the full-length virus and the *GP* and *NP* genes ($\sim$1.5–7.5 $\times$ 10^{-4} substitutions per site per year) we would expect between 3 and 15 substitutions to accumulate across the entire ZEBOV genome over the course of a year. The observed divergence of 11 substitutions between the 2007 and 2008 viruses fits this well, raising the possibility that the 2008 virus is a direct descendent of the 2007 virus and that the latter continued to be transmitted in the area around Luebo. However, great apes were not present, signs of wildlife mortality were noticeably absent [21], and it is unlikely that human cases in the area would have been missed over a period of several months, posing the question of how the virus could have persisted so inconspicuously during this period. Following viral introduction into the area in 2007, subsequent transmission among local, nonmigrating bats could provide a possible explanation, especially as local bats species include *Hypsignatus monstrosus* and *Epomops franqueti* (E. Leroy, personal observations), both of which are suspected of being natural ZEBOV reservoir species [22].

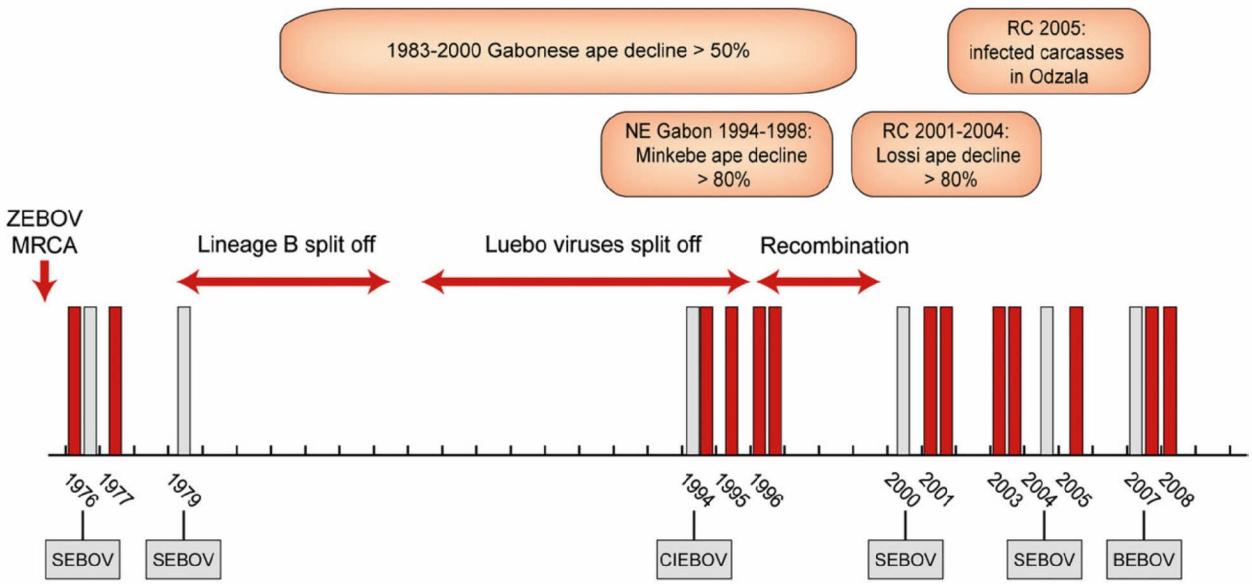

Figure 5. *Zaire ebolavirus* (ZEBOV) epizootics and human outbreaks (red line) are reported, along with outbreaks due to other *Ebolavirus* species (grey line). Time of occurrence of the main genetic events in *Zaire ebolavirus* (ZEBOV) evolution are reported in red; MRCA: most recent common ancestor. SEBOV: *Sudan ebolavirus*; CIEBOV: *Côte d'Ivoire ebolavirus*; BEBOV: Bundibugyo Ebola virus.

More generally, an increasing number of epidemiological events due to Ebola viruses have occurred over the last 20 years, contrasting with the prior apparent silent period [1978–1993] (Figure 5). Since 1994, 15 Ebola virus outbreaks have been recorded, of which 11 were due to ZEBOV, also responsible for massive local mortality among great apes [11, 12, 14, 16]. The absence of great apes and of noticeable mortality among other wildlife species in Luebo indicates that transmission of ZEBOV can occur in the absence of any indicators. Whereas the mechanisms underlying such cryptic circulation are elusive, these observations may help to explain the prolonged periods of apparent epidemiological silence, during which ZEBOV genetic diversity increases (Figure 5). However, the possibility remains that isolated cases, limited outbreaks, or epizootics may have been missed.

In conclusion, this study highlights that the Luebo outbreaks were phylogenetically, temporally and spatially distinct from all previous outbreaks. In contrast to the continuous ladder-like evolution of ZEBOV observed until 1996, the most recent trees display a more bushlike cladogenetic picture, with coexistence of distinct lineages. The emergence of a novel strain of ZEBOV, with the recent discovery of BEBOV, point to the depth of viral genetic diversity in the underlying natural animal hosts and suggests the possibility of future outbreaks with additional novel viruses or strains. Spillovers and the potential for recombination in ZEBOV raise the possibility—hypothetical at this stage—that viruses with increased transmissibility from the natural host to other animals or humans may emerge. Identifying the environmental drivers that trigger emergence of Ebola viruses from their reservoirs, which may differ from one viral species to another, after they circulate silently for years or decades, remains one of the key research challenges.

Supplementary Data

Supplementary Data are available at *The Journal of Infectious Diseases* online.

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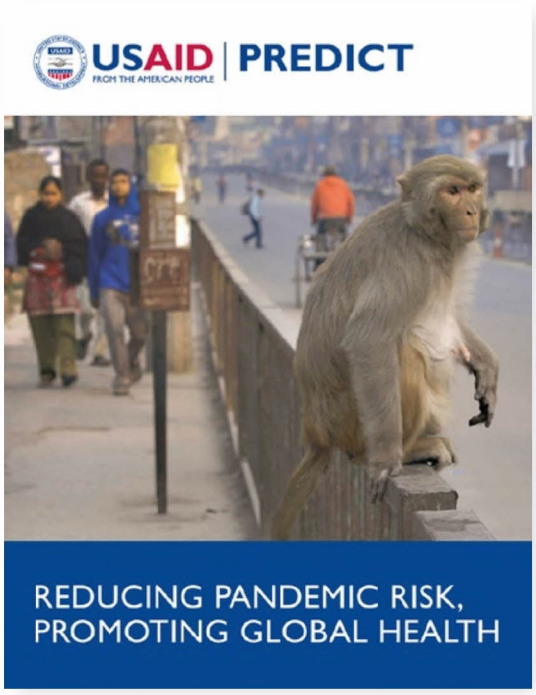

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In Tanzania, rapid population growth and land conversion have led to greater sharing of resources and increased interactions among wildlife, domestic animals, and humans.

PHOTOS BY LIZ VANWORMER (LEFT) AND BY WOUTRINA SMITH (RIGHT)

TANZANIA

In Tanzania, human livelihoods are often directly dependent on natural resources. Rapid population growth has increased land-use change, the demand for natural resources, and the potential for contact between wildlife and people. Tanzania has diverse wildlife-livestock-human interfaces, which offer ideal targets for zoonotic disease surveillance. Tanzania is home to millions of wild animals, many of which migrate across borders to other countries and ecosystems. Ongoing human and environmental changes are altering landscapes and connections between people and animals. For example, in the more arid areas of the Rift Valley, which join the Congo Basin in the west, water diversion and land conversion for agriculture coupled with continued population growth are restricting once vast livestock grazing areas for traditional livestock keepers (Copolillo et al. 2008; Walsh 2012). The result is an increase of livestock populations on remaining grazing land, often bordering villages and wildlife protected areas. The increasing density of livestock near wildlife-rich protected areas coupled with water scarcity may change the dynamics of current zoonotic threats and may facilitate transmission of diseases of pandemic potential across this interface (Mazet et al. 2009). Additionally, Tanzania possesses many islands of rainforest habitat, most notably the Eastern Arc Mountains, which are biodiversity hotspots due to the high degree of endemism and small mammal diversity (Burgess et al. 2007). These remnant forests provide an exceptional opportunity to assess zoonotic pathogen potential in rodent, bat, and primate species. Rapidly growing urban centers like Dar es Salaam on the Indian Ocean coast present another unique environment where pathogens from bats and rodents have the potential to be transmitted to and spread in dense human populations.

FOR A SUMMARY OF THIS SECTION GO TO WWW.TANZANIA.PREDICT.GLOBAL

Despite the wealth of biodiversity and close interaction of human and animal populations at these interfaces, Tanzania's capacity to conduct wildlife disease surveillance at the regional and national level is still developing. Existing collaborations between Sokoine University of Agriculture (SUA) and the ministries and institutions responsible for livestock, wildlife, and human health enabled the PREDICT team to increase and expand wildlife surveillance activities at high-risk wildlife-livestock-human interfaces throughout the country. These collaborations were strengthened through PREDICT surveillance work, as well as capacity building activities, in partnership with the Tanzania Wildlife Research Institute and other local stakeholders.

The PREDICT Project in Tanzania, in collaboration with in-country partners, conducted active surveillance of viruses of wildlife at critical human-wildlife interfaces. Thorough site assessment and sample collection from diverse bat, rodent, and nonhuman primate species allowed the PREDICT team to examine the potential for zoonotic transmission at many high-risk disease transmission interfaces.

LOCAL PARTNERS

In Tanzania, PREDICT established partnerships with national ministries, universities, and NGOs to conduct surveillance activities and inform key stakeholders on progress on a quarterly basis. Partners in Tanzania and East Africa included:

- Sokoine University of Agriculture (SUA)
- USAID
- Ministry of Livestock and Fisheries Development
- Ministry of Health and Social Welfare
- Ministry of Natural Resources and Tourism
- Tanzania Wildlife Research Institute (TAWIRI)
- Tanzania National Parks (TANAPA)
- National Institute of Medical Research (NIMR)
- Muhimbili University of Health and Allied Sciences
- Central Veterinary Laboratory and Regional and District Veterinary Offices
- Centers for Disease Control and Prevention Laboratory, Dar es Salaam
- Centers for Disease Control Global Disease Detection Group, Nairobi, Kenya
- Makerere University Walter Reed Project (MUWRP), Uganda

Udzungwa red colobus was one of the diverse primate species sampled at ecotourism interfaces in the Udzungwa Mountains.

PHOTO BY PREDICT TANZANIA TEAM

MAJOR ACHIEVEMENTS

- Implemented the first systematic and national-level viral surveillance approach for wildlife in Tanzania (see Success Stories for more information).
- Trained a One Health Workforce by providing a variety of educational and training opportunities at multiple levels (see Success Stories for more information).
- Developed and refined the PREDICT local media surveillance system to identify reports of human and animal disease in local Tanzanian news sources not currently integrated into digital disease detection systems like HealthMap, providing proof of concept for the expansion of local media surveillance to seven other PREDICT countries.
- Partnered with CDC representatives and local district chief medical officers to plan paired human-animal surveillance activities at sugar cane plantations in central Tanzania. Pilot data from this collaboration will provide information on the risk of viral transmission from rodents to sugar cane plantation workers and could spur future One Health programs.
- Conducted 319 active wildlife sampling events, and collected samples from a total of 1,753 animals (650 bats, 632 rodents, 251 nonhuman primates, and 220 samples from other taxa, primarily samples collected from bushmeat).
- Developed the first viral detection laboratory for wildlife in Tanzania, trained laboratory technicians at Sokoine University of Agriculture (SUA) in consensus-based PCR diagnostics, and initiated screening for arenaviruses, a high priority viral family due to the detection of novel arenaviruses in commensal rodents in Tanzania.
- Increased government, non-governmental organization, and community stakeholder awareness of approaches to prevent disease transmission at human-wildlife interfaces through presentations and distribution of calendars with educational messages on zoonotic diseases.
- With support from the Emerging Pandemic Threats DELIVER project, installed a liquid nitrogen generating plant in south-central Tanzania to strengthen cold chain for biological materials and wildlife samples to enhance local disease surveillance activities.

SUCCESS STORIES

Building SMART Surveillance in Tanzania

PREDICT implemented the first systematic and national-level viral surveillance approach for wildlife in Tanzania, improving awareness of high-risk human-animal interfaces, targeting animal sampling in key taxa at prioritized sites representing Tanzania's biological and geographic diversity and building the infrastructure and human resource base for the detection of emerging pathogens of pandemic potential.

Further, PREDICT-Tanzania partnered with diverse stakeholders at multiple levels of Tanzanian health systems, including community game officers, health workers, livestock extension officers, university researchers, veterinary professionals, and ministry level officials to characterize disease transmission risk, conduct wildlife surveillance activities, and share results. In Tanzania, PREDICT incorporated indigenous knowledge and participation, local media systems, and online intelligence to design and optimize surveillance activities reflecting the country's broad ecological and behavioral diversity.

PHOTOS BY PREDICT TANZANIA TEAM

Photos: A maize field crop raiding interface where diverse species of rodents were sampled (left). A PREDICT field team member collects an oral swab sample from an anesthetized rodent captured at an agricultural market.

In 2009, preliminary capacity assessments identified few national and donor investments in wildlife disease surveillance, and those that did exist primarily emphasized known diseases of conservation concern. Outside of major urban centers, limited infrastructure existed to support collection of wildlife samples for molecular diagnostics. By January 2014, liquid nitrogen cold chain capability was extended to central areas of the country, and personnel from universities, the Tanzania Wildlife Research Institute (TAWIRI), government health workers, veterinary and livestock extension officers, and community members were trained and actively participated in the identification of priority human-animal interfaces and in safe animal capture and sampling of high-risk wildlife groups.

Strategic. Working at the community level with village councils, elders, government representatives, and wildlife professionals and at the global level through real-time disease outbreak alerts, PREDICT-Tanzania characterized human-animal interfaces by type of risk and strategically prioritized interfaces for surveillance to ensure coverage of high-risk taxonomic groups and a diversity of human-animal contact.

Measurable. PREDICT-Tanzania successfully sampled over 1700 animals from 12 areas in Tanzania representing diverse interfaces for human-animal contact and engaged with stakeholders in surveillance and risk reduction activities. PREDICT-Tanzania also tested priority specimens from over 1,200 individuals, building the first baseline data on potential emerging viral threats from wildlife to vulnerable populations.

Adaptive. PREDICT-Tanzania used site assessments and feedback from diverse stakeholders to support and adapt surveillance to interfaces and locations of highest risk for disease emergence and spillover. Surveillance of local media sources also identified disease reports, risky behaviors, and human-animal contact used to adapt surveillance strategies.

Responsive. PREDICT-Tanzania cooperated closely with local and regional partners to coordinate surveillance with existing wildlife, livestock, and human disease surveillance and monitoring activities. In collaboration with veterinary researchers from the Tanzania Wildlife Research Institute, PREDICT sampled bats in northern Tanzania's fragmented forests. At the request of the Centers for Disease Control and Prevention's Global Disease Detection group, PREDICT sampled rodents in the Kilombero Valley as a first step towards paired human-animal disease surveillance and assessments of viral sharing and occupational hazard in sugarcane plantations.

PHOTO BY PREDICT TANZANIA TEAM

PREDICT-Tanzania and Tanzania Wildlife Research Institute team members conduct fruit bat trapping and sampling at Muheza in Tanga region.

Targeted. PREDICT-Tanzania targeted surveillance at interfaces where people have high contact with key wildlife groups. In close coordination with the surveillance and pathogen detection teams, priority samples from each animal were identified according to interface and potential transmission pathway and tested for viral families representing potential spillover and pandemic threat.

Training a One Health Workforce

In Tanzania, PREDICT provided a variety of educational and training opportunities at multiple levels, from community members to university students and health professionals. A total of 110 individuals (74 male, 36 female), including project research staff, were trained in topics ranging from disease surveillance (animal capture and handling, sample collection, and cold chain) to laboratory systems (biosafety, diagnostics, and information management), and disease prevention, enhancing Tanzania's disease surveillance system and One Health workforce.

PREDICT utilized a cooperative and participatory framework for surveillance site selection, working with local community members and district game and livestock officers to identify locations of wildlife habitat and collect information on human-animal contact to characterize interfaces. In return, PREDICT provided training to an estimated 19 district level officials and community members in zoonotic disease transmission and prevention and on-the-job training in safe animal handling and sampling to multiple high-risk occupational groups (game officers, wildlife professionals, veterinarians, livestock officers, and hunters).

In partnership with RESPOND's One Health Central East Africa (OHCEA) University Network, PREDICT trained 47 future One Health leaders and scientists from Sokoine University of Agriculture and Muhimbili University of Health and Allied Sciences. Participants received hands-on field training in biosafety, safe wildlife capture and sampling, cold chain management, and One Health surveillance approaches. Training was also conducted in Morogoro, Tanzania at the SUA campus, including an overview of diagnostic methods for detecting zoonotic and emerging disease threats.

PREDICT sponsored staff participation in multiple capacity building opportunities, including:

- Wildlife Capture Africa course in Zimbabwe focusing on the safe use of chemical and physical restraint in wildlife immobilizations.
- Professional exchange and training for Tanzania laboratory technicians from SUA at Makerere University Walter Reed Project (MUWRP) in Uganda on DNA/RNA extraction, PCR, and data management to standardize pathogen detection regionally.
- Molecular diagnostic training for a SUA laboratory technician at the UC Davis One Health Diagnostic and Surveillance Laboratory in California on viral nucleic acid extraction, PCR techniques, consensus PCR viral family testing, and information management.
- One Health Summer School training at Sokoine University of Agriculture organized by Southern African Centre for Infectious Disease Surveillance (SACIDS) focused on the One

Health approach, disease and vector ecology, molecular epidemiology, biosafety, disease surveillance, and outbreak investigations.

- Safe capture, handling, and sample collection training for nonhuman primate disease surveillance with the PREDICT-Rwanda and Mountain Gorilla Veterinary Project field staff.
- Good Clinical Practices courses for researchers at the National Institute for Medical Research (NIMR), organized by Family Health International (FHI) and Kenya AIDS Vaccine Initiative Project (KAVI) Institute.

PHOTO BY PREDICT TANZANIA TEAM

One Health Central and Eastern Africa University Network Students at a rodent capture and sampling training with PREDICT Tanzania team members.

As a result of these training investments, PREDICT contributed to an enhanced social network of human resources for disease surveillance in Tanzania and encouraged greater transdisciplinary cooperation in the health system among Tanzania's future leaders and scientists.

CAPACITY BUILDING

Infrastructure improvements

At the Sokoine University of Agriculture's Faculty of Veterinary Medicine in Morogoro, Tanzania, PREDICT developed the first viral detection laboratory for wildlife in the country. The laboratory is equipped with trained personnel and advanced molecular technology to screen wildlife samples for known and unknown viruses within priority viral families (e.g. arenaviruses) and genera of potential pandemic potential and is networked to regional and global laboratory diagnostic centers of excellence in Uganda and the US for support and confirmatory testing. Technicians at the SUA lab regularly consult and engage in refresher trainings with mentors in the US with telemedicine-based knowledge and skill transfer, enabling acquisition and adoption of new techniques, protocols, and diagnostic testing on demand.

Additionally, with support from the EPT program partner DELIVER, PREDICT-Tanzania strengthened capacity for disease surveillance, sample collection, and storage through installation of a liquid nitrogen generating plant, the sole source of liquid nitrogen in the South-central area. The liquid nitrogen generator provides the means for maintaining cold-chain during sampling efforts and has enhanced capacity for other surveillance activities in Tanzania.

Operationalizing One Health

One Health research and disease surveillance capacity was improved nationally in cooperation with 10 partner organizations and agencies, including the Tanzania Wildlife Research Institute (TAWIRI), and through training programs to improve wildlife surveillance and disease diagnostics. Sharing of PREDICT activities and results with National Institute for Medical Research, Ministry of Livestock and Fisheries Development, and Tanzania Wildlife Research

PHOTO BY PREDICT TANZANIA TEAM

PREDICT team members assess a fruit bat colony near human dwellings in Kilombero Valley in central Tanzania.

Institute increased discussion and awareness of wildlife viral surveillance and detection, promoting opportunities for future one health surveillance initiatives.

PREDICT-Tanzania also worked with Sokoine University of Agriculture and the Health for Animals and Livelihood Improvement (HALI) project to expand the One Health Workforce in Tanzania through a center of excellence in ecosystem and wildlife health. PREDICT and RESPOND in Tanzania effectively catalyzed wildlife health momentum at Sokoine University, where plans are in place to offer a new master's program in wildlife and ecosystem health, building on current graduate programs in One Health and molecular biology supported though the Southern African Centre for Infectious Disease Surveillance (SACIDS). Through these programs, veterinary medicine, agriculture, animal science, forestry, wildlife, and social science expertise will support transdisciplinary education and training using a One Health approach, with options for field-based training at the HALI project Iringa station, PREDICT Tanzania's surveillance team base. This center will provide a long-term training ground for students, faculty, researchers, and government personnel in One Health approaches to solving complex problems and will foster and encourage greater local, regional, and national cooperation and coordination of surveillance and health research.

SURVEILLANCE

PREDICT conducted wildlife surveillance at a number of high-risk wildlife-human contact interfaces in the following areas in Tanzania (Figure 1):

- South-central (Eastern Arc Mountains – Udzungwa Mountains National Park and surrounding forest reserves and villages, Kilombero Valley, Ruaha National Park and surrounding areas, Mbeya region, and Mbinga district)
- North-west (Geita – Industrial and artisanal mining areas)
- North-east (Eastern Arc – Amboni caves, Tanga region, and surrounding areas)
- Eastern (Coastal, Dar es Salaam and surrounding areas, Morogoro, and Mtwara)

High-risk interfaces targeted for wildlife pathogen surveillance in Tanzania included (Figure 2; Table 1):

1. Hunting/bushmeat interfaces where wild animals, most commonly rodents, ungulates, and carnivores, are consumed after being hunted, killed, and slaughtered under poor sanitary conditions;
2. Crop raiding interfaces where wild animals interact with humans by feeding on crops in local agricultural fields or in fruit and vegetable markets;
3. Peri-domestic settings and areas near extractive industries where wild animals (i.e. rodents and bats) commonly interact with humans in and around dwellings, villages, urban centers, and work sites or camps;
4. Ecotourism interfaces where tourists from around the world travel to see wildlife in unique environments including savannas, forests, mountains, and caves; and

5. High traffic interfaces where construction of infrastructure including roads, railways, and buildings has enhanced human encroachment into wildlife habitat, and the rapid flow of people could facilitate rapid spread of zoonotic pathogens.

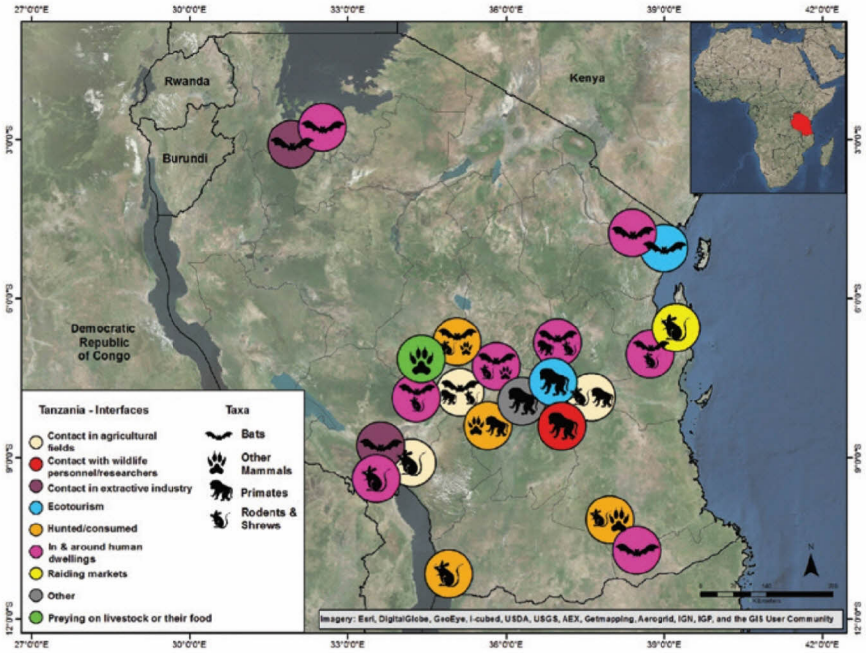

Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

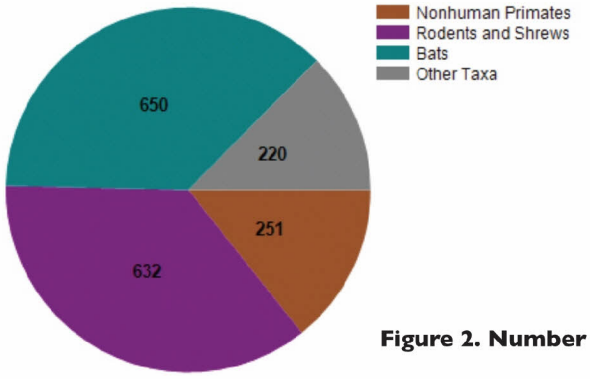

Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	17	247	0	1
Ecotourism and recreational activities	30	0	59	0
Extractive industries	0	0	48	2
In or near human dwellings	22	306	543	7
Hunted wildlife	4	70	0	195
Wildlife preying on livestock or their food	0	0	0	15
Wildlife raiding markets	0	9	0	0
Protected areas	122	0	0	0
Other high-risk interfaces	56	0	0	0
Total:	**251**	**632**	**650**	**220**

Bushmeat hunting and consumption project. The PREDICT Tanzania team launched a bushmeat surveillance study in the Ruaha Ecosystem of South-central Tanzania within nine villages where both legal and illegal hunting are practiced. Working with a community surveillance network, PREDICT collected muscle and organ tissue samples from over 200 fresh-killed wild animals. The study revealed that consumption of bushmeat in the area is very common, with animals hunted and slaughtered under poor sanitary conditions, which may expose hunters to wildlife pathogens. Specimens from hunted animals will be tested for priority viral families using the consensus PCR approach. Additionally, we are conducting a survey of hunters and bushmeat consumers in the villages to assess the behavioral and socioeconomic factors associated with subsistence poaching in the area. Data obtained from this study will improve the understanding of the subsistence poaching in the area along with factors potentially implicated in zoonotic disease transmission risk.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

The PREDICT Tanzania team communicated with the Tanzania Wildlife Research Institute (TAWIRI), Ministry of Health and Social Welfare, and Ministry of Livestock and Fisheries Development to offer support in investigating numerous outbreaks in wildlife, domestic animal, and human populations, including outbreaks of African Swine Fever in Mbeya, undiagnosed illnesses in people in Gombe, and an outbreak of unknown origin killing goats and chickens on Musira Island in Bukoba. PREDICT surveillance and diagnostic support was not utilized in outbreak investigation and response efforts, but communications were strengthened with government partners on integrating PREDICT wildlife disease investigation support in national disease outbreak response planning.

The PREDICT Tanzania team collaborated with Tanzania National Parks (TANAPA) to jointly sample giraffe in Ruaha National Park, which were showing signs of an undiagnosed skin disease. PREDICT and TANAPA worked together to use advanced molecular diagnostics (deep sequencing) to identify the cause of this disease.

PHOTO BY PREDICT TANZANIA TEAM

PREDICT Tanzania director Professor Rudovick Kazwala (bottom right) works with a team of researchers from Tanzania National Parks and Sokoine University of Agriculture to safely collect samples from an anesthetized giraffe.

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Tuberculosis infection in wildlife from the Ruaha ecosystem Tanzania: implications for wildlife, domestic animals, and human health

D. L. CLIFFORD[1,2]*, R. R. KAZWALA[3], H. SADIKI[3], A. ROUG[1], E. A. MUSE[4], P. C. COPPOLILLO[5,6] AND J. A. K. MAZET[1]

[1] *Wildlife Health Center, One Health Institute, School of Veterinary Medicine, University of California, Davis, CA, USA*
[2] *Wildlife Investigations Laboratory, California Department of Fish and Wildlife, Rancho Cordova, CA, USA*
[3] *Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania*
[4] *Ruaha National Park, Tanzania National Parks, Arusha, Tanzania*
[5] *Ruaha Landscape Programme, Wildlife Conservation Society, Iringa, Tanzania*
[6] *Working Dogs for Conservation, Three Forks, MT, USA*

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SUMMARY

Mycobacterium bovis, a pathogen of conservation, livestock, and public health concern, was detected in eight species of wildlife inhabiting protected areas bordering endemic livestock grazing lands. We tested tissues from 179 opportunistically sampled hunter-killed, depredation, road-killed, and live-captured wild animals, representing 30 species, in and adjacent to Ruaha National Park in south-central Tanzania. Tissue culture and PCR were used to detect 12 (8·1%) *M. bovis*-infected animals and 15 (10·1%) animals infected with non-tuberculosis complex mycobacteria. Kirk's dik-dik, vervet monkey, and yellow baboon were confirmed infected for the first time. The *M. bovis* spoligotype isolated from infected wildlife was identical to local livestock, providing evidence for livestock–wildlife pathogen transmission. Thus we advocate an ecosystem-based approach for bovine tuberculosis management that improves critical ecological functions in protected areas and grazing lands, reduces focal population density build-up along the edges of protected areas, and minimizes ecological stressors that increase animals' susceptibility to bovine tuberculosis.

Key words: One health, tuberculosis (TB), veterinary epidemiology and bacteriology, wildlife disease, zoonoses.

INTRODUCTION

Tuberculosis (TB) is one of the most widespread infectious diseases and a leading cause of death for adults worldwide [1]. Although much attention has focused on treatment and prevention of human TB caused by *Mycobacterium tuberculosis*, zoonotic TB due to *Mycobacterium bovis*, known as bovine tuberculosis (bTB), has become an important re-emerging public health concern in developing countries. A large number of livestock keepers (pastoralists) combined with poor public health infrastructure, limited bTB control measures for cattle and animal products, and a large immunocompromised human population due to HIV/AIDS make Africa particularly vulnerable to the health impacts of bTB [2]. Zoonotic bTB infection is significant, as *M. bovis* is naturally resistant to

* Author for correspondence: Dr D. L. Clifford, Wildlife Health Center, One Shields Ave. University of California Davis, Davis, CA 95616, USA.
(Email: dlclifford@ucdavis.edu)

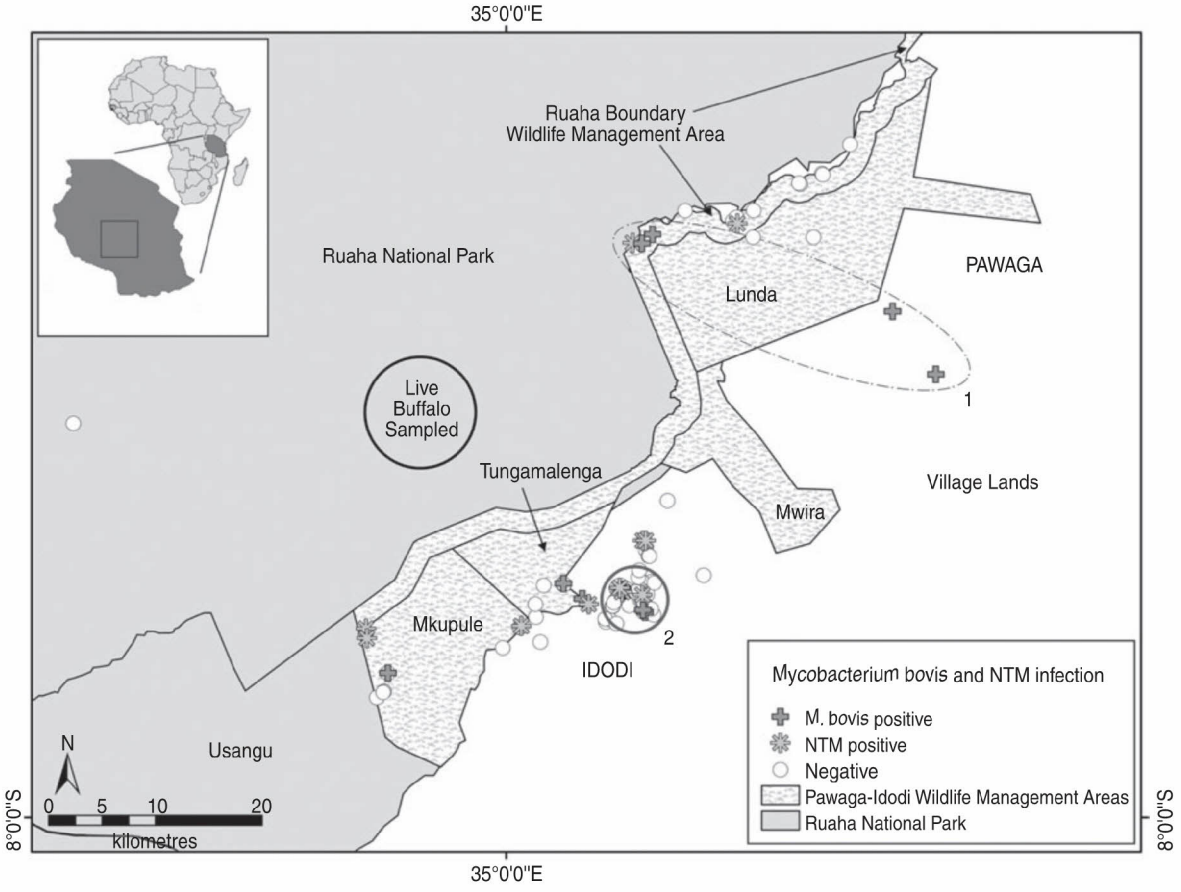

Fig. 1. Spatial distribution of *M. bovis* and non-tuberculosis complex mycobacteria (NTM) infected and uninfected wildlife carcasses ($n = 121$), and the approximate sampling area where 30 live buffalo were tested for bovine tuberculosis in a livestock–wildlife interface area in the southern portion of the Ruaha ecosystem, south-central Tanzania. Although not statistically significant, an elliptical (no. 1, $P = 0{\cdot}057$) and circular (no. 2, $P = 0{\cdot}066$) shaped region of a higher than expected number of *M. bovis* cases (nos. 1 and 2), as indicated by spatial scan statistics are shown. The geopolitical division of village lands (Idodi or Pawaga) is shown in capital letters.

pyrazinamide, a first-line TB treatment drug [1]. Both of these TB-complex mycobacteria have demonstrated the ability to move between animal (including wildlife) and human populations [3, 4].

Bovine TB has also emerged as a disease of concern for wildlife conservation. Spillover from bTB-infected cattle herds was thought to have resulted in bTB infections in African buffalo (*Syncerus caffer*) inhabiting the southern portion of Kruger National Park (KNP), South Africa (Kloeck, 1998 cited in [5]). Within 15 years of the discovery of the first cases in KNP in 1990, bTB had spread through most of park's buffalo herds [6], and infections were documented in 10 other species [5]. Reported impacts of bTB in KNP and other South African parks included decreased body condition and drought tolerance of infected buffalo [7], lowered buffalo reproductive success [8], and mortality and disruption of pride dynamics in infected lion prides [5]. In East Africa, bTB infections have been documented since the 1960s in buffalo in Uganda [9, 10] and recently in wildebeest (*Connochaetes taurinus*), topi (*Damaliscus lunatus*), and one lesser kudu (*Tragelaphus imberbis*) in northern Tanzania [11]. However, little is known about whether or not bTB is present in other ecosystems, and population-level impacts of bTB have not been characterized.

The Ruaha ecosystem of south-central Tanzania encompasses vast wildlife protected areas of high conservation value bordered by lands inhabited by large numbers of livestock. Farmers, traditional livestock keepers, livestock, and wildlife inhabiting the southern portion of the Ruaha ecosystem all depend upon water from the Great Ruaha River and its tributaries (Fig. 1). Over time, human migration due to protected area creation and other government resettlement

programmes [12] coupled with the seasonal drying of the Great Ruaha River largely due to upstream diversion for agriculture [13] has altered the distribution of people, livestock, and wildlife. Heightened competition for land and water resources has increased wildlife conflict and concerns that increased overlap among human, wildlife, and livestock populations in Ruaha may be increasing the risk of zoonotic disease transmission including bTB [14, 15].

Despite data indicating widespread bTB infection in cattle surrounding Ruaha's wildlife protected areas, it was not known if wildlife in the Ruaha ecosystem had been infected. Prevalence of bTB in cattle grazing the southeastern portion of the Ruaha ecosystem was estimated to be 13%, with 51% of sampled herds containing one or more positive reactors [16]. Previous wildlife data from Ruaha were limited to results from a sample of six wild animals [five elands (*Taurotragus oryx*) and one roan antelope (*Hippotragus equinus*)] that were serologically negative by enzyme immunoassay [11]. Accordingly, we set out to determine if bTB was present in Ruaha's wildlife as part of a large-scale project assessing the impact of zoonotic diseases at the rapidly changing environmental interface of human, livestock, and wildlife populations in the Ruaha ecosystem.

We hypothesized that given widespread bTB presence in cattle, bTB would also be present in wildlife species inhabiting the livestock–wildlife interface areas south of Ruaha National Park (RNP) and that wildlife species would be infected with the same strain of *M. bovis* found in local cattle. We also examined the species and spatial distribution of *M. bovis* wildlife infections to determine potential for pathogen persistence in wildlife maintenance hosts, and identify potential high-risk areas for transmission that could be targeted as part of an ecosystem-based approach to reduce transmission of disease at the livestock–wildlife interface and improve animal and human health.

METHODS

Study area

The southern extent of the Ruaha ecosystem lies in northern Iringa District, south-central Tanzania (07°19′S to 07°36′S and from 35°05′E to 35°29′E). It covers about 30 000 km^2 of different rangeland-use areas, RNP, the Rungwa, Kisigio and Muhesi game reserves, the Lunda-Mkwambi Game Controlled Area, the recently formed community-based Pawaga–Idodi Wildlife Management Area (PIWMA), and village lands. The area is internationally significant in terms of biodiversity conservation because it contains the only protected area system covering the transition between the vegetation communities of the Sudanian *Acacia-Commiphora* zone of East Africa and the *Brachystegia* (miombo) woodlands of southern Africa [12]. Our study was concentrated in the southernmost portion of the Ruaha ecosystem, comprised of rural villages, the PIWMA, and RNP (Fig. 1). Habitat consisted of patchily distributed semi-arid woodland and brushland, and active and fallow agriculture fields. Lands bordering the wildlife protected areas are heavily grazed, as evidenced by denuded vegetation, bare patches of soil, and the presence of many livestock and livestock faeces.

Ethics statement

All research activities in Tanzania were reviewed, approved, and permitted by the Tanzania Commission on Science and Technology (COSTECH), the Tanzania Wildlife Research Institute (TAWIRI), Tanzania National Parks (TANAPA), and University of California Davis Institutional Animal Care and Use Protocols nos. 12394 and 15919. No animals were killed for the purposes of this study.

Animal sampling

From 2006–2010, lungs, mediastinal and mesenteric lymph nodes, and other tissue samples from hunter-killed wildlife, opportunistically found carcasses, and wildlife depredated for causing crop damage in and around the PIWMA and RNP were collected by community game scouts trained to safely collect specimens, project veterinarians (D. Clifford, H. Sadiki), or technicians. The date, sex, age, and species were recorded for each animal sampled. Whenever possible the location of the carcass (latitude and longitude in decimal degrees, World Geographic System 1984 datum) was recorded using a handheld global positioning system (GPS) unit. For carcasses where GPS locations were not recorded, the nearest village or a locally known place name within the protected area was recorded by the scout. Collected tissues were examined for gross lesions and subsectioned: the outer surface of the tissue was seared, and then a sample for culture was collected using forceps that were chemically disinfected and a new sterile disposable scalpel blade for each animal. Specimens for

culture were placed into sterile Whirlpak® plastic collection bags (Nasco, USA). Samples were frozen on average within 24 h of collection and then stored frozen at −20 °C until testing.

Additionally, we received heparinized blood samples from 30 live African buffalo immobilized in RNP in 2011 during foot-and-mouth disease (FMD) surveillance efforts conducted by the South African Development Community and the Tanzanian Ministry for Livestock. Buffalo belonging to the Msembe herd that ranges in the southern portion of RNP were opportunistically immobilized via dart gun delivered from a helicopter. Additional buffalo herds inhabiting other areas of RNP were not sampled. Only buffalo aged 2–4 years were captured and sampled as they were the target age group for FMD testing.

Diagnostic testing

Culture and molecular diagnostics – wildlife tissues

Frozen tissues were thawed to room temperature, then pooled lung and lymphoid tissues were homogenized, decontaminated, and neutralized using standard methods [17]. Resulting sediments were inoculated onto Lowenstein–Jensen media with pyruvate and Lowenstein–Jensen media with glycerol and incubated at 37 °C for up to 12 weeks. Positive cultures with appropriate colony morphology [18] were subcultured onto another set of the same media for 3–4 weeks to obtain pure culture and then examined by microscope for the presence of acid-fast-bacilli (AFB) using Ziehl–Neelsen stain.

Heat-killed AFB-positive samples were further characterized by multiplex polymerase chain reaction (PCR), using primers to the 16S rRNA gene specific for the *Mycobacterium* genus and able to distinguish between *M. avium* and *M. intracellulare*, and primers aimed at the MPB70 gene of *M. tuberculosis* complex (MTC) organisms [19]. Samples with an amplification product of 1030 bp indicative of the genus *Mycobacterium* and of 372 bp were considered positive signals for MTC.

Spoligotyping was used to delineate the *Mycobacterium* species for animals with MTC and distinguish unique strain types. Briefly, PCR products representing all the spacer sequences in an isolate's genome were amplified using primers specific for the direct-repeat sequences of the direct repeat locus of the MTC chromosome. Hybridization of the amplification product mixture against a membrane to which the 43 individual spacer sequences were covalently linked was used to generate a specific pattern of positive and negative hybridization signals [20]. The absence of spacers 3, 9, 16, and 39–43 was used to classify isolates as *M. bovis* [20, 21]. The *M. bovis* spoligotype patterns from wildlife samples were coded [22] and compared to spoligotypes SB0133 and SB0425 which were dominant in the cattle isolates from a study in an adjacent area in Mbeya and Iringa regions [23], and in 2007 from a cow from a village in Pawaga Division on the southern border of the PIWMA and RNP (Fig. 1).

Serology – live buffalo samples

Blood samples from live-captured buffalo were tested for bTB infection using a commercially available *M. bovis* gamma interferon (INF-γ) test kit, that utilizes a monoclonal antibody-based sandwich enzyme immunoassay to detect the production of INF-γ (Bovigam®, Prionics, Switzerland). Within 8 h of collection, heparinized blood samples from each animal were subdivided into three aliquots that were mixed with phosphate-buffered saline (nil antigen), bovine and avian purified protein derivative (tuberculin; Veterinary Laboratory Agency Weybridge, UK), respectively, and then incubated for approximately 20 h at 37 °C. After incubation, plasma was harvested by centrifugation, frozen at −20 °C, and transported to Sokoine University of Agriculture where the Bovigam assay was performed according to the manufacturer's instructions. All samples were tested in duplicate and positive and negative controls supplied by the manufacturer included on each 96-well plate. Animals were classified as positive bTB reactors if $OD_{bovine} - OD_{control}$ was $\geqslant 0{\cdot}049$ and if OD_{bovine} was greater than OD_{avian} according to Whipple *et al.* [24], with additional consideration given to samples with $OD_{bovine} \geqslant 0{\cdot}385$, as this value optimized test predictive value for sampled buffalo in South Africa [25].

Data analysis

Field and laboratory data were entered into an electronic database (Microsoft Excel, USA). The proportion of AFB-positive, *M. bovis*-infected, and non-TB complex mycobacteria (NTM) infected animals was calculated for all animals tested and for each species. Associations between *M. bovis* and

NTM infection proportions, species group (defined as hoofstock, carnivores, primates, or small mammals), age (young *vs.* adult), sex, and sampling location (inside *vs.* outside of a wildlife protected area) were examined using Fisher's exact tests using Stata version 11.2 software (StataCorp., USA).

For carcasses that had known locations, a spatial scan statistic was used to determine if *M. bovis* infections were distributed randomly over space in our sampling area, and if not, to evaluate any spatial infection clusters for statistical significance (SaTScan version 9.1.1, M. Kulldorff, Harvard Medical School, Boston, MA, USA) [26]. A Bernoulli model utilizing case-control (0/1) data was used, as it does not assume a homogenous distribution of the underlying population. A circular or elliptically shaped moving window was used to scan for spatial clusters encompassing from zero to not more than 50% of the locations by comparing the observed infections inside *vs.* outside the window using likelihood functions [27]. Both the circular and elliptical window options were evaluated because the elliptical window allows for a better fit to linear geographical features, including roads and rivers, which may be associated with the location of carcass recovery due to ease of access [28]. Maximum-likelihood estimates were generated by Monte Carlo simulations of 999 iterations and clusters evaluated for significance with $P=0{\cdot}05$. Spatial scan statistics were not used to evaluate clusters of NTM infections as location data was not known for all NTM-infected individuals.

RESULTS

Culture and molecular diagnostics – wildlife tissues

Tissue samples from 149 animals comprising at least 30 different species were collected. The exact number of species represented in the sample was not known as the specific species was not identified for three squirrels and 10/11 sampled mongooses. Kirk's dik-dik (*Madoqua kirkii*) and impala (*Aepyceros melampus*) were the two most commonly sampled species, comprising 36% of the total sample (Table 1). Hoofstock species comprised 59% of the sample ($n=88$), with the remainder of sampled animals comprised of 24% carnivores ($n=36$), 12% primates ($n=18$), 4% small mammals ($n=6$) and a single elephant. Adult animals were most commonly sampled ($n=125$, 84%); 15 young animals were sampled, and age was not recorded for nine animals. Male animals comprised 70% of the animals sampled ($n=105$); 33 females and 11 animals not having sex identified comprised the remainder of the sample. Variable numbers of samples were collected each year [2006 ($n=19$); 2007 ($n=20$), 2008 ($n=6$), 2009 ($n=96$), 2010 ($n=8$)]. Of 124 animals whose exact or approximate locations were known, 38 (31%) were located within a wildlife protected area [PIWMA ($n=37$), RNP ($n=1$)]; while the remaining 86 individuals were located outside protected areas on village lands. Hoofstock were more likely to be sampled within protected areas than carnivores (one-sided Fisher's exact $P=0{\cdot}048$) and primates (one-sided Fisher's exact $P=0{\cdot}009$). All small mammals were sampled outside of protected areas.

Cultures from 34 animals had positive AFB growth and were further characterized using the mycogenus PCR (Table 1). Of the 27 samples that produced amplification products, 12 belonged to the MTC, and 15 were NTM. *M. avium* and *M. intracellulare* were not identified. Yellow, white, or grey nodules were noted on gross examination of tissues from eight animals, but only one animal with gross lesions, an African lion (*Panthera leo*), had AFB growth on culture (Table 1).

Spoligotyping allowed classification of all 12 MTC isolates as *M. bovis* as they lacked spacers 3–7, 9, 16, and 39–43 (Table 2) [20, 21]. *M. bovis* was isolated from 8% (12/149) of individuals sampled comprising eight species (Table 1). All 12 *M. bovis* isolates were spoligotype pattern SB0133 (Table 2), a previously recognized pattern in the *M. bovis* online spoligotype database belonging to the African 2 clonal complex of *M. bovis* [29]. The spoligotype pattern of wildlife samples was the same as those previously isolated from cattle sampled in Iringa and adjacent Mbeya region from 1993 to 1995 [23], and the single positive cow from our study area that was slaughtered and sampled in 2007 (Table 2).

Although *M. bovis* infection was documented in 17% (3/18) of sampled primates, the primate infection proportion was not significantly greater than that of sampled hoofstock (8%, 7/88, one-sided Fisher's exact $P=0{\cdot}227$), or carnivores (6%, 2/36, one-sided Fisher's exact $P=0{\cdot}200$). *M. bovis* infection proportions were equivalent between sampled males (9%) and females (9%, Fisher's exact $P=1{\cdot}00$) and there was no difference in *M. bovis* infection proportions between animals sampled inside (8%, 3/38) and outside (10%, 9/86) wildlife protected areas (Fisher's exact $P=0{\cdot}754$). Infection with *M. bovis* was only detected in adult animals.

Table 1. *Results of acid-fast bacilli culture and mycobacterium PCR for 149 wild animals sampled from 2006 to 2010 in a livestock–wildlife interface area in and around the Pawaga–Idodi Wildlife Management Area and Ruaha National Park, Iringa Region, south-central Tanzania*

Species	No. tested	No. AFB positive	No. NTM	No. MTC
African buffalo (*Syncerus caffer*)	5	2 (40)	1 (20)	1 (20)
Bushbuck (*Tragelaphus scriptus*)	5	2 (40)	0 (0)	0 (0)
Bush duiker (*Sylvicapra grimmia*)	2	0 (0)	0 (0)	0 (0)
Eland (*Taurotragus oryx*)	1	0 (0)	0 (0)	0 (0)
Greater kudu (*Tragelaphus strepsiceros*)	3	1 (33·3)	1 (33·3)	0 (0)
Kirk's dik-dik (*Madoqua kirkii*)	30	4 (13·3)	2 (6·7)	2 (6·7)
Impala (*Aepyceros melampus*)	24	9 (37·5)	3 (12·5)	3 (12·5)
Lesser kudu (*Tragelaphus imberbis*)	1	1 (100)	0 (0)	1 (100)
Sable antelope (*Hippotragus niger*)	2	0 (0)	0 (0)	0 (0)
Waterbuck (*Kobus ellipsiprymnus*)	1	1 (100)	1 (100)	0 (0)
Bush pig (*Potamochoerus larvatus*)	6	1 (16·7)	1 (16·7)	0 (0)
Common warthog (*Phacochoerus africanus*)	2	0 (0)	0 (0)	0 (0)
Giraffe (*Giraffa camelopardalis*)	2	0 (0)	0 (0)	0 (0)
Common zebra (*Equus quagga*)	4	0 (0)	0 (0)	0 (0)
African elephant (*Loxodonta africana*)	1	0 (0)	0 (0)	0 (0)
Vervet monkey (*Chlorocebus pygerethrus*)	11	2 (18·2)	0 (0)	2 (18·2)
Yellow baboon (*Papio cynocephalus*)	7	2 (28·6)	0 (0)	1 (14·3)
Aardwolf (*Proteles cristata*)	1	0 (0)	0 (0)	0 (0)
African civet (*Civettictis civetta*)	2	0 (0)	0 (0)	0 (0)
Black-backed jackal (*Canis mesomelas*)	1	0 (0)	0 (0)	0 (0)
Blotched genet (*Genetta tigrina*)	11	3 (27·3)	2 (18·2)	1 (9·1)
Caracal (*Felis caracal*)	1	1 (100)	1 (100)	0 (0)
Leopard (*Panthera pardus*)	2	1 (50)	0 (0)	0 (0)
Lion (*Panthera leo*)	3	1 (33·3)	1 (33·3)	0 (0)
Mongoose (*Herpestes* or *Mungos* spp.)	11	2 (18·2)	1 (9·1)	1 (9·1)
Spotted hyena (*Crocuta crocuta*)	1	0 (0)	0 (0)	0 (0)
Zorilla (*Ictonyx striatus*)	3	0 (0)	0 (0)	0 (0)
Squirrel spp. (Sciuridae)	3	1 (33·3)	1 (33·3)	0 (0)
Porcupine (*Hystrix* spp.)	1	0 (0)	0 (0)	0 (0)
Spring hare (*Pedetes capensis*)	2	0 (0)	0 (0)	0 (0)
Total	**149**	**34 (22·8)**	**15 (10·1)**	**12 (8·1)**

AFB, Acid-fast bacilli; NTM, non-tuberculosis complex mycobacteria; MTC, *Mycobacterium* tuberculosis complex.
The number of animals positive/total tested (% positive) for acid-fast bacilli growth on culture, and tuberculosis and NTM is reported. All tuberculosis complex mycobacteria isolated were classified as *Mycobacterium bovis* by spoligotyping.

NTM infection was detected in 10% (15/149) of sampled animals belong to 11 species (Table 1). There was no difference in NTM infection proportion in species groups: 17% (1/6) of small mammals, 14% (5/36) of carnivores, and 10% (9/88) of hoofstock sampled were infected. No NTM infections were documented in sampled primates. NTM infection proportions were similar between sampled males (10%) and females (12%, Fisher's exact $P=0{\cdot}758$), and between sampled adults (12%) and young animals (15%, Fisher's exact $P=0{\cdot}677$). There was also no difference in NTM infection proportion between animals sampled inside (16%, 6/38) and outside (12%, 7/86) protected areas (Fisher's exact $P=0{\cdot}215$).

Exact locations for 12 *M. bovis*-infected animals and 109 uninfected animals were available for spatial analyses. A geographical cluster of four *M. bovis*-infected animals was detected inside a 143 km^2 elliptical area that extended along the road from grazing lands through the Lunda portion of the PIWMA and up to the PIWMA/RNP border (no. 1, Fig. 1), but was not statistically significant ($P=0{\cdot}057$). A second smaller 10·9 km^2 circular geographical cluster of three *M. bovis*-infected animals was detected in village lands (no. 2, Fig. 1), but was also not statistically significant ($P=0{\cdot}066$). Although neither cluster was statistically significant, less than one infected animal would be expected to have

Table 2. *Spoligotype pattern (SB0133; www.mbovis.org) of 12* Mycobacterium bovis *isolates obtained from a sample of 149 wild animals and a domestic cow inhabiting a livestock–wildlife interface area in the southern portion of the Ruaha ecosystem, south-central Tanzania*

Species	Location	Spacers 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
African buffalo	PIWMA(Lunda)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Kirk's dik-dik	Village (Idodi)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Kirk's dik-dik	Village (Lunda)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Impala	PIWMA (Lunda)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Impala	PIWMA (Mkpule)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Impala	Village (Tungamalenga)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Lesser kudu	Village (Lunda)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Vervet monkey	Village (Idodi)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Vervet monkey	Village (Idodi)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Yellow baboon	Village (Tungamalenga)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Blotched genet	Village (Idodi)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Mongoose sp.	Village (Tungamalenga)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Domestic cow	Village (Pawaga)	■	■	■					■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

The approximate location of each case; Pawaga–Idodi Wildlife Management Area (PIWMA) or village lands (Village), and the nearest place name (in parentheses) with reference to Figure 1 is specified.

occurred in each of these areas if *M. bovis* infections were randomly distributed throughout the area where carcasses were found.

Serology – live-sampled buffalo

All 30 sampled buffalo were noted to be apparently healthy at the time of capture. Three buffalo (10%, 2 male, 1 female) were positive reactors according to test criteria established by Whipple *et al.* [24]. Two of three positive reactors also had mean OD_{bovine} values $\geqslant 0{\cdot}385$ and thus would be considered positive using an alternate cut-off designed to maximize test predictive value in African buffalo developed by Michel *et al.* [25].

DISCUSSION

We document that bTB is present in wild animals inhabiting protected areas and village lands in the Ruaha ecosystem of south-central Tanzania. Our findings demonstrating *M. bovis* infection in 8% of sampled wild animals are consistent with findings from northern Tanzania [11]. However, the opportunistic nature of the sampling effort in this study limits our ability to estimate the true prevalence of bTB in this population. Although weaker animals may be more likely to be successfully hunted, opportunistically found, or depredated, and thus potentially more likely to be infected than the general population, this potential bias in our data may be balanced by the fact that infected wildlife sampled in this study lacked clinical bTB lesions, and that trophy hunters prefer to select larger healthy males.

An unexpected finding was the documentation of bTB infection in eight different species occupying different ecological niches. To our knowledge, this is the first published report of *M. bovis* infection in free-ranging vervet monkey, yellow baboon, and Kirk's dik-dik in Africa, and the first published isolation of *M. bovis* in African buffalo and impala in Tanzania. *M. bovis* infection has been documented previously in chacma baboons (*Papio ursinus*) in South Africa [5] and in olive baboons (*Papio anubis*) from Kenya that were feeding on slaughterhouse offal from *M. bovis*-infected cows [30, 31]. Neither of these baboon species is present in the Ruaha ecosystem, but the yellow baboon fills a similar niche, being abundant both in protected areas and in village lands where they are adept at utilizing anthropogenic food sources. No published record of *M. bovis* infection was found for vervet monkeys, but *M. tuberculosis* infection has

been reported in vervet monkeys held at a wildlife rehabilitation centre in South Africa [32].

Spoligotype similarity between wildlife sampled in this study and local livestock supports the hypothesis that livestock and wildlife are sharing pathogens. Infection of small carnivores and primates may indicate more recent spillover transmission from cattle as these species often live close to human settlements, have limited potential to maintain bTB in the absence of an alternate infection source (i.e. dead-end hosts), and often have short duration of illness [33]. The infected mongoose, blotched genet, vervet monkeys, yellow baboon, and Kirk's dik-dik in this study were all sampled near villages.

In addition to spillover, documentation of *M. bovis* infection in three species of large bodied, long-lived, gregarious herbivores (African buffalo, lesser kudu, impala) emphasizes the possibility for pathogen persistence in one or more wildlife maintenance hosts. Buffalo are the major wildlife bTB maintenance host in Africa, transmitting bTB within their herds without repeated spillover from cattle and serving as a source of bTB to other sensitive species, especially large carnivores [34]. Although there is no evidence indicating lesser kudu and impala are maintenance hosts, greater kudu with advanced bTB can shed large numbers of bacteria and may be able to maintain a separate infection cycle, as documented in KNP [33]. Once established, bTB infection in wildlife maintenance hosts can be a source of infection for livestock, thereby complicating control efforts or resulting in the re-emergence of bTB in livestock populations where the disease was formerly eradicated [35, 36].

Our documentation of infected buffaloes in the community-based wildlife management area and in RNP has conservation and economic significance. Large herds of healthy buffalo attract revenue from both non-consumptive tourists and hunters coming to the game reserves surrounding RNP. Seasonal drying of the Great Ruaha River may reduce the spatial distribution of buffalo and cattle, compressing herds into a smaller area where potential for disease transmission is higher and forage competition is more severe. In addition, forage limitation, due to increased frequency and severity of bush fires, may be an ecological stressor that acts synergistically to cause disease (P. Coppolillo, unpublished data).

We did not identify a unique buffalo-only strain of *M. bovis* in Ruaha's buffalo, thus it is possible that the infections found were solely due to spillover from livestock. However, the fact that 3/30 young buffalo from a single herd sampled inside RNP were serological reactors strongly suggests that bTB is being transmitted buffalo-to-buffalo. Given the continued ecological threats, a wider systematic buffalo health assessment of multiple herds and age groups, coupled with updated herd demographic and spatial distribution data is needed to estimate the population prevalence of bTB, determine if bTB is widespread in buffalo herds in Ruaha, and determine if bTB or other disease could be contributing to any spatial range contraction or demographic changes.

In addition to conservation impact, bTB infection in wildlife has implications for human health. Buffalo, kudu, and impala are important hunted species, and dik-dik are commonly consumed bushmeat. Although the risk of contracting bTB from consumption of well-cooked meat is minimal for most people, organ meat (including lungs) is commonly consumed and most carcasses are processed in the field with little or no sanitary precautions. These factors coupled with a relatively high prevalence of infection with HIV/AIDS may put people processing and consuming hunted wildlife at greater risk of contracting and developing clinical TB [1].

Implications for *M. bovis* control at the wildlife–livestock interface

We provide supportive evidence of a multi-host transmission cycle of *M. bovis* in the Ruaha ecosystem, involving spillover between livestock and wildlife, as well as pathogen persistence in wildlife maintenance hosts. Unfortunately, eradication strategies in and around other protected areas where similar pathogen dynamics have been documented involve resource-intensive actions, such as fencing, culling, or large-scale test-and-slaughter programmes, which are not compatible with larger ecosystem conservation goals. Even with intense interventions, these efforts have met with limited success due to logistical challenges and the existence of other possible maintenance hosts that preclude a single-species intervention approach [33].

Perhaps what is most achievable in an ecosystem like Ruaha is to recognize that bTB is a multi-host disease affecting both wildlife and livestock and that a myriad of factors including spatio-temporal population overlap, animal density, drought, human-induced habitat change, and cultural practices will affect bTB transmission and should be considered when designing efforts to control it. An alternative approach to control and better understand bTB

in this resource-poor system could include targeting testing of cattle and wildlife in shared grazing lands to identify areas or sites with increased spillover risk for management. For example, spatial analysis revealed two areas of higher than expected numbers of *M. bovis* infections within our sampling area. Even though these infection clusters were not statistically significant, potentially due to small sample size and low infection proportion, they may indicate a localized region within our sampling area that warrants additional field investigation. If resources were available to support more systematic bTB surveillance in both wildlife and livestock, focused testing in high-risk areas identified by spatial statistics could reveal a source of spillover or a highly infected local livestock or wildlife population for targeted intervention. Additionally, a better understanding of livestock grazing strategies, locations, and densities would also help identify transmission risk and control points.

At this time, there is no large-scale control or eradication programme for bTB in Tanzanian cattle or wildlife. Accordingly, the best approach to control the disease may be to focus on preserving the ecological functions of both protected and grazing areas to minimize both species overlap and the ecological stressors that increase an animal's susceptibility to bTB. For example, provision of adequate grazing and water resources for livestock would help minimize livestock incursions into wildlife protected areas during times of resource scarcity and would reduce interspecies transmission of not only bTB but potentially many other diseases. Programmes to facilitate market access for rural cattle could be coupled with efforts to promote sustainable livestock herd densities to optimize health, carcass condition, and efficient use of grazing resources. For wildlife population resilience, restoration of sustainable agricultural practices could support adequate dry-season water resources and minimize habitat degradation and encroachment.

CONCLUSION

A cattle strain of *M. bovis* infects multiple wildlife species inhabiting protected areas at the livestock–wildlife interface in the Ruaha ecosystem of Tanzania. Determining the species and spatial distribution of infection in both wildlife and livestock could enable an ecosystem-based approach to reduce disease transmission and improve opportunities for conservation interventions, tourism growth, livestock productivity, and livestock and human health security.

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DECLARATION OF INTEREST

None.

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EcoHealth

Short Communication

Bat Predation by *Cercopithecus* Monkeys: Implications for Zoonotic Disease Transmission

Elizabeth Tapanes,[1] Kate M. Detwiler,[1] and Marina Cords[2]

[1]*Department of Anthropology, Florida Atlantic University, 777 Glades Rd., Social Science Building 187, Boca Raton, FL 33431*
[2]*Department of Ecology, Evolution and Environmental Biology and New York Consortium in Evolutionary Primatology, Columbia University, New York, NY 10027*

Abstract: The relationship between bats and primates, which may contribute to zoonotic disease transmission, is poorly documented. We provide the first behavioral accounts of predation on bats by *Cercopithecus* monkeys, both of which are known to harbor zoonotic disease. We witnessed 13 bat predation events over 6.5 years in two forests in Kenya and Tanzania. Monkeys sometimes had prolonged contact with the bat carcass, consuming it entirely. All predation events occurred in forest-edge or plantation habitat. Predator–prey relations between bats and primates are little considered by disease ecologists, but may contribute to transmission of zoonotic disease, including Ebolavirus.

Keywords: Africa, disease ecology, emerging infectious disease, Ebola virus, guenon, zoonoses

Forest-dwelling primates and bats share habitat and food resources, but behavioral interactions between them are poorly documented. Bats are reservoirs for zoonotic disease, which has led researchers to hypothesize that primate consumption of fruits contaminated with an infected bat's saliva or feces facilitates zoonotic disease transmission (Dobson 2005; Alexander et al. 2015; Rodhain 2015). Primates and bats may also interact directly, however, although reports of such behavior are rare. In the recent *Mammals of Africa* compendium, for example, there is not a single mention of primates as a known predator of any African bat (Happold and Happold 2013). Nevertheless, there have been a few reports of primates preying on bats (*Pan paniscus*, Bermejo et al. 1994; *Saimiri oerstedi*, Boinski and Timm 1985; *Cebus capucinus,* Fedigan 1990; *Papio anubis* Palombit 2013; *Perodicticus potto* Pimley and Bearder 2013). Primate consumption of bats would likely constitute another mechanism of disease transmission, but descriptions of this behavioral interaction are limited and provide little detail (Lima and O'Keefe 2013).

Guenons in the genus *Cercopithecus* prefer fruits, but are opportunistic omnivores, consuming leaves, invertebrates, and occasionally vertebrate prey (Chapman et al. 2002; Gautier-Hion 1988). *Cercopithecus mitis* consumes the most diverse set of vertebrate species reported to date, including lizards and snakes, birds, galagos, flying squirrels, and mice (Lawes et al. 2013); two reports briefly mention bat feeding as well (Cords and Fuller 2010; Lawes et al. 2013). Here, we provide the first behavioral details, accompanied by photographic and video documentation, of predation on bats by *Cercopithecus* monkeys, including

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Correspondence to: Elizabeth Tapanes, e-mail: etapanes@fau.edu

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C. mitis and a *C. mitis* x *C. ascanius* hybrid. These observations suggest an alternative pathway for bat-*Cercopithecus* disease transmission that has implications for zoonotic disease transmission to humans.

Observations came from studies of (1) *C. mitis* in the Kakamega Forest, Kenya, studied since 1979, with ≤6 habituated groups followed regularly at a time (Cords 2012); and (2) *C. mitis*, *C. ascanius*, and their hybrids in Gombe National Park, Tanzania, studied intermittently since 1994 (Detwiler 2002). We collected data on bat predation opportunistically while researching *Cercopithecus* behavior. At Gombe, we documented bat predation events with photography and video. We also surveyed colleagues by email for similar reports from other field sites.

We report 13 bat predation events involving *C. m. stuhlmanni* (n = 11), *C. m. doggetti* (*n* = 1), and a *C. m. doggetti* × *C. a. schmidti* hybrid (*n* = 1). Eleven events occurred in Kakamega between April 2007 and October 2013, and two in Gombe in October 2014. No bat predation was reported at other research sites where *Cercopithecus* are common (Table 1).

Eleven of 13 predation events were successful. In eight cases, we encountered monkeys eating bats, but did not observe the kill. In two events at Kakamega, we observed a monkey snatch a bat from its day-roosting tree before consuming it. These trees were *Bischofia javanica*, an exotic that monkeys commonly visited for fruit, and *Grevillea robusta*, an exotic occasionally visited for flowers. We did not detect other bats nearby: the roosting bat appeared to be alone. In a third Kakamega case, we encountered a juvenile female *C. mitis* struggling to kill a bat that vocalized.

Table 1. Field Sites and Researchers Surveyed for Guenon Predation on Bats.

Site	Researcher(s)
East and Central Africa	
Laikipia, Kenya	Isbell L
Kibale National Park, Uganda	Brown M, Butynski T, Chapman C, Rothman J, Struhsaker T
Nyungwe National Park, Rwanda	Kaplan B
Ugalla, Tanzania	Piel A
Ituri Forest and Lomami National Park, Democratic Republic of Congo	Hart J
West Africa	
Lama Forest, Republic of Benin	Goodwin R
South Africa	
Lajuma, South Africa	Linden B*, Emerson S

* *C. mitis* flushed a bat out of its roosting tree: recorded as accidental, not a direct predation attempt.

Bat species identification was difficult because carcass consumption was often well under way when first detected (Fig. 1a). However, we confirmed three bats as Pteropodidae and a fourth as Molossidae (Fig. 1b). In four additional events, we recorded three small (13–18 cm body length) and one large (approximate 50 cm total wingspan) bat. We also found that individual monkeys spent from 10 (small bat) to ≥ 66 min (large bat) consuming a single bat from data on four events. In at least some cases, monkeys consumed the entire carcass, including bones.

During three cases of bat feeding, we observed monkeys exchange aggressive behavior, including supplants, hits, chases, and/or aggressive growls. This behavior seemed aimed at retaining or securing access to the bat carcass. During two other incidents, group-mates aggregated around a feeding individual, watching it closely. At Gombe, we encountered a solitary adult male *C. mitis* eating a bat (Fig. 2). During his >60 min feeding bout, approximately 10 researchers and tourists gathered, triggering the male to emit repeated *ant* calls for 20 minutes without fleeing, which is uncommon (Fuller 2014; Supplementary material S1).

We observed two unsuccessful bat predation events in Kakamega involving several monkeys chasing a bat simultaneously. In one chase, an adult female grabbed and bit the bat, but it escaped, made a distress call and climbed 2 m up a nearby tree before flying off with a bloody wing. In the second case, several monkeys chased a bat while emitting aggressive vocalizations.

All cases of monkeys hunting and/or feeding on bats occurred in or near human-modified or forest-edge habitats. Both Gombe events occurred within 100 m of the forest/lake edge. One of 11 Kakamega cases occurred in a human settlement (forest station) on the forest edge, five in exotic plantations of *Pinus, Cupressus,* or *Grevillea,* and another five in old (ca. 60 years, Weist et al. 2013) mixed plantation forest. Kakamega study groups did not regularly use these plantation habitats until 2008. All observations of monkeys preying on bats, except for the one in the settlement, occurred after June 2009; thus, these events seemed to track the monkeys' occupancy of plantation habitats, and despite somewhat greater observation effort, none was observed in the three study groups that did not use plan-

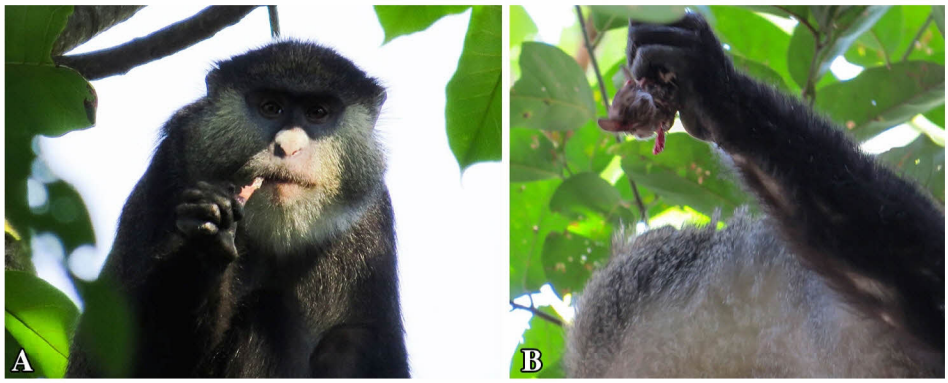

Fig. 1. *C. mitis* × *C. ascanius* hybrid feeding on a Molossid bat (**a**), and facial view of bat (**b**). Photos by Felix Angwella.

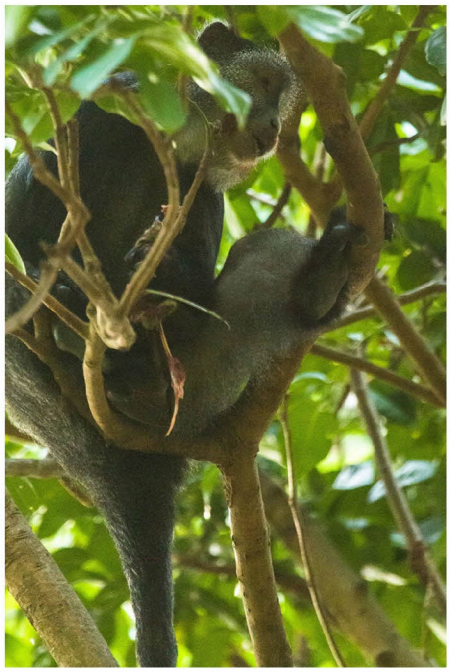

Fig. 2. *C. mitis* feeding on a large bat, likely in the family Pteropodidae. Photo by first author.

tations. Observation conditions were similar in plantation and more natural forest. We therefore think it is unlikely that habitat-related visibility differences explain why all but one of the Kakamega observations occurred in plantation forest.

Guenons as Bat Predators

Our data indicate that *Cercopithecus* monkeys opportunistically prey on bats at Kakamega and Gombe. Aggressive behavior and close visual attention to conspecifics, and an uncommonly long bout of alarm calls by a monkey that persisted in eating a bat rather than flee from an alarming stimulus indicate bats are desirable food items. Roosting bats are easy prey for predators who can reach them while torpid or asleep (Estók et al. 2010). In three predation events, bats were roosting in leaves when monkeys preyed upon them. We suspect that the lack of evidence of *Cercopithecus* predation on bats at other sites does not indicate the behavior's absence, as observations of predators hunting bats in their roosts are generally rare (Lima and O'Keefe 2013), and may be detected rarely even when extensive longitudinal behavioral data, like those examined here, are available. Conditions at Kakamega (multiple-habituated groups) and Gombe (forest-edge habitat) may have facilitated observation of this rare behavior.

Over 36 years of research at Kakamega, observed predation on bats coincided with the monkeys' increased use of plantation forests, both exotic and mixed indigenous. Both the Gombe and Kakamega ecosystems have experienced forest fragmentation and loss over recent decades (Mitchell et al. 2006; Pintea et al. 2011). These habitat changes have led more primates to use forest edges and adjacent-modified habitat like the plantation forest where all observed predation events occurred. While effects of habitat change on bats are unknown and merit further study, our observations suggest that *Cercopithecus* preying on bats may be habitat specific, and possibly affected by anthropogenic habitat change.

Implications for Zoonotic Disease Transmission

Fruit bats are hypothesized reservoirs for Ebola and confirmed reservoirs for Marburg and Henipa viruses (Olival and Hayman 2014; Yob et al. 2001). Insectivorous bats are

now also implicated as possible Ebola hosts. The most widespread Ebola epidemic to date has been hypothesized to have spread from a child playing in a tree inhabited by *Mops condylurus*, a common insectivorous bat found throughout sub-Saharan Africa, in which Ebolavirus antibodies have been documented (Pourrut et al. 2009; Saéz et al. 2014). Other viruses of potential zoonotic significance have been identified in African bats (e.g., Mortlock et al. 2015; Pernet et al. 2014; Tong et al. 2009).

Although disease transmission from bats to non-human primates may occur when primates contact infected bat feces or saliva, our observations of *Cercopithecus* handling and eating bats suggest that direct predation may also be an important pathway of disease transmission for guenons. For example, while bats are likely the primary reservoirs for Ebolaviruses, human infection has occurred primarily via contact with infected primates (Olival and Hayman 2014). In fact, four people are hypothesized to have contracted Ebola in 2001 from the contaminated meat of *Cercopithecus nictitans*, a close relative of *C. mitis* (Boumandouki et al. 2005). In a sample of 107 wild-caught captive *Cercopithecus* monkeys, one tested positive for Zaire Ebolavirus antibodies (Leroy et al. 2004). *Cercopithecus* monkeys are highly prized as bushmeat by humans and are also hunted by chimpanzees (Linder and Oates 2011; Moussoun et al. 2015; Watts and Mitani 2002). Chimpanzee meat is also valued as bushmeat (Hicks et al. 2010), and directly implicated in viral transmission to humans (Olival and Hayman 2014). Thus, predation on bats by *Cercopithecus* monkeys specifically may have implications for zoonotic disease transmission to humans.

All applicable institutional and/or national guidelines for the care and use of animals were followed.

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Review

Filoviruses in Bats: Current Knowledge and Future Directions

Kevin J. Olival [1,†,*] and David T. S. Hayman [2,3,4,†]

[1] EcoHealth Alliance, 460 W. 34th Street, New York, NY 10001, USA

[2] Department of Biology, Colorado State University, Fort Collins, CO 80523, USA; E-Mail: davidtshayman@gmail.com

[3] Department of Biology, University of Florida, Gainesville, FL 32611, USA

[4] Molecular Epidemiology and Public Health Laboratory, Hopkirk Research Institute, Massey University, Private Bag 11 222, Palmerston North 4442, New Zealand

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: olival@ecohealthalliance.org; Tel.: +1-212-380-4460; Fax: +1-212-380-4465.

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Abstract: Filoviruses, including *Ebolavirus* and *Marburgvirus*, pose significant threats to public health and species conservation by causing hemorrhagic fever outbreaks with high mortality rates. Since the first outbreak in 1967, their origins, natural history, and ecology remained elusive until recent studies linked them through molecular, serological, and virological studies to bats. We review the ecology, epidemiology, and natural history of these systems, drawing on examples from other bat-borne zoonoses, and highlight key areas for future research. We compare and contrast results from ecological and virological studies of bats and filoviruses with those of other systems. We also highlight how advanced methods, such as more recent serological assays, can be interlinked with flexible statistical methods and experimental studies to inform the field studies necessary to understand filovirus persistence in wildlife populations and cross-species transmission leading to outbreaks. We highlight the need for a more unified, global surveillance strategy for filoviruses in wildlife, and advocate for more integrated, multi-disciplinary approaches to understand dynamics in bat populations to ultimately mitigate or prevent potentially devastating disease outbreaks.

Keywords: bats; Chiroptera; disease ecology; emerging infectious diseases; Ebola; Filovirus; Lloviu; Marburg; Ravn; review

1. Introduction and Background

Filoviruses, including Ebola and Marburg viruses, are recognized as a significant threat to public health and conservation as they cause periodic human and non-human primate outbreaks with high mortality rates. Since 1967 when *Marburgvirus* first emerged in humans, their importance as lethal pathogens causing hemorrhagic fever has been appreciated, but their origins, natural history, and ecology remained elusive for decades. In 2005, the first direct evidence from field studies that bats were reservoir hosts for *Ebolavirus* was reported [1], and research has since been growing to understand the role that bats play in the maintenance, transmission, and evolution of filoviruses. There are a number of excellent reviews on the history of filoviruses, their virology, molecular biology, and vaccine development [2–4], including a special volume published in this journal "Advances in Filovirus Research 2012" [5]. We do not wish to replicate those previous reviews here and those subjects are not the focus of our paper. Thus, we only briefly review key aspects of filovirus biology before focusing our review on the issue of filoviruses in bats, with a focus on understanding the ecology, epidemiology, and natural history of this system. Through extensive review of the published literature and by drawing examples from research on other bat-borne zoonoses, we will specifically examine the current state of knowledge regarding Marburgviruses and Ebolaviruses in bats and highlight key areas for future research to better understand these associations.

1.1. Basic Virology

The *Filoviridae* family in the order Mononegavirales is separated from other Mononegavirales on the basis of morphological, physiochemical, and biological features [6,7] and more latterly genomic analyses [8]. Filoviruses are non-segmented, negative-strand RNA viruses. The viruses are filamentous (Filo- derived from the Latin *filum* or thread) enveloped particles of variable length. The filovirus genomes are typically approximately 19 kb in length [6,9]. The proteins expressed by the filoviruses are: nucleoprotein (NP), glycoprotein (GP), RNA-dependent RNA polymerase (L), and four structural proteins: VP24, VP30, VP35, and VP40 [9,10]. *Ebolavirus* is able to express a truncated soluble glycoprotein (sGP) through RNA editing. The ribonucleoprotein is derived from the RNA genome, NP, VP30, VP35, and L protein, though *Marburgvirus* is reported to be able replicate in the absence of VP30. The VP35 protein is known to block interferon induction in both Marburg and Ebola viruses [11], and the discovery of the open reading frame for this protein integrated into bat genomes is an area for future research exploration to better understand host-virus interactions and immunity [12]. The two proteins VP40 and VP24 form the internal viral membranes and the surface of the viral membranes are spiked with GP trimers. The trimers are formed from GP1 and GP2, which are cleaved from the GP precursor. The GP trimers mediate receptor binding and are the target for neutralizing antibodies [13].

1.2. Viral Taxonomy and Phylogeny

In this article, we defer to the revised filovirus taxonomy of the 9th report of the International Committee on Taxonomy of Viruses (ICTV) including proposals by Kuhn *et al.* [14,15]. Ebolavirus and Marburgvirus are the two currently recognized genera of the family Filoviridae. Lloviu virus [16] may be classified as a distinct genus, Cuevavirus, and species Lloviu cuevavirus [14]. The two classified genera are divided into increasing numbers of species, as more viruses are discovered. Within the genus Ebolavirus, Zaire ebolavirus, Sudan ebolavirus, Reston ebolavirus, Taï Forest ebolavirus (formerly Côte d'Ivoire ebolavirus), and Bundibugyo ebolavirus are recognized species. Within the genus Marburgvirus there is a single species, Marburgvirus marburgvirus (formerly Lake Victoria marburgvirus), which consists of two very divergent "viruses": Marburg virus and Ravn virus, approximately 20% divergent at a genetic level [8,14,15,17–19]. This is in contrast to the known diversity for Ebolavirus species, with Zaire ebolavirus having only a 2.7% nucleotide difference between sequences, Sudan ebolavirus 5.2%, and Reston ebolavirus 4.5% [8,20]. Despite increasing numbers of viruses being detected, some species are represented by single viral lineage (e.g., Taï Forest ebolavirus by Tai Forest Virus and Lloviu cuevavirus by Lloviu virus). These taxonomic classifications will continue to change as increased surveillance in wildlife hosts and humans and genome sequencing will uncover more divergent lineages within Filoviridae, from new localities and new hosts. While viral taxonomy ultimately relies on formal proposals and expert review by the ICTV [11,12], it will also be important to have flexible and more rapid classification schemes in place to assess the taxonomy of new lineages as our knowledge of filovirus diversity grows [20,21].

Phylogenetic techniques, in particular coalescent-based models, have also been used to estimate the ages of filoviruses. Interestingly, common ancestor age estimates have ranged from thousands to millions of years [12,16,22,23], suggesting both novel techniques and increased sample sizes are needed, and that better understanding of filovirus evolution (e.g., purifying selection, integration into host genomes, *etc.*) must be gained before reliable dates can be obtained. For individual species, some models have suggested *Zaire ebolavirus* viruses diverged from a common ancestor very recently [24–27]. Recent analyses using Bayesian coalescent phylogenetic analyses on 97 whole-genome sequences have been able to estimate nucleotide substitutions/site/year for different viruses (ranging from 0.46×10^{-4} for *Sudan ebolavirus* to 8.21×10^{-4} for *Reston ebolavirus*) [8]. The analysis by Carroll *et al.* estimates recent common ancestry (approximately 50 years ago) for *Reston ebolavirus* and *Zaire ebolavirus*, and the authors suggest these species may have experienced recent genetic bottlenecks. *Marburg marburgvirus* and *Sudan ebolavirus* species were estimated to have common ancestors less than 1000 years ago (approximately 700 and 850 years ago, respectively), whereas the *Filoviridae* were estimated to share common ancestry 10,000 years ago [8].

1.3. Filovirus Outbreaks in Humans—Brief History Including Known Links to Bat Exposure

Lake Victoria marburgvirus was the first filovirus discovered in 1967, when laboratory workers in Marburg, Germany and Belgrade, Yugoslavia (now Republic of Serbia) were exposed to the virus after contact with infected, imported green monkeys (Chlorocebus spp.). Subsequently, a number of small human outbreaks of Marburgvirus (both Marburg virus and Ravn virus) occurred sporadically between

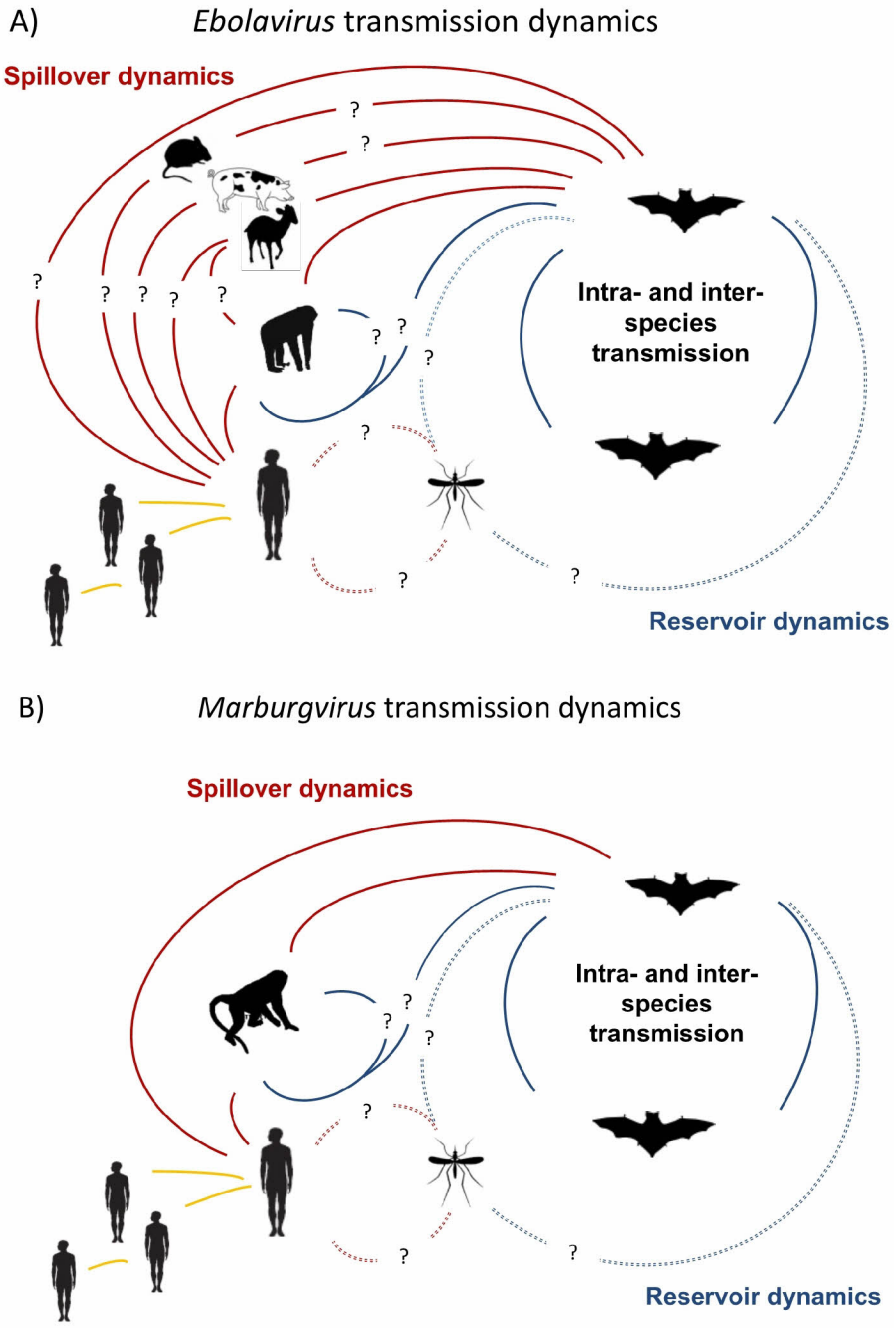
1975–1997, some of which had some link to bat caves [11,28]. The two largest outbreaks of Marburg virus occurred in the Democratic Republic of Congo (DRC) 1998–2000 where 128/154 infected people died; and in Angola in 2004–2005 where 227/252 patients succumbed to the virus [11]. The DRC outbreak was linked to gold mining in Goroumbwa cave [29], and origins of the Angola outbreak are not certain. Three small outbreaks occurred in Uganda between 2007–2008, one associated with gold mining from Kitaka cave, and two single human cases were Western tourists visiting Python Cave in Uganda while on vacation [28]. Both Kitaka and Python cave are known to harbor large bat populations, and have been sites for follow up studies on Marburg ecology [19,28].

The history of Ebolavirus outbreaks in Africa have also been previously reviewed including an excellent summary of outbreaks up until 2005 [30]. Briefly, as described in that review, in 1976 two outbreaks occurred around the same period—one in Eastern Sudan and one in Eastern Zaire—resulting in 53% and 89% mortality and the first discoveries of Sudan and Zaire ebolaviruses, respectively. Subsequently there was one human Ebolavirus case in 1977 in DRC, and a cluster of 34 cases in E. Sudan in 1979. No Ebolavirus outbreaks occurred again until 1994, when there were a series of outbreaks between 1994–1997 and more again between 2000–2004 [30]. There has only been a single, non-fatal case of Taï Forest ebolavirus in humans, a veterinarian who was infected after performing a necropsy on a chimpanzee in 1994 [31]. Bundibugyo ebolavirus was discovered after human cases of hemorrhagic fever in late 2007 in Western Uganda, but the links to an animal reservoir are not clear [32]. A large Ebolavirus outbreak occurred in DRC in 2007 (186 deaths out of 260 cases, 71.5% mortality), and the initial human "index case" was later speculated to have been linked to purchasing freshly killed fruit bats for consumption [33]. Most recently in 2012, there were four distinct outbreaks in Uganda and DRC, one caused by Marburgvirus that was discovered to be nearly genetically identical to sequences collected from bats a few years prior [34]. Currently, in March 2014, there is an ongoing outbreak of *Ebolavirus* in Guinea. At the time of writing, the WHO reported 103 cases or suspected cases with 66 deaths. Polymerase (L) gene sequence analysis suggests that this outbreak is caused by *Zaire ebolavirus*, which is the first time that this virus has been detected in W. Africa [35].

Reston ebolavirus was first discovered in 1989 from laboratory macaques exported from the Philippines to the USA [36,37]. Subsequent detections of the same virus were made in primates in 1992 and 1996 [38], and Reston ebolavirus was found to be circulating in pigs in the Philippines in 2008 [39]. A small percentage of people (1% of 458 exposed individuals) from the 1989 and 1996 events were found to have IgG antibodies to Reston ebolavirus, but were asymptomatic [38]. Reston ebolavirus infection in humans is rare and not known to cause any human disease.

As noted by others, one interesting feature of filovirus epidemics is that genetic analyses show epidemics can happen as a result of single introduction events into human populations with subsequent human-to-human transmission, or as a result of multiple introductions with less human-to-human transmission (Figure 1), but higher genetic diversity [8,17,40]. Thus, rapid genetic characterization of human and non-human primate outbreaks will continue to be critical in order to better understand the zoonotic and epidemiological origins of filovirus outbreaks [32,34]. Given that molecular tools and high-throughput sequencing (HTS) continue to get cheaper and more efficient, the time from outbreak to full viral genome sequence ready for analysis will continue to decrease and mostly likely be limited by infrastructure for cold-chain and transport of specimens.

Figure 1. (**A**) The multiple transmission pathways are shown for Ebolavirus genera viruses. The role of vectors is unlikely, but not known (dashed line). Those pathways with epidemiological uncertainty are shown with question marks. Potential reservoir dynamics are shown in blue, spillover epidemics in small mammals (Africa), pigs (Reston ebolavirus only), duikers (Africa), primates and humans shown in red and ongoing human transmission in orange; (**B**) The multiple transmission pathways are shown for Marburgvirus genera viruses. The role of vectors is unlikely, but not known (dashed line). Those with epidemiological uncertainty are shown with question marks. Potential reservoir dynamics are shown in blue, spillover epidemics in primates and humans shown in red and ongoing human transmission in orange.



2. Natural Reservoirs

2.1. Investigations to Find the Natural Reservoir—Elusive for Decades and Ongoing

While there is no consensus on how to unambiguously define an infection "reservoir", a number of criteria can be applied to identify potential animal reservoirs during epidemiological investigations, and to generally classify when a host species may act as a "reservoir" *vs.* an "accidental host", see Box 14.1 "What Is a Natural Reservoir for a Pathogen?" in [41]. The natural reservoir for *Marburg-* and *Ebolavirus* remained elusive for decades. Very diverse taxa have been suggested as potential reservoirs for filoviruses over the years, including bats, rodents, arthropods, and plants [42–46]. In a massive field investigation to find the natural reservoir following the 1995 Kikwit, DRC outbreak over 3000 animals were collected primarily from forest areas near the home of the index case, but no evidence of *Ebolavirus* was found [44]. The sampling included 78 mammal species, 51 bird species, and 22 reptiles and amphibians species were collected, and 18 species and approximately 1/5 of all the animals collected were bats. However sample sizes per species were low, with only 4 bat species having greater than 20 individuals collected [44]. Swanepoel *et al.* demonstrated that plants, reptiles, invertebrates and some vertebrates were unlikely reservoirs, because experimentally they were refractory to infections [18]. However the bats they tested (see below) were able to survive infection, support replication, and mount an adaptive immune response. Despite years of investigations, it took nearly forty years from the discovery of *Marburgvirus* in the late 1960s to identify fruit bats as (at least one of) the primary natural reservoir for this virus.

2.2. Role of Primates—Potential Reservoirs or Dead-End Hosts?

Primates are known to have a role in filovirus, ion, as the first known human cases were linked to exposure to lab primates in Europe in 1967. Viruses in the genera *Ebolavirus* and *Marburgvirus* have been isolated from infected primates [27,47–49], however the role of primates in the natural ecology of filoviruses is still poorly understood and their role as part of a reservoir complex is unknown (Figure 1). Human disease is frequently linked to contact with infected primate carcasses, though direct contact with other infected hosts is reported [18,19,33,50,51] (Figure 1). It is uncertain whether there is primate-to-primate transmission, or if primates are "dead-end" hosts and R_0 (the number of infections one infected individual causes on average over the duration of the infectious period in a naïve population) is always close to 0. However, it is noticeable that primates, especially great apes, appear to have been severely affected by Ebola (*Zaire ebolavirus*) and populations of western lowland gorillas (*Gorilla gorilla gorilla*) and common chimpanzees (*Pan troglodytes*) have declined by approximately 80% in parts Central Africa and these declines are linked (chronologically and through a small number of molecular studies) to *Ebolavirus* [50,52,53]. Following a human *Ebolavirus* outbreak in Gabon and Congo over a five month period 130/143 gorillas disappeared, with 10/12 gorillas and 3/3 common chimpanzees testing positive to *Ebolavirus* by PCR, antigen capture or immunohistochemical staining post-mortem [52]. These observations suggest that even if R_0 is less than 1, ape-to-ape transmission may be prolonged enough to cause significant epidemics. Given the many years these ape populations will take to recover after these mass mortality events [53] it suggests that African apes are unlikely to be able act as sole reservoirs for infection.

In Asia, *Reston ebolavirus* has been isolated from captive primates (*Macaca fascicularis*) in the Philippines (131/1051 were antigen positive) [36,37]. Nidom *et al.* reported anti-*Ebolavirus* antibodies in orangutans (*Pongo pygmaeus*), however, there was substantial variation in titers in orangutans and the study lacked both positive and negative controls [54] that are essential standards required to interpret serological findings [55]. These antibody findings in otherwise healthy orangutans could mean that the filovirus circulating in Asia is less virulent in apes or that orangutans are more resistant to disease (but not infection). Either of which might lead to them being able to act as hosts for filoviruses. Although, like *Zaire ebolavirus*, Reston virus has caused disease and killed primates [36,49,56–60], so if there is an intermediate or novel filovirus circulating in Asian apes it would likely need to be much less pathogenic and cause less disease in apes to persist within these populations. Moreover, a key issue is having a susceptible pool of hosts large enough for pathogens to persist within, which would likely make low density solitary apes, such as orangutans, unlikely reservoirs for acute immunizing infections [61], though they could form part of a complex of multiple species forming a reservoir [62]. Recent evidence for *Ebolavirus* infection in Asian fruit bats species could potentially support the idea that multiple hosts may be involved [63,64]. What is clear is that in Africa apes are susceptible to *Ebolavirus* and may suffer severe disease [52,65,66]. The susceptibility of African apes is both a problem for human health when human–ape contact occurs, as well as a major conservation concern for already threatened species.

2.3. Evidence of Bats as Key Reservoirs—Ebola Viruses and Marburg in Africa

The evidence for bats as reservoirs of ebolaviruses comes from numerous epidemiological and ecological studies. We summarize the known bat host species, methods of detection, and key references for each filovirus species with available data in Table 1. Prior to the detection of Ebolavirus RNA from healthy bats in the field, there were several reasons epidemiologists thought bats may be a reservoirs for ebolavirus. Index cases during Marburgvirus epidemics in Kenya [51,67] gave researchers an epidemiological link between bats and filoviruses when multiple transmission events occurred in mines [68–70]. Ecological niche models were used to provide regional perspective on the geographic and ecological distributions of Ebolavirus and Marburgvirus and suggested that various bat, mouse, rat, dormice, and shrew species may be sources of the infection as their distributions overlapped those of all four (then known) African filoviruses [71–73]. Other virological studies also suggested small mammals, comprising rodents and shrews, might be reservoirs [74]. Arthropod vectors were also considered, but viral replication in arthropod cell lines was unsuccessful [9,45].

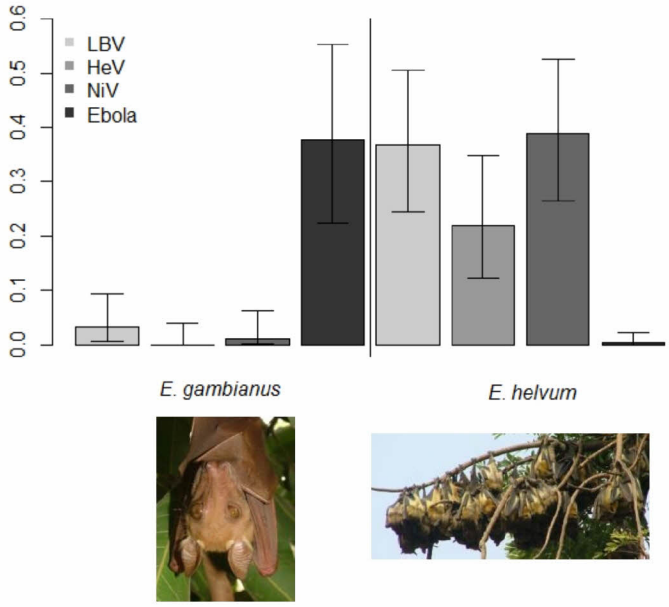
To test some of these hypotheses, a wide range of hosts were infected with ebolavirus experimentally in 1996, and bats stood out because they got infected, replicated virus, and survived infection [18]. Finally, in 2005 Leroy *et al.* managed to detect anti-*Ebolavirus* antibodies and *Ebolavirus* RNA in three fruit bat species: *Hypsignathus monstrosus* (24%, 4/17), *Epomops franqueti* (7%, 8/117) and *Myonycteris torquata* (7%, 4/58) after sampling 1,030 animals, including 679 bats, 222 birds and 129 small terrestrial vertebrates [1]. Viral nucleotide sequences were detected in liver and spleen samples (but not other tissues) from *H. monstrosus* (19%, 4/21), *E. franqueti* (4%, 5/117) and *M. torquata* (3%, 4/141). Subsequently anti-*Ebolavirus* antibodies have been detected in numerous other bat species in Africa (Table 1), including high seroprevalences in *E. franqueti* (37%, 10/27),

Epomophorus gambianus (38%, 14/37, Figure 2), *H. monstrosus* (44%, 7/16), and *Nanonycteris veldkampii* (25%, 1/4) species [75], but notably not in another common fruit bat species [76] in West Africa (Figure 2). Compelling evidence that *Rousettus aegyptiacus* was a key reservoir for *Marburgvirus* came from several studies; and is still the only filovirus to have been isolated from bats [28,30].

Table 1. Bat species found filovirus positive by serology or PCR. Bat species listed here for each virus were used to generate the geographic range maps in Figure 3. There are no currently known bat hosts for *Bundibugyo*, *Sudan*, or *Tai Forest ebolaviruses*. PCR = polymerase chain reaction; HTS = high-throughput sequencing. Species synonyms for *Myotis pilosus* and *Tadarida condylura* are used but original host name is retained from original publication.

Virus	Bat Species	Detection Method	References
Marburgvirus	*Epomops franqueti*	Antibodies	[77]
	Hypsignathus monstrosus	Antibodies	[77]
	Miniopterus inflatus	Antibodies; PCR	[18,77]
	Rhinolophus eloquens	Antibodies; PCR	[18]
	Rousettus aegyptiacus	Antibodies; PCR; Viral Isolation	[18,19,28,77–79]
Lloviu virus	*Miniopterus schreibersii*	PCR; HTS	[16]
Reston ebolavirus	*Cynopterus sphinx*	Antibodies	[80]
	Hipposideros pomona	Antibodies	[80]
	Miniopterus schreibersii	Antibodies	[80]
	Myotis pilosus (=Myotis ricketti)	Antibodies	[80]
	Pipistrellus pipistrellus	Antibodies	[80]
	Rousettus amplexicaudatus	Antibodies	[64]
	Rousettus leschenaultii	Antibodies	[63,80]
Zaire ebolavirus	*Eidolon helvum*	Antibodies	[76]
	Epomops franqueti	Antibodies; PCR	[30,75,77,81]
	Epomophorus gambianus	Antibodies	[75]
	Hypsignathus monstrosus	Antibodies; PCR	[30,75,77,81]
	Micropteropus pusillus	Antibodies	[77]
	Tadarida condylura (=Mops condylurus)	Antibodies	[77]
	Myonycteris torquata	Antibodies; PCR	[30,77,81]
	Rousettus aegyptiacus	Antibodies	[77]
	Rousettus leschenaultii	Antibodies	[63]

Figure 2. Differing antibody prevalence (as a proportion) from cross-sectional studies of two bat species from Ghana, West Africa. *Epomophorus gambianus* (**left**, Gambian epauletted fruit bat) roosts in low density, is non-migratory and has a high seroprevalence of anti-*Ebolavirus* antibodies. *Eidolon helvum* (**right**, Straw-colored fruit bat) roosts in high density, is migratory and has a low seroprevalence of anti-Ebolavirus antibodies, but high seroprevalence of antibodies against other RNA viruses. The viruses are: Lagos bat virus (LBV), Hendra virus (HeV), Nipah virus (NiV), and *Ebolavirus* (Ebola). Results are adapted from [75,76,82].

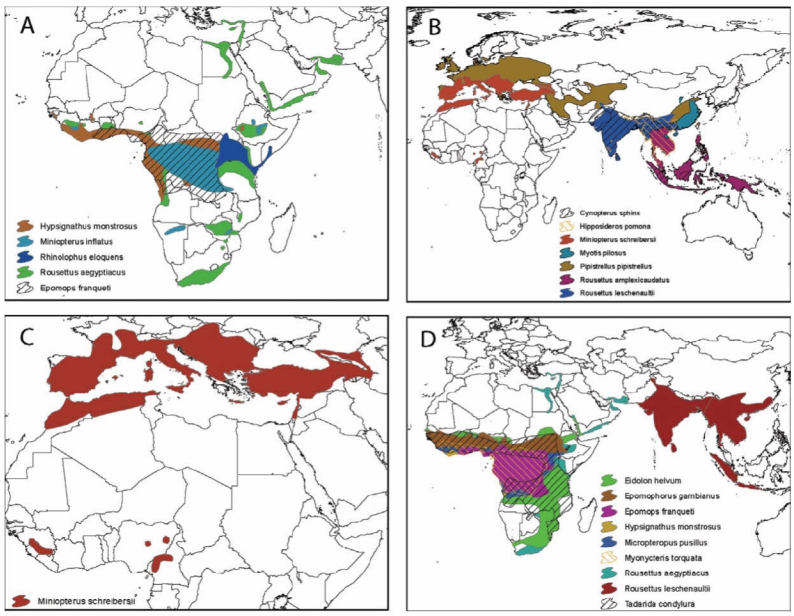


2.4. Evidence of Filoviruses from Bats in Asia and Europe

In just the past few years, antibody reactive with *Reston ebolavirus* and *Zaire ebolavirus* antigen have been detected in bats from the Philippines, China, Bangladesh, and orangutans from Indonesia (as previously mentioned). Though not conclusive evidence of the presence of these infections, the presence of these or related viruses are not entirely surprising considering the recent discoveries of *Marburgvirus* and *Ebolavirus* from congeneric species (*Rousettus spp.*) in Africa, and considering the large and overlapping geographic ranges for many of these bat species (Figure 3). *Rousettus amplexicaudatus* bats in the Philippines were found seropositive for *Reston ebolavirus* and implicating as the potential reservoir host for this virus in Asia [64]. Additional efforts to identify more solid evidence for *Reston ebolavirus* bat reservoirs in the Philippines and to understand the ecology of bats in this region are underway [83]. In Bangladesh, Olival *et al.* found serological evidence to both *Reston* and *Zaire ebolavirus* in *Rousettus leschenautii* [63]. This was the first evidence for a filovirus infecting wildlife in mainland Asia and suggested that an as-of-yet identified virus, perhaps genetically intermediate between *Reston* and *Zaire ebolavirus*, may be circulating in bat populations there. This Bangladesh bat species was also of particular interest because, along with several other frugivorous bat species in the region, it has close contact with humans and a potential transmission interface through a shared food resource (date palm sap) [63,84]. Yuan *et al.* similarly found *R. leschenautii* to be

seropositive for *Reston* and *Zaire ebolavirus* antibodies in China, along with several other insectivorous bat species (Table 1) [80].

Figure 3. Geographic range for potential bat host species for (**A**) *Marburgvirus marburgvirus*; (**B**) *Reston ebolavirus*; (**C**) *Lloviu virus*; and (**D**) *Zaire ebolavirus*.



In 2002, widespread die-offs of Schreiber's Bent-winged bats (*Miniopterus schreibersii*, Family Vespertilionidae) in the Iberian Peninsula (France, Spain, and Portugal) prompted a wildlife disease investigation. Tissue microscopy from bats collected from a cave in Northern Spain (Cueva del Lloviu) suggested that the bats died from viral pneumonia, and subsequent pathogen screening found that individuals were infected with a novel filovirus, named Lloviu virus [16]. This is the only described filovirus not known to infect humans. This finding was also highly significant as it was the first discovery of an *Ebolavirus* outside of Africa or Asia, and although causation was never proved, it has been speculated that mortality was from Lloviu virus infection.

2.5. Experimental Research Supporting Bats as Reservoirs

Experimental studies supporting the role of bats as reservoirs are few, but two key studies have investigated the capacity for bats to become infected with filoviruses and to survive infection. As mentioned above, Swanepoel *et al.* showed that Zaire ebolavirus could replicate and lead to seroconversion without disease in three species of bats infected (*Tadarida condylura*, *T. pumila*, and *Epomophorus wahlbergi*) and that virus could be isolated from feces [45]. Using captive bred *R. aegyptiacus* bats of known serological and infection status Paweska *et al.* demonstrated that viremia could be induced and Marburg virus detected in multiple tissues 2 to 9 days post infection [85]. Following viremia, IgG antibody could be detected 9 to 21 days post infection. Marburg virus could also be detected in numerous tissues, including lung, intestines, kidney, bladder, salivary glands, and female reproductive tract. None of the bats showed clinical symptoms, nor was gross pathology seen. However, it is worth noting that these studies in *R. aegyptiacus* could not induce infection following

oral or intra-nasal inoculation (the above results were following intra-dermal or intra-peritoneal inoculation), nor could virus be isolated from secretions. Similarly, the study by Swanepoel *et al.* inoculated *Tadarida* spp. bats by sub-cutaneous injection; however, fecal shedding was observed in one individual. Thus, while these results are consistent with *R. aegyptiacus* being a reservoir host, they do not shed light on the potential mechanisms for bat-to-bat transmission [85]. Additional experiments underway using a captive *Rousettus* colony housed at CDC Atlanta will likely shed more light on some of these unresolved issues [86]. Lastly, Albarino and colleagues point out that the virus used by Paweska *et al.* was passaged almost 40 times in primate Vero cells prior to infecting bats [87], and it is not known how this may affect the infectivity or virulence of this virus. Reverse genetics can now be used to reconstruct "wild type" *Marburgvirus* strains from genome sequences obtained directly from bats, even in the absence of a viral isolate, and may be a useful tool more relevant than using human or vero-adapted viruses to understand viral dynamics in bats [87].

The recent establishment of bat cell lines [88], including those of the most likely primary reservoir host for Marburgviruses, *Rousettus aegyptiacus* [89], has been invaluable to further unravel the molecular mechanisms of filovirus cell entry and host range in bats. A recent study expressing filovirus envelope GPs on the surface of vesicular stomatitis virus suggest that *Lloviu virus* GP allows viral entry into bat cells more easily than other filoviruses, and thus may be an exceptionally bat-adapted virus [90]. This finding of evidence for adaptation suggests that the bat mortality that prompted the discovery of Lloviu virus may be less likely due to this highly adapted virus, although lyssaviruses are a prime example of host-adapted viruses that remain highly virulent to bat hosts [91]. Additional investigations of host range *in vitro* also using vesicular stomatitis virus expressing GP surface protein, found that *Marburgvirus* was able to infect 6 different bat cell lines from 4 divergent bat genera (*Eidolon*, *Rhinolophus*, *Carollia*, *Tadarida*) [92].

3. Filovirus Dynamics and Ecology in Bats—What We Know and Don't Know

3.1. Lessons to Learn from Other Bat Zoonoses

Overall, filovirus ecology remains a neglected area of research, which is understandable as potential reservoirs are still being discovered and for many years remained elusive. Understanding zoonotic disease emergence and cross-species pathogen transmission require multi-disciplinary, process-based approaches that integrate ecological and evolutionary dynamics [93,94]. Several frameworks have been proposed to improve how ecological studies relating to bats and emerging infectious diseases can be performed [95]. Below we highlight some key areas with existing, but limited, information available regarding filovirus ecology and dynamics in bats, and give examples from other bat zoonoses investigations, e.g., research over the past decade into the ecology of Henipaviruses in Malaysia [96–101], which may be able to contribute valuable tools or approaches to filovirus ecology research in these areas.

3.2. Seasonality of Infection Dynamics in Bats

The most prominent study to test hypotheses regarding bat-filovirus ecology using field approaches and longitudinal sampling is by Amman *et al.*, who looked at breeding cycles and their relationship to

Marburgvirus prevalence [28]. Given that many aspects of bat biology, such as mating, birthing, and migration (e.g., [28,76,102–104]) are seasonal, Amman *et al.* were the first to test the hypothesis that birthing might be linked to increases in infection prevalence and ultimately spillover for Marburgvirus in bats. Prevalence of other bat derived viruses, including coronaviruses and rabies, are reported to show seasonal dynamics [105–107] and the increase in susceptible hosts and contact rates during the birthing period may drive infection dynamics [108]. Their study of Marburgviruses in *R. aegyptiacus* bats in Python Cave, Uganda discovered 2.5% of the bats were actively infected by PCR (and some yielding *Marburgvirus* isolates) [28]. Their analyses suggested *Marburgvirus* infection occurred in distinct pulses in older juvenile bats (approximately 6 months old), coinciding with twice yearly birthing seasons. The authors also reviewed previous human infections and found that most (83%, 54/65) occurred during this same high prevalence/seasonal birth period. Relatedly, Pourrut *et al.* 2009 found that pregnant females bats were statistically more likely to be seropositive for Ebola virus [77]. As many bats have synchronous mating and birthing [103,104,109–112] and births increase population size and contact rates, the influx of susceptible juveniles may be a central driver of bat infection dynamics. Recent theoretical studies using stochastic epidemiological models with a seasonal birth pulse suggest increased synchrony of birthing increases the necessary critical community size necessary for infection persistence [113]. Thus, seasonal birthing may decrease the probability of pathogens persisting in a colony, but lead to increased periods of infection prevalence following birthing. Whether this is true of all filoviruses in all locations is unknown and further field studies, integrated with modeling, are necessary to understand the role of host ecology on the persistence and emergence of filoviruses in bats [93,95].

Evidence from other bat-infection systems suggests that RNA virus shedding may be linked to host ecology and seasonality. Drexler *et al.* studied a maternal colony of *Myotis myotis* bats for three years and showed that RNA viruses (coronaviruses and astroviruses), but not DNA viruses (adenoviruses) were increasingly detected in greater numbers (by quantitative PCR) during colony formation and after parturition [106]. Wacharapluesadee *et al.* showed that Nipah Virus (NiV) in *Pteropus lylei* bats has seasonal dynamics, but with different dynamics for different strains, with a Bangladesh NiV strain more frequently observed April to June and a Malaysian NiV strain found from December to June [114]. These more complex patterns are also suggested by Plowright *et al.* who modeled the transmission dynamics of Hendra virus (HeV) in Australian Pteropid bats and found that their models fit the available data better when population connectivity and immunity (including waning maternal immunity) interact, suggesting more complex dynamics than a simple increase in susceptible juveniles providing enough young for persistence [99].

There remain, however, uncertainties about how strong the effects of seasonal birthing are for other filoviruses, and how much coloniality (as shown by *R. aegyptiacus*) and other factors drive infection dynamics. Further still, it has recently been demonstrated that host population structure may be a useful tool to predict infection presence [115] and this remains to be seen for the potential reservoirs of filoviruses. Interestingly, the sub-Saharan African species, *Eidolon helvum*, has been shown to have a high seroprevalence of antibodies against several RNA viruses, but not filoviruses compared to other species in the same locations [76,82] (Figure 2). Given this species is ecologically similar in some ways to *R. aegyptiacus* (seasonal, synchronous birthing; colonial; frugivorous), it poses the question as

to whether the ecological differences prevent filovirus circulation (*E. helvum* is migratory; tree roosting) or if there are underlying genetic host restrictions.

3.3. Viral Shedding and Immunity in Bats

There is little understood about filovirus shedding and persistence in bats, though several key studies [1,45,85] suggest that the within-host infection dynamics are the classical "susceptible—infected—immune[recovered]" (SIR) cycle [108]. Swanepoel *et al.* showed that in experimental infection studies Ebola virus replicated in the three species of bats infected (*Tadarida condylura*, *Tadarida pumila*, and *Epomophorus wahlbergi*) with virus isolated from feces 21 days after infection [45]. The bats also seroconverted, suggesting recovery with an adaptive immune response. Leroy *et al.* showed that anti-*Ebolavirus* IgG-positive animals were not *Ebolavirus* PCR-positive, and *vice versa*, suggesting again that infection occurs and is followed by seroconversion [1]. In Amman *et al.* showed that *R. aegyptiacus* bats were discovered to have Marburg virus PCR-positive lung, kidney, colon and reproductive tissues, which may suggest transmission by oral, urine, fecal, or sexual means [28]. The finding of widespread antibody positive bats (Table 1) suggests that survival following filovirus infection is common among bat species. The most compelling evidence for the long-term survival of free-ranging bats following Ebola virus infection is a study by Hayman *et al.*, in which a seropositive bat was known to be alive 13 months after release with a radio collar [76].

3.4. Multi-Host and Multi-Pathogen Dynamics in Bats

Multi-species interactions are critical to understand in order to accurately model viral dynamics in bat populations. To date, there is evidence for filovirus infection in a total of 17 bat species for (Marburgvirus, Zaire ebolavirus, Reston ebolavirus, and Lloviu virus), but no currently known bat hosts for Bundibugyo, Sudan, or Tai Forest ebolavirus (Table 1). Virus has only been detected via PCR and sequencing in 7 (41%) of these potential bat reservoir species, and some serological findings listed in Table 1 are sparse (e.g., only 2/679 Epomops franqueti seropositive for Marburg virus [77]. Multiple bat species could potentially act as reservoirs for Zaire ebolavirus, Reston ebolavirus, and Marburg virus, but only one host species is currently known for Lloviu virus (Table 1). Many of these species have overlapping geographic ranges, and have the potential (at a geographic, not necessarily ecological, scale) to interact and share pathogens (Figure 3). However, while either fragments of virus (PCR) or antibodies were detected in these hosts, their true role as reservoirs *versus* incidental hosts and the relative contribution of each species to interspecific host dynamics is currently unknown. Multiple circulating pathogens can also change within-host and within-population dynamics and could confer cross-species immunity [93]. For example, multiple divergent Marburgvirus strains circulate within a single roost of R. aegyptiacus [19,28]. This poses interesting questions regarding how these pathogens interact, such as is there cross-immunity and do divergent viruses have the same infection dynamics? Though cross-reactivity is shown among ebolaviruses, it is unknown how this translates to immunity within the hosts [116]. Leroy *et al.* demonstrated numerous bats infected (detected by PCR) with similar Zaire ebolavirus species PCR fragments some years apart, but within the species, these short genomic fragments differed between species and collection time [1]. In both cases, multiple hosts

and circulating pathogens can complicate our understanding of virus-host interactions and should be considered during study design [93].

3.5. Meta Populations and Connectivity

Another key aspect of ecological theory that must be investigated further is the role that meta-population dynamics may play in the ecology and evolution of filoviruses. Amman *et al.* provided evidence of direct movement between different caves for *R. aegyptiacus* and have found that there is genetic similarity between viruses detected in geographically distant locations [28]. They suggest that *R. aegyptiacus* exist as a large meta-population with virus circulation over broad geographic ranges. Population genetic studies using mitochondrial and microsatellite markers have confirmed that a congeneric species, *Rousettus leschenaultia*, is highly vagile and panmictic across large areas (e.g., from India throughout China) [117]. Further investigations to understand host movement and connectivity of potential filovirus reservoirs are warranted.

Several previous studies have investigated the relationship between host population structure and bat viral dynamics. Olival *et al.* showed that *Pteropus vampyrus*, the primary natural reservoir for NiV in mainland Southeast Asia, was highly vagile and panmictic using both host and parasite genetics, and was likely the primary player in NiV transmission and circulation [97,98,118,119]. Plowright *et al.* suggested meta-population dynamics were necessary for HeV persistence in Australian Pteropid bats and they predicted reduced connectivity leads to larger epidemics within bat colonies due to a greater loss of herd immunity in colonies with lower levels of connectivity [99]. More recently, Peel *et al.* have used host panmixia to predict infection dynamics across sub-Saharan Africa and shown similar antibody prevalences against two viruses, Lagos bat virus (a lyssavirus) and an as yet undetermined henipa-like paramyxovirus [115]. This species has been shown to both breed freely enough that there is panmixia [115] and travel between roosts over shorter time spans [104], suggesting movement between colonies within the period short enough for infection to occur and for a bat to become infectious [45]. These meta-population dynamics will be important to consider when designing future ecological studies and modeling bat-filovirus data.

4. Future Directions in Bat Filovirus Research

4.1. Unexplored Diversity and Geographic Gaps—A More Unified Surveillance Strategy

There are over 1200 bat species globally and only a small fraction (~15%) have been targeted for viral discovery to date [41]. That said, pathogen discovery in bats is becoming a widespread activity globally, and this presents an opportunity for researchers to screen specimens for filoviruses while running other routine assays. Global surveillance programs like CDC's Global Disease Detection centers, or United States Agency for International Development's (USAID) Emerging Pandemic Threat Program have established laboratory protocols for screening specimens from a diversity of wild mammal hosts. For example, the USAID PREDICT project uses degenerate PCR primers to screen bats, rodents, and primates across multiple (~10–20) viral families including Filoviruses in 20 countries around the world [94]. Through capacity building in emerging infectious disease "hotspots" globally [120], these efforts have the potential to establish a new baseline for the "unknown" zoonotic

pool in wildlife and redraw the biogeographic boundaries of pathogen distribution and host range [94,121]. These global, coordinated efforts may allow us to identify novel viruses that have not yet emerged into human populations and develop prevention strategies to ensure that they do not. Lloviu virus is a good example of this, as it was picked up during wildlife surveillance after a die-off in a bat population [16] and is now part of follow-up studies to better understand its genome [8], molecular biology, and cell entry [90]—in part to be able to predict its potential to spillover and infect humans.

While it is important to survey wildlife showing clinical signs of disease, most viruses are discovered in bats from asymptomatic animals, and a two-pronged approach of screening both healthy and diseased animals is required [122]. Modeling approaches to target bat host species based on life-history traits [123,124] or viral "habitat" suitability using ecological niche models [71–73] can both be used to refine the taxonomic and geographic scale of surveillance for novel filoviruses or novel filovirus host species.

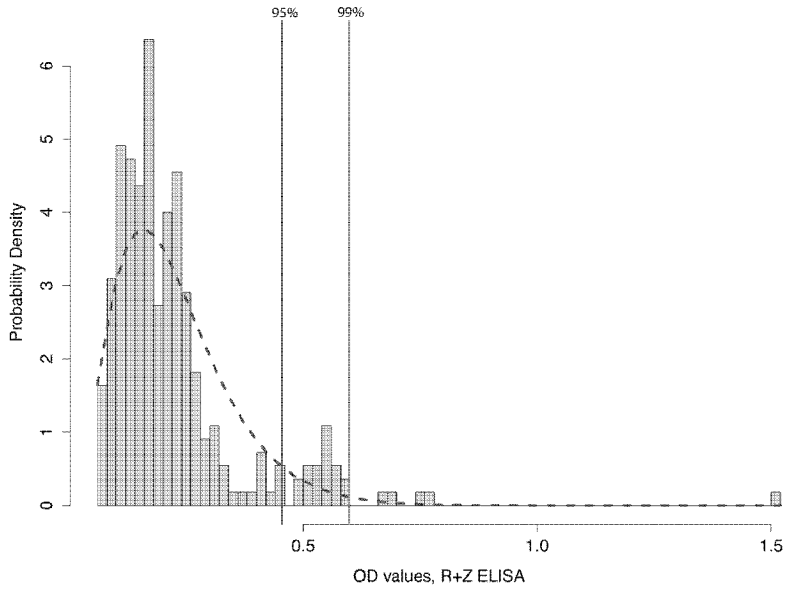
4.2. Develop More Sensitive, Non-Invasive Tools for Longitudinal Monitoring of Bat Populations

As part of a more unified filovirus surveillance strategy in bats, it will also be necessary to develop non-invasive sampling protocols *and* better detection methods for viral discovery [121,125]. Following an experimental inoculation, Swanepoel *et al.* demonstrated that *Zaire ebolavirus* could be detected in bat feces, but few studies to date have routinely screened bat excreta by PCR in the field. There are also limited data comparing viral detection from organ specimens with data from excreta collected from the same animals. Developing more sensitive assays to detect antibodies or virus from small quantities of blood [126] or bat excreta [121], respectively, has two potential benefits. First, bats (of which many species are threatened) do not need to be killed to identify potential filovirus reservoirs, or study the distribution and the seasonality of viral shedding or infection. Second, for management interventions, it is most important to understand the routes of viral shedding in bats and the seasonality of this shedding, rather than the presence or absence of a virus in a given animal or tissue type. Thus, there may be more value in detecting a virus in bat feces, urine, or saliva than there would be in bat tissue *if* transmission is occurring indirectly in bat habitat (caves or mines). However, if the risk interface is through bushmeat hunting and direct butchering of bats [33], then understanding prevalence and viral load in tissues and blood would be most relevant.

There is also a need for better studies of immunological responses in bats [127]. Understanding bat immune responses to filovriruses will help understand the ecology of these viruses within the natural setting because it can be challenging to interpret antibody data in wild species and difficult to use these data to decide whether or not a species is a reservoir (see Figure 4). More specific and sensitive assays, such as Luminex technology [128] and pseudotype assays [129] may help resolve some of these issues. Baker *et al.* demonstrated how accurate quantification of antibody responses using Luminex technology was able to demonstrate the potential effects of pregnancy on henipavirus transmission in a captive study of *Eidolon helvum* that would not have been possible with assays that used dilution series or provide binary responses [130]. These assays still require positive and negative controls, but Peel *et al.* have shown how similar data can be analyzed in the absence of validated gold standard assays from the appropriate species and population (and applied these methods to bat sera) [131].

These approaches, however, remain problematic without better knowledge of the immune response of the bat species to a particular virus. Depending on how cut-off values are determined, some studies can easily overestimate the seroprevalence of a given populations or species. Statistical tools that consider antibodies as noisy populations of antibodies, rather than a binary process, and seek to delineate cut-offs for epidemiological studies, are useful tools for understanding serological assays and have used by several authors including Peel, Pourrut, and Olival [63,77,131] (Figure 4). However, ultimately researchers should aim to understand the dynamic antibody responses in the appropriate species infected with the appropriate virus by an optimal assay before interpreting field data, despite this being difficult in practice.

Figure 4. Methodology for determining potentially positive (*i.e.*, cut-off values) for bat individuals using serological data. This figure highlights some of the challenges in interpreting filovirus serology (cut-off values) in bats, and why these data should be examined carefully. Distribution of Optical Density (OD) values from ELISA assay using 1:1 mixture of recombinant nucleoproteins for *Reston* + *Zaire* (R+Z) *ebolavirus* in *Rousettus leschenautlii* fruit bats. Data from [63], using methodology adapted from [77]. Cut-off values were determined to be >0.454 for the R+Z ELISA using a maximum likelihood estimator, gamma distribution, and 95% risk of error. Pourrut *et al.* 2009 used an exponential distribution, but the data here are better fitted to a gamma distribution. This approach is less arbitrary than the standard approach of using a value 3× the OD value of negative control, as it uses the distribution of the data itself and a statistical framework to identify potential positive cut-off values. Grey bars = OD values from individual bats for the R+Z ELISA (without positive or negative controls); red line = gamma distribution; blue = 95% confidence of cutoff values; green = 99% confidence. After initial screening, 15 (11%) of 141 *R. leschenaultii*, 6 (8%) of 75 *Cynopterus* spp., and 4 (7%) of 56 *Megaderma lyra* bats were potentially positive at the 95% confidence level. However, only 5 (3.5%) of 141 (95% CI 1.5%–8.0%) of *R. leschenaultii* bats were reported as seropositive after additional testing by ELISAs and Western Blot [63].



4.3. Genomics and Viral Fossils

The number of vertebrate genomes available for bioinformatic studies will continue to rise in the next decade as the both the cost and effort needed for sequencing them continue to decline. Advances in HTS can also offer insights into viral evolution, by offering rapid, culture free methods that allow analyses of whole viral genomes (e.g. [8]), as well as characterizing the host genome of potential reservoir species. The recent HTS of two bat genomes offers tantalizing, but preliminary, insights into how bats may be adapted for flight and perhaps have altered innate immune systems that suggest bats may respond to viral infections in a subtly different way to other mammalian hosts studies to date [132]. Understanding host responses to filovirus infection and details of host-viral interactions at a genetic level may improve understanding of field data and enable researchers to develop more nuanced methods of interpreting serological assays, and modeling infection dynamics.

Several *in silica* studies published in the past few years have used genomic data and identified filovirus genes (endogenous viral elements, EVEs) integrated into the genomes of several mammalian species, including bats [12,23,133,134]. One particularly interesting avenue for future research is whether integration of these viral genes confer some immunological advantage to hosts [134]. Evidence for this is supported by the fact that in some cases long open reading frames for these endogenous viruses have been preserved in host genomes for over thousands of years and that their presence correlates with the absence of disease in host species [134]. With the availability of more data, additional comparative genomic studies that seek to understand the phylogenetic distribution of these endogenous viruses in mammalian hosts may help to inform why some bat species appear to be resistant to infection; but also could be used to identify potential filovirus reservoir hosts that are not yet known [133]. For example, Katzourakis *et al.* found a strong association of endogenous filoviruses elements in both rodents and marsupials—pointing to these groups as potentially important reservoirs, although currently not known to harbor exogenous filoviruses [133]. These *in silica* analyses may be of use to help target which of the ~5000 mammals species to focus efforts for exogenous filovirus discovery and can be part of a more unified strategy for global filovirus surveillance.

4.4. Better Understanding Viral Shedding and Transmission in Bats

While we have a decent understanding of the progression of infection and immunity in individual humans [3], little is known about antibody persistence and viremia in bats. Experimental infections studies in captive bats and long-term monitoring of bat populations in the field using mark-recapture should help to inform this. As previously mentioned, a large number of outbreaks have been directly linked to mining activities or cave exposure [40,51,67,135–137]. However, the route of transmission is uncertain—is infection through aerosolized droplets of bat excreta and inhalation, or through some other mechanism? Experimental studies will shed light on these mechanisms and routes of exposure and can be used to guide policy to mitigate spillover.

While experimental studies with BSL-4 agents such as filoviruses can be challenging, captive studies can be used to understand infection and antibody dynamics in the absence of experimental challenge. Two studies of henipavirus infected or seropositive fruit bats have been undertaken and show the temporal dynamics of antibodies. Though both studies raise many additional questions,

they allow researchers to better understand the results of field studies [130,138]. Experimental studies of filoviruses in primates have been useful to describe filovirus infection, including the symptoms, inflammatory response, viral shedding and therapeutic potential of immunoglobulins in primates [139,140].

Experimental studies of other bat derived viruses and their non-bat hosts have been used to try to tease apart spillover transmission mechanisms. Horses can be infected with HeV through intranasal infection, suggesting inhalation may be a potential route of infection [141], but epidemiological studies of human filovirus infection suggest more close contact is required for human transmission. Pigs, hypothesized to have been infected with NiV following ingestion of excreta contaminated/partially eaten fruit, have been shown experimentally to be susceptible to infection following ingestion of NiV, with nasopharyngeal shedding [142]. Following the discovery of swine as a potential host for *Reston ebolavirus* [39], pigs have been used as experimental models. The significance of pigs in filovirus transmission has been discussed elsewhere [143,144], however, experimental studies have shown that *Zaire ebolavirus* can be transmitted from pigs to cynomolgus macaques without direct contact [145]. The mechanism(s) of transmission to primates, which are epidemiologically linked to several filovirus outbreaks and are severely affected by infection, remain unknown. Again, these studies are useful for understanding whether transmission to target, novel hosts is possible, but do not necessarily elucidate the mechanisms for transmission of filoviruses between putative reservoir bat hosts or bats and non-bat species. Studies of transmission mechanisms between and from bats to target species, such as pigs and primates, are a priority for experimental studies. The examples from other systems, in particular the henipaviruses HeV and NiV, suggest that similar studies could be used to identify potential transmission pathways (Figure 1). While there are many inherent difficulties with performing such studies for filoviruses, including extensive field situations, BSL-4 level facilities, and ethical issues, these experiments could greatly improve our understanding of filovirus ecology.

4.5. Better Understanding Host Ecology and Spillover Potential to Humans

While there is evidence to support specific instances of viral spillover, the epidemiological links between bats, Ebolaviruses, and human and primate infection are not clear. Recent epidemiological surveys following an outbreak reported increased bat activity through bat migration and hunting prior to an outbreak of Ebola virus in DRC [33]. One recent study found a high prevalence (15%) of IgG antibodies to Zaire ebolavirus in human populations in Gabon, and that populations living in forest areas were at a higher risk to being seropositive as compared to human populations in the grassland, savannah, and lake area [146]. Interestingly, no significant differences in seroprevalence were found between populations that hunted or had contact with animals *vs.* those that did not.

Several authors have speculated that, like that suspected proximate cause for the NiV outbreak in Malaysia [147], bats may drop partially eaten, *Ebolavirus*-contaminated fruits that terrestrial mammals eat and become infected [148]. In Bangladesh video surveillance has shown bats having direct contact to palm sap, an epidemiological link to NiV infection in human [84] and studies have shown NiV can survive on the surface of mango flesh for up to 2 days [149]. The role of fruit tree masting in inter-species interactions and filovirus spillover, e.g., between frugivorous bats, ungulates (duikers), and primates in the forest, is suspected but not known. Similar video studies to those in Bangladesh

have shown how apes in Africa share fruit resources [61], but it is currently unknown if partially eaten fruits can lead to infection with filoviruses. Greater use of such surveillance technology may shed further light on transmission pathways in the filovirus-bat systems.

Models using the SIR structure have been used for human epidemic dynamics [150–152] but not for wildlife. Multi-species SIR models [108] could be developed to describe filovirus transmission within bats and between bats and other host species (e.g., gorillas) and could be parameterized using data from field and experimental investigations. These epidemiological studies could be used to answer questions regarding the transmission processes, including if the virus(es) could persist within specific populations or species alone. These models may also be used to highlight which aspects of host and virus biology may be important and require further study, through the use of sensitivity analyses [95]. As we have previously described, population genetic tools can be used to define geographic limits of populations and quantify connectivity between bat populations for each host species known to harbor a given filovirus species. Fine scale gene flow data can be combined with GPS or satellite telemetry and GIS modeling of landuse change—to assess if environmental features (available habitat) spatially correlate with observed breaks in gene flow and population limits. Satellite telemetry studies in the Philippines, as part of multi-disciplinary investigations of viruses have shown non-random foraging and increased roosts compared to previous knowledge [83]. These types of studies can highlight important aspects of host ecology, as well as the impacts and distributions of infected or previously infected individuals [76].

Collectively, these ecological studies will be critical to inform disease management options. For example, management options that reduce human–bat contact during seasonal periods of high risk viral shedding, or at key interfaces, will likely be the most effective approaches and can balance both conservation and human health needs [63,95]. The need to better understand the ecology of filoviruses in their natural hosts and factors that facilitate transmission could not be timelier, as an unprecedentedly large human Ebola virus outbreak is currently ravaging Guinea [35]. We advocate for more integrated, multi-disciplinary approaches to understand filovirus dynamics in bat populations, and to mitigate and control these potentially devastating disease outbreaks.

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Author Contributions

K.J.O. and D.T.S.H. contributed equally in writing this review and producing its figures.

Conflicts of Interest

The authors declare no conflict of interest.

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Zoonoses 1

Ecology of zoonoses: natural and unnatural histories

William B Karesh, Andy Dobson, James O Lloyd-Smith, Juan Lubroth, Matthew A Dixon, Malcolm Bennett, Stephen Aldrich, Todd Harrington, Pierre Formenty, Elizabeth H Loh, Catherine C Machalaba, Mathew Jason Thomas, David L Heymann

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See Comment pages 1883 and 1884

This is the first in a Series of three papers about zoonoses

EcoHealth Alliance, New York, NY, USA (W B Karesh DVM, E H Loh MS, C C Machalaba MPH); **IUCN Species Survival Commission Wildlife Health Specialist Group, Gland, Switzerland** (W B Karesh); **World Organisation for Animal Health Working Group on Wildlife Diseases, Paris, France** (W B Karesh); **Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA** (Prof A Dobson DPhil); **Santa Fe Institute, Santa Fe, NM, USA** (A Dobson); **University of California, Los Angeles, Los Angeles, CA, USA** (Prof J O Lloyd-Smith PhD); **Fogarty International Center, National Institutes of Health, Bethesda, MD, USA** (J O Lloyd-Smith); **Animal Health Service, Food and Agriculture Organization of the**

More than 60% of human infectious diseases are caused by pathogens shared with wild or domestic animals. Zoonotic disease organisms include those that are endemic in human populations or enzootic in animal populations with frequent cross-species transmission to people. Some of these diseases have only emerged recently. Together, these organisms are responsible for a substantial burden of disease, with endemic and enzootic zoonoses causing about a billion cases of illness in people and millions of deaths every year. Emerging zoonoses are a growing threat to global health and have caused hundreds of billions of US dollars of economic damage in the past 20 years. We aimed to review how zoonotic diseases result from natural pathogen ecology, and how other circumstances, such as animal production, extraction of natural resources, and antimicrobial application change the dynamics of disease exposure to human beings. In view of present anthropogenic trends, a more effective approach to zoonotic disease prevention and control will require a broad view of medicine that emphasises evidence-based decision making and integrates ecological and evolutionary principles of animal, human, and environmental factors. This broad view is essential for the successful development of policies and practices that reduce probability of future zoonotic emergence, targeted surveillance and strategic prevention, and engagement of partners outside the medical community to help improve health outcomes and reduce disease threats.

Introduction

Pathogens shared with wild or domestic animals cause more than 60% of infectious diseases in man.[1] Such pathogens and diseases include leptospirosis, cysticercosis and echinococcosis, toxoplasmosis, anthrax, brucellosis, rabies, Q fever, Chagas disease, type A influenzas, Rift Valley fever, severe acute respiratory syndrome (SARS), Ebola haemorrhagic fever, and the original emergence of HIV.[2–6] Zoonotic diseases are often categorised according to their route of transmission (eg, vector-borne or foodborne), pathogen type (eg, microparasites, macroparasites, viruses, bacteria, protozoa, worms, ticks, or fleas), or degree of person-to-person transmissibility.[7] The greatest burden on human health and livelihoods, amounting to about 1 billion cases of illness and millions of deaths every year, is caused by endemic zoonoses that are persistent regional health problems around the world.[2] Many of these infections are enzootic (ie, stably established) in animal populations, and transmit from animals to people with little or no subsequent person-to-person transmission—for example, rabies or trypanosomiasis.

Key messages

- Nearly two-thirds of human infectious diseases arise from pathogens shared with wild or domestic animals
- Endemic and enzootic zoonoses cause about a billion cases of illness in people and millions of deaths every year, and emerging zoonoses are a rising threat to global health, having caused hundreds of billions of US dollars of economic damage in the past 20 years
- Ecological and evolutionary perspectives can provide valuable insights into pathogen ecology and can inform zoonotic disease-control programmes
- Anthropogenic practices, such as changes in land use and extractive industry actions, animal production systems, and widespread antimicrobial applications affect zoonotic disease transmission
- Risks are not limited to low-income countries; as global trade and travel expands, zoonoses are increasingly posing health concerns for the global medical community
- Ecological, evolutionary, social, economic, and epidemiological mechanisms affecting zoonoses' persistence and emergence are not well understood; such information could inform evidence-based policies, practices, and targeted zoonotic disease surveillance, and prevention and control efforts
- Multisectoral collaboration, including clinicians, public health scientists, ecologists and disease ecologists, veterinarians, economists, and others is necessary for effective management of the causes and prevention of zoonotic diseases

Search strategy and selection criteria

We selected high-quality references that showed rigorous scientific methodologies in their research and analyses. We searched Web of Science for reviews and research articles published between Jan 1, 1990, and June 1, 2012, with the search terms "zoonotic disease" and "antimicrobial resistance", and filtered results for "animals", "wildlife", or "wild animals". We chiefly selected publications from the past decade but did not exclude commonly referenced or highly regarded older publications. We also searched reference lists of articles identified by this search and selected those we judged relevant. Review articles and book chapters are cited to provide readers with more details and more references. Non-peer-reviewed sources such as reports from the World Organization for Animal Health, the Food and Agriculture Organization, and WHO were also reviewed to provide direct information or additional supporting references. Additional references and materials were suggested by anonymous reviewers and additional reviewers invited by the authors.

Other zoonotic pathogens can spread efficiently between people once introduced from an animal reservoir, leading to localised outbreaks (eg, Ebola virus) or global spread (eg, pandemic influenza). Zoonoses made up most of the emerging infectious diseases identified in people in the past 70 years which, although relatively rare compared with endemic zoonoses, are a substantial threat to global health and have caused economic damage exceeding hundreds of billions of US dollars in the past 20 years.[8,9] Apart from the appearance of a pathogen for the first time in human beings, the distinction between endemic and emerging zoonoses can be viewed as temporal or geographical. An endemic disease in one location would be regarded as an emerging disease if it crossed from its natural reservoir and entered the human or animal populations in a new geographical area, or if an endemic pathogen evolved new traits that created an epidemic (eg, drug resistance).

Transmission of pathogens into human populations from other species is a natural product of our relation with animals and the environment. The emergence of zoonoses, both recent and historical, can be considered as a logical consequence of pathogen ecology and evolution, as microbes exploit new niches and adapt to new hosts. The underlying causes that create or provide access to these new niches seem to be mediated by human action in most cases, and include changes in land use, extraction of natural resources, animal production systems, modern transportation, antimicrobial drug use, and global trade. Although underlying ecological principles that shape how these pathogens survive and change have remained similar, people have changed the environment in which these principles operate. Domestication of animals, clearing of land for farming and grazing, and hunting of wildlife in new habitats, have resulted in zoonotic human infection with microorganisms that cause diseases such as rabies, echinococcosis, and the progenitors of measles and smallpox that had historically affected only animal populations through changes in contact and increased transmission opportunities from animals to people.[10–12] As human societies have developed, each era of livestock revolution presented new health challenges and new opportunities for emergence of zoonotic pathogens.[13]

In the past few decades, accelerating global changes linked to an expanding global population have led to the emergence of a striking number of newly described zoonoses, including hantavirus pulmonary syndrome, monkeypox, SARS, and simian immunodeficiency virus (the animal precursor to HIV). Some of these zoonoses, such as HIV, have become established as substantial new human pathogens that circulate persistently without repeat animal-to-person transmission. SARS could have established, but was contained by rapid global response to its emergence;[14] other zoonoses, such as Ebola virus and Nipah virus, have not become established because of local control efforts or their intrinsic inability to transmit efficiently between people. However, others such as hantavirus pulmonary syndrome, which is enzootic in rodents in many locations, cause sporadic and infrequent clusters of infections in human beings.[15] In all cases, these emerging zoonoses are defined by their relatively recent appearance (or detection) in a population or, in some cases, an amplification of transmission that increases the incidence, prevalence, or geographical distribution of previously rare pathogens.[15]

Emergence of a zoonosis depends on several factors that often act simultaneously to change pathogen dynamics. The capacity of a pathogen to transmit or spread in a population is commonly quantified by the basic reproduction number, or R_0 (panel 1). In addition to inherent properties of the pathogen, factors affecting emergence or spread include environmental factors or changes in land use, human population growth, changes to human behaviour or social structure, international travel or trade, microbial adaptation to drug or vaccine use or to new host species, and breakdown in public health infrastructure.[17] With more than a billion international travellers every year, infected individuals could potentially spread zoonotic diseases anywhere in the world. Thus, with the emergence of new infectious diseases and the chronic presence of known zoonotic diseases in many low-income and middle-income countries that might or might not be adequately diagnosed or reported, zoonoses are increasingly relevant to the global medical community.

Panel 1: Basic reproduction number (R_0)

The ability of a pathogen to transmit in a population is commonly quantified by the basic reproduction number (R_0), which can be described mathematically. Formally, R_0 is the average number of secondary cases an infected individual can cause in a specific population in which all individuals are susceptible. If R_0 is greater than 1, the number of cases caused by a pathogen will increase and cause an epidemic. By contrast, when R_0 is less than 1, the number of cases will diminish and the pathogen will eventually become extinct. For many pathogens, R_0 is correlated with density of susceptible hosts (and contacts between them), thus one way that a new zoonosis can fail to become endemic in people is if the human population is sparse. This straightforward relation between population density and the ability of new zoonoses to colonise people might underpin the emergence of a series of endemic diseases thousands of years ago (eg, the Egyptian plagues, smallpox, and rubella), when populations aggregated into towns or cities and thus reached the density at which R_0 for person-to-person transmission of pathogens introduced from animals exceeded 1, or could exceed 1 by evolving person-specific adaptations.[16]

UN, Rome, Italy (J Lubroth DVM); **Chatham House Centre on Global Health Security, London, UK** (M A Dixon MSc, Prof D L Heymann MD); **Institute of Infection and Global Health, School of Veterinary Science, University of Liverpool, Leahurst, Neston, UK** (Prof M Bennett PhD); **Bio-Economic Research Associates (bio-era), Stockbridge, VT, USA** (S Aldrich BA, T Harrington MBA); **Strategies for Epidemic and Emerging Diseases, Pandemic and Epidemic Diseases Department, WHO, Geneva, Switzerland** (P Formenty DVM); **International Influenza Unit, Office of Global Affairs, US Department of Health and Human Services, Washington, DC, USA** (M J Thomas MPH); **and London School of Hygiene and Tropical Medicine, London, UK** (D L Heymann)

Correspondence to:
Dr William B Karesh, EcoHealth Alliance, 460 West 34th Street, New York, NY 10001, USA
karesh@ecohealthalliance.org

Ecology of zoonoses: why pathogens do what they do

Understanding infectious diseases beyond the scale of individual clinical cases requires assessment of ecological and evolutionary perspectives. An epidemic is fundamentally an interaction between populations of two species, pathogen and host, and hence has formal similarities to predator–prey and other consumer–resource systems that ecologists have studied for decades. Multiyear cycles of immunising diseases such as measles have been understood by direct analogy to predator–prey cycles, and are driven by alternating periods of predator population growth (when prey are abundant) and decline (when prey are depleted).[18] Similarly, interactions between pathogen strains can be understood through assessment of principles of ecological competition: one recent study[19] explained the striking diversity of pneumococcal serotypes, and the epidemiological effect of the polyvalent conjugate vaccine, by interpretation of components of the acquired immune response in terms of stabilising and fitness-equalising ecological mechanisms. Such parallels are intrinsic and pervade all aspects of infectious disease—even the central epidemiological concept of R_0 is borrowed from population ecology.[20] Similarities apply to both macroparasites (helminths and arthropod ectoparasites) and microparasites (viruses, bacteria, and protozoa). One difference is that microparasites have short generation times and can be subject to strong selection pressures from host immunity, other organisms present in the microbiome, and antimicrobial drugs, all of which are key potential components of the ecosystem in which the microbes live. As a result, pathogen evolution can occur in very short timescales;[21,22] significant evolutionary changes can occur in the course of one epidemic or even during individual infections. A conspicuous example is the development of resistance in bacteria in response to antimicrobial therapy and, in a slightly longer timescale, the antigenic change in influenza viruses that results in the need for frequent updating of the influenza vaccine formulation.[23]

The dynamics of zoonotic disease transmission are deeply embedded in the ecology and evolutionary biology of their hosts. A zoonosis comprises interaction between at least three species: one pathogen and two host species, with people and another animal species acting as the reservoir of the infection. For vector-borne zoonoses,[24] the ecology is complicated because the ecology of numerous other vector and reservoir host species can change transmission dynamics.[24] Directly transmitted zoonoses can also have several reservoir hosts, potentially serving different roles in pathogen dynamics, such as amplification or transmission to human beings.[25] For example, the zoonotic paramyxovirus Nipah virus has fruit bat reservoir hosts in Malaysia. The virus became established in domestic pig populations, amplifying viral transmission and leading to a large outbreak in human beings in 1998–99.[26] More than 100 people died during this outbreak and more than 1 million pigs were killed to control the disease.

Changes in abundance of animal hosts can strikingly affect disease incidence in people. A decrease in the abundance of a preferred animal host can cause an arthropod vector to shift feeding patterns to human beings, leading to a disease outbreak. For example, when rinderpest was first introduced to east Africa, cattle and wildebeest populations depleted rapidly and tsetse flies switched to feeding on people, causing a large epidemic of sleeping sickness.[27] Environmental changes (including anthropogenic effects) might change the abundance of a wildlife reservoir host, increasing transmission within the reservoir and the risk of zoonotic transmission. El Niño events in 1991–92 and 1997–98 led to human hantavirus cases in the southwestern USA via an ecological cascade: increased precipitation caused vegetation growth, allowing rodent densities to rise, allowing an increase in hantavirus infections in rodents. This increase did not cause population declines in rodents because, like many wildlife reservoirs of zoonotic pathogens, hantavirus causes mild or subclinical infections in this group. However, the increased prevalence in rodents increased the risk of infection in people.[28]

Ecological principles also apply at the scale of individuals. Infected hosts contain a population of pathogens that grows and evolves according to the same principles as a free-living plant or animal population. Processes of viral replication, immune clearance, and tissue tropism can be understood by analogy to ecological processes of reproduction, mortality, and dispersal between habitats.[29,30] The microbial ecology of zoonotic pathogens within their reservoir hosts can be a key determinant of risk to human health. For example, feeding different diets to beef cattle before slaughter leads to different environmental conditions within the gut, and a shift in the balance of competition among microbial species, which can change the abundance of human pathogens such as *Escherichia coli* O157:H7.[31] The ecological principle of competitive exclusion is the basis for common approaches to control of zoonotic pathogens in livestock and poultry.[32,33]

Meta-genomic studies show that the community of commensal bacteria within healthy hosts plays an important part in defence against pathogens.[34] Furthermore, disruption of this community through changes in diet or use of antimicrobials can allow the growth of other organisms, some of which might be pathogenic. This mechanism underlies differential susceptibility to *Clostridium difficile* infection and might also increase the risk of zoonotic infections (as reported for salmonella).[35,36] This factor underscores the importance of study of the full microbial community within hosts (microbiome), and not just pathogens.[34]

Zoonotic disease risk and global demand for food

Increasing demand for food due to an expanding global population has led to a substantial susceptibility of our populations to food-borne zoonoses.[37] Pathogens in the livestock production chain are a particular risk, with repeated outbreaks from meat, eggs, milk, and cheese, or meat byproducts incorporated into foods as flavouring, oils, or stock.[38] Globally, most types of domesticated and wild vertebrates and many invertebrates are foods for people; such foods are capable of harbouring zoonotic bacteria, viruses, or parasites.[38]

Knowledge of the ecology of many foodborne pathogens and their range of hosts is poor. When disease outbreaks occur in people, the animal source is often difficult to identify, restricting epidemiological investigation and ecological understanding. As for many zoonoses, foodborne pathogens often cause mild or subclinical disease in reservoir hosts, and because surveillance systems for wildlife and domestic animals are not universally adequate for detection of clinical disease or pathogen presence, humans beings often act as sentinel populations for zoonoses.[39]

The volume of consumption of wildlife products for food is at least an order of magnitude lower than it is for domestic livestock.[40] However, human being–animal contact associated with hunting, preparation, and consumption of wild animals has led to transmission of notable diseases. Such diseases include HIV/AIDS, which was linked to the butchering of hunted chimpanzees,[41] SARS, which emerged in wildlife market and restaurant workers in southern China,[42] and Ebola haemorrhagic fever linked to the hunting or handling of infected great apes or other wild animals.[43] All these disease transmissions are examples of organisms or pathogens exploiting new host opportunities resulting from human behaviour. For central African countries alone, estimates of annual wild meat consumption total 1 billion kg.[4] Solutions to increased demand for bushmeat are not straightforward, and although substitution of protein from domestic animal production might seem logical, increased livestock production in developing countries without adequate disease-management practices might lead to the emergence of other pathogens due to the introduction of new hosts.

Many foodborne zoonoses are enzootic in livestock (eg, bovine tuberculosis, brucellosis, salmonellosis, and some helminth infections), especially in low-income and middle-income countries, and result in endemic infections and outbreaks of disease in people. Cultural and farming practices such as stocking rates, mixing of species, methods of confinement, and feeding, and lack of proper implementation of disease-control methods—because of weak veterinary infrastructures and insufficient public–private partnerships to support and strengthen them—can serve to maintain or spread zoonotic diseases in livestock and provide a source of new infections in susceptible human populations (panel 2).[45,46] The techniques with which animals are slaughtered and processed, and how products are stored, packed, transported, and prepared at the place they are consumed, also enable foodborne disease outbreaks.[37] Outbreaks of trichinosis in people are often linked to the consumption of incompletely cooked meat from pigs and wild boars and, occasionally, wild game.[37] Cysticercosis (caused by the pig tapeworm *Taenia solium*) affects 50 million people every year.[2] Echinococcosis (caused by the larval stages of the dog tapeworm *Echinococcus granulosus* for which ungulates serve as the intermediate host) affects 200 000 people every year, resulting in relative economic impacts equivalent to US$4·1 billion annually for treatment and control in humans and animals.[6] Other notable foodborne parasites include trematodes (liver, lung, and intestinal flukes), which are a neglected disease group despite contributing to a substantial disease burden in southeast Asia and posing a serious impediment to public health and economic prosperity in the region.[47]

Panel 2: Emergence of highly pathogenic avian influenza A H5N1

Although smallholder herds and flocks remain important for the livelihoods and food security of millions of people, intensification of livestock production is rapidly occurring worldwide. This process has inherent advantages in terms of increased productivity, economies of scale, ease and efficiency of surveillance, and application of herd health. However, ecological risks of intensified production (eg, increased host density and contact rates, reduction of genetic diversity within populations, and selection of genetic stock for improved feed conversion rather than disease resistance) without effective disease-control practices, were shown by the emergence of highly pathogenic avian influenza A H5N1. This form of avian influenza evolved from a less virulent strain in domestic poultry to become very virulent, probably as a result of increased mixing between flocks and species in an environment where biosecurity improvements have not kept pace with the rate of livestock intensification.[44] The organism expanded its geographical range through various movement and marketing practices, contamination of inanimate objects and environments, and in some cases transmission back to migratory birds.[44] More than 579 cases of H5N1 influenza in people have been reported globally, resulting in 341 deaths, and more than 230 million birds have been killed by the disease or culled in counter-epizootic measures. However, the virus continues to circulate in avian populations. More effective control of this disease in poultry, such as improved surveillance, prevention, and response programmes, could have prevented cases of disease in people and protected livelihoods.

Land-use change, extractive industries, and zoonoses

Many zoonoses can be linked to large-scale changes in land use that affect biodiversity and relations between animal hosts, people, and pathogens. Land modification, irrespective of reason, changes vegetation patterns, vector and host species dynamics (eg, abundance, distribution, and demographics), microclimates, and human contact with domestic and wild animals. All these factors are crucial in disease ecology. The effects have been well studied and described for vector-borne diseases such as malaria and Lyme disease.[48] In northeastern USA, a historical cycle of deforestation, reforestation, and habitat fragmentation changed predator–prey populations and led to the emergence of Lyme disease.[24] Prevalence of human alveolar echinococcosis (caused by *Echinococcus multilocularis*, a tapeworm of wild and domestic canids, with small mammals serving as intermediate hosts) in Tibet is correlated with overgrazing and degradation of pastures and the resulting increase in small mammal densities.[49]

In tropical regions, changes in land use have been linked to the occurrence of Chagas disease,[50] yellow fever,[51] and leishmaniasis.[51] Such changes are particularly intense in tropical regions where primary forest is opened up to mining, logging, plantation development, and oil and gas extraction. This deforestation poses a threat to global health because many of these regions are emerging disease hotspots—rich in wildlife biodiversity and probably rich in the diversity of microbes, many of which have not yet been encountered by people.[8] Increased access to tropical forests for these extractive industries might increase the risk of zoonotic disease by changing habitat and vector community composition, modifying the distribution of wildlife populations and domestic animals, and increasing exposure to pathogens through increased human contact with animals.[48,50]

Human contact with wildlife is increased on a large scale through road building, establishment of settlements, and increased mobility of people, and the extractive process itself.[51] Where these changes take place, hunting, consumption, and trade in wildlife for food often increases.[4,52] If sites are poorly managed, increased populations can strain existing infrastructure, leading to overcrowding, poor sanitary conditions, improper disposal of waste, and a lack of potable water.[53] All of these changes increase the risk of cross-species transmission of pathogens, resulting in zoonotic disease. Additionally, new human inhabitants (recent immigrants) might not have immunity to zoonotic diseases endemic to the area, making them particularly susceptible to infection.

Extractive industry companies often have to do assessments of the environmental and social effect of their processes. However, assessments of the health effect that include principles of disease ecology are rarely done because standard operating procedures in developing countries and specific laws or regulations often do not require an assessment for health risks at a community level.[54] Furthermore, although some guidelines include zoonotic disease from domestic animals in their intended scope, few adequately address the range of potential zoonotic pathogens.

Antimicrobial drug resistance and zoonoses

Antimicrobial resistance is an important clinical problem in veterinary and human medicine. Better regulation of antimicrobial use in animals and more judicious use by human beings is needed than exists at present.[55] Use of antibiotics is the most direct mechanism for the evolution of antimicrobial-resistant infectious diseases in people. However, because many organisms carried by livestock are zoonotic and the transmission of drug-resistant genetic material between bacterial populations by phages can occur by other means, the widespread use of antimicrobial drugs for prophylaxis and as growth promoters in livestock production has led to worries about a possible route for the emergence of antibiotic resistance in people.[56]

From an ecological perspective, antimicrobial resistance is a natural occurrence; genes conferring resistance probably originated as an evolutionary response to antimicrobial drugs produced by free-living bacteria, fungi, and plants to protect themselves from infection or competition (panel 3).[63,64] The early antibiotics used in human medicine were all derived from natural bacterial and fungal sources. In turn, the use of these compounds would have resulted in selection for resistance in bacteria, and horizontal transfer via transposons and plasmids allowed these genes to spread rapidly through microbial populations and communities. Resistance is emerging today on the same evolutionary principles. Microbial populations are adapting subject to the same forces of competition and selection, but the current widespread use of antimicrobial agents in people far exceeds that of any time since their development as drugs.

Increased intensification of livestock production during the 20th century led to problems with infectious diseases that transmitted easily in dense host populations. In response, agricultural industries introduced a range of antimicrobial drugs because of their prophylactic qualities.[65] Some of these antibiotics are also used extensively in animal feed, to enhance growth rates, improve feeding efficiency, and decrease waste production of animals.[66] Whether or not the use of antibiotics in agriculture has exacerbated drug resistance in people has been debated widely.[67] Farmworkers exposed occupationally to antibiotics have an increased prevalence of resistant gut bacteria, and resistant pathogens of relevance to human medicine—including meticillin-resistant *Staphylococcus aureus*—have been identified in farm animals, although the transfer of

these bacteria from people to farm animals is also a plausible explanation.[56,68] Several pathways exist through which antimicrobial-resistant zoonotic pathogens could be transmitted from livestock to people, including through food consumption, direct contact with treated animals, waste management, use of manure as fertiliser, faecal contamination of run-off, movement of fomites through water and wind, and translocation or migration of animals.[63,69,70] Moreover, 30–90% of veterinary antibiotics are excreted after administration to livestock, mostly in unmetabolised form, presenting a direct route for environmental contamination.[56,69]

Although known to occur, the extent of transfer of antimicrobial-resistant organisms from animals to people is unclear.[56] Reduction of the use of antimicrobial drugs in animals might not be a complete solution, because diversity in antimicrobial resistance in people is unlikely to be always related to geographical overlap with livestock.[71] Furthermore, the potential for reversal of resistance is unknown, as is whether it would occur in clinical settings after a change in antimicrobial use. Substantial reductions in levels of resistant strains have been shown after termination of drug use,[67] although persistence has been noted.[72] Thus, reversion to drug susceptibility probably depends on occurrence of natural dilution of microbial populations with susceptible strains and fitness costs of resistance.[72]

Perspectives

The continuing effect of the HIV/AIDS pandemic is a reminder of the risk of zoonotic pathogens spreading from their natural reservoirs to man. What is far less broadly appreciated is that none of the approaches commonly used to search for potential new human pathogens—such as tracing back the source host of a human disease—probably would have identified simian immunodeficiency virus as a potential risk to man. Thus, bold new approaches are needed.[73] According to estimates from the UN, the global population will be more than 9 billion by 2050, and more than half the global population already lives in urban areas. Changes to food production systems provide more food security for growing populations, but also change zoonotic disease risks in ways that challenge disease control. The effect of endemic zoonotic diseases results in an annually recurring burden to the health and livelihoods of people worldwide, but disproportionately burdens low-income and middle-income countries.[2,5] Costs of zoonotic diseases are not restricted to expenses of human or animal treatment and control efforts. The disruptions to commerce and society caused by disease outbreaks can account for a large share (and in some cases almost all) of the economic costs from disease. For example, SARS cost an estimated $30–50 billion despite causing illness in fewer than 9000 people.[9]

Panel 3: Ecology of antimicrobial resistance

Antimicrobial-resistant bacteria occur in many wild mammals and birds in numerous geographical locations.[57–59] Although such bacteria are expected to exist wherever they are exposed to antimicrobials naturally produced by bacteria, fungi, or plants, resistance noted in wildlife can also be a result of either transmission of resistant organisms from domestic animals or people, or anthropogenic contamination of the environment with antimicrobials or their metabolites. Analysis of genes conferring antimicrobial resistance from bacteria found in non-human primates, people, and livestock shows that resistant bacteria from non-human primates that live close to people and livestock are genetically more similar than are bacteria found in non-human primates from areas with little or no geographical overlap with people and livestock.[60] The study also shows the natural occurrence of antimicrobial-resistant organisms and similarities in resistance patterns where wildlife, livestock, and people are in contact.

Studies of antimicrobial resistance in faecal *Escherichia coli* from rodents on pig and poultry farms in the UK suggested that resistance patterns, and the genes encoding resistance, are much the same in both wildlife and livestock (Bennett M, unpublished). Another study showed different patterns of resistance in *E coli* in bank voles (*Myodes glareolus*), wood mice (*Apodemus sylvaticus*), and cattle on dairy farms in the UK.[61] Moreover, prevalence of vancomycin resistance in *E coli* between these two rodent species changes throughout the year.[61,62] This finding suggests that, whatever the original sources of resistant bacteria and genes, differences in the ecology of wildlife species (eg, their diet and physiology) produce selection pressure on the microbes, rather than differential exposure to anthropogenic antimicrobials or presence of different resistant strains in the environment.

The dynamics of antimicrobial resistance in wildlife, both naturally occurring and arising from anthropogenic influences, are not well established. Long-term multicentre studies could provide an improved understanding of natural variation, changes with time, and interspecies transfer. In addition to observational studies, experimental work with wildlife could provide valuable insights to understanding of population and community effects of antimicrobial use and persistence of changes.

Understanding the ecology of zoonotic diseases at the human being–animal interface is a complex challenge. It requires knowledge of animal and human medicine, ecology, sociology, microbial ecology, and evolution, and the underlying issues that drive increased transmission of pathogens in humans, wildlife, and livestock: an idea described as a One Health perspective.[13,40] Meeting the challenge will also require an understanding of how the environment is changing, and how these changes affect microbial dynamics across the system. Therefore, prevention and

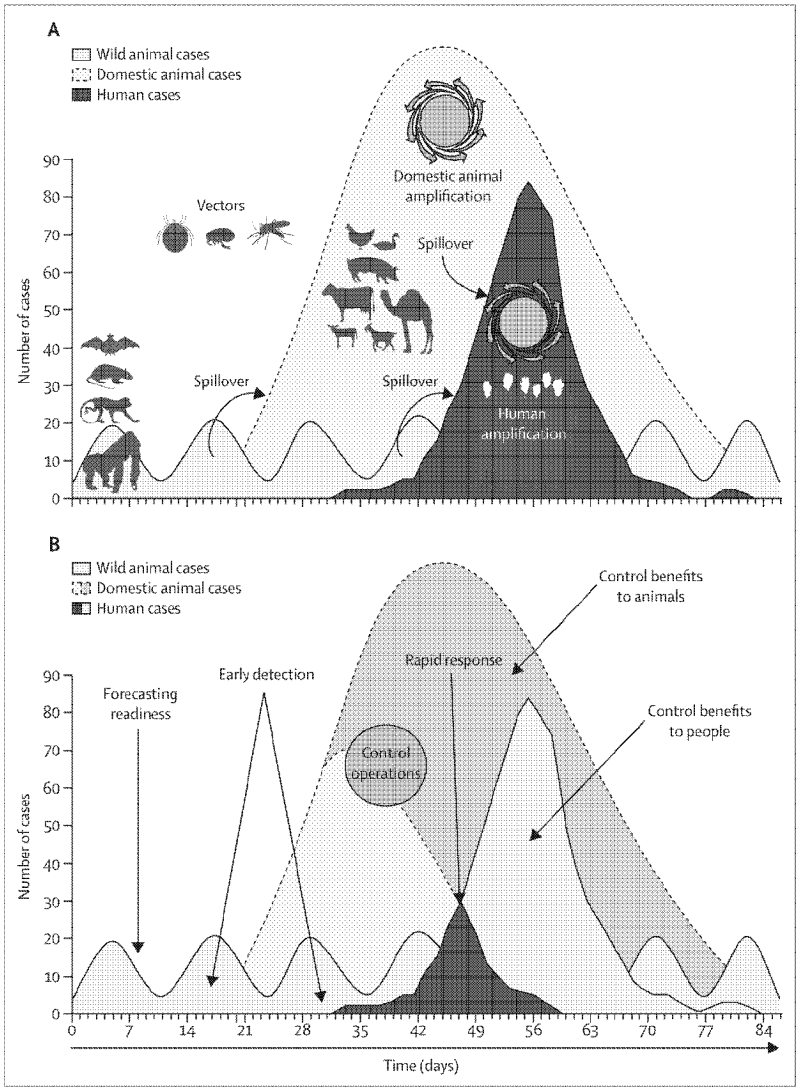

Figure: **Clinical relevance of disease ecology**
(A) Transmission of infection and amplification in people (bright red) occurs after a pathogen from wild animals (pink) moves into livestock to cause an outbreak (light green) that amplifies the capacity for pathogen transmission to people. (B) Early detection and control efforts reduce disease incidence in people (light blue) and animals (dark green). Spillover arrows shows cross-species transmission.

response to zoonotic diseases and elimination or mitigation of transmission routes to prevent their emergence will need multisectoral collaboration.[5,40] Because zoonoses affect developed and developing countries alike, and spread readily across national boundaries, mitigation and control needs collaboration between ministries of health, environment and agriculture, and intergovernmental agencies involved in health, trade, food production, and the environment. International disease-prevention efforts will be enhanced by the implementation of WHO's International Health Regulations, which allow for reporting of a broad range of human disease events, and through support of implementation of international standards for animal health and zoonoses produced by the World Organization for Animal Health, which includes reporting obligations for animal diseases including zoonoses. The need for improved veterinary services in many low-income and middle-income countries is implied by the gap in broad awareness of zoonotic diseases and their ability for detection and prevention in animals, and the ability to quantify and report their occurrences. Because disruptive effects to commerce and society can account for a large share of the economic costs of disease, integration of control strategies in animals into zoonotic disease control efforts might prove more cost effective than would control in people alone.[74]

Recent advances in understanding of patterns of zoonotic disease emergence and spread have begun to be integrated into human infectious-disease-control programmes, although substantial progress needs to be made.[75] Enhancing the role ecologists play in control programmes could include production of more accurate mathematical model outputs by collaboration with clinicians with real-time data, participation in both prospective and retrospective study design, and field studies to identify key risk factors to target surveillance and interventions.[7] Collaboration between public health scientists, who normally use epidemiological techniques with human case data, and disease ecologists who often work with wildlife or livestock data to model risk in human beings, should be encouraged. These disease ecology approaches might be particularly useful in driving advances in prediction of the emergence and spread of novel zoonoses.[73] Understanding of the relation between environmental changes, wildlife population dynamics, and the dynamics of their microbes can be used to forecast risk of human infection with enzootic or endemic zoonoses (figure). All zoonoses have non-human reservoir hosts, and the dynamics of the pathogen in these hosts often determines the risk of outbreaks in people. This risk can vary with geography, seasons, or through multiyear cycles, and can depend on factors such as changes in land use, weather, climate, or environment. Investigations into the dynamics of zoonotic pathogens in their wildlife reservoir could act as an early warning system to better inform the risk of an outbreak in livestock or people, and reduce the number of cases of human disease. For example, satellite tracking of vegetation density correlates with breeding sites for the vector of Rift Valley fever, and has been used to successfully forecast cases of disease in human beings, and the necessity for vaccine supply.[76] These approaches can be developed further to ultimately predict the risk of future disease emergence.[73]

Study of the ecological, evolutionary, social, economic, and epidemiological mechanisms that facilitate the persistence of common endemic zoonoses and those that drive zoonotic disease emergence in people has

intrinsic value. Although studies of common endemic zoonoses are often underfunded and regarded as neglected tropical diseases, studies of zoonotic disease emergence are challenged because they are often intensive, retrospective, and sometimes expensive (eg, studies to understand the cause of Nipah virus emergence or wildlife reservoirs of Ebola virus). Furthermore, emerging zoonotic disease studies are often considered as animal-focused or academic research (eg, studies to understand how dynamics of a pathogen in a wildlife host can change seasonally), when they are actually translational research efforts essential to guide clinical or public health interventions (eg, seasonal variation in dynamics drives variation in risk to people).

The complex ecology of antimicrobial resistance and foodborne zoonoses suggests new avenues for research, including an understanding of the microbiome from people and that of the animals they contact, and what causes zoonotic microbes to proliferate in some conditions. Effects of the use of antibiotics in animal production are not well understood, and the translation of this science could be enhanced by involvement of physicians, veterinarians, and ecologists in the design and interpretation of studies. Standardised data collection and long-term monitoring are needed, as are risk assessments for development of multidrug resistance or multibacterial infections in human beings resulting from antimicrobial use in food animals and from wildlife.[63,67,69] Exploration of alternatives such as probiotics, diets to promote healthy or protective gastrointestinal flora, new methods of immune-system modulation, bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides is warranted to help reduce the need for antimicrobial use in people and animals.[56,77]

Industries based on the extraction of natural resources provide materials and economic incentives, but might lead to the release of pathogens that are new to human hosts. Guidelines for safe or best practices that include ecological knowledge to reduce the risk of disease emergence or occurrence are urgently needed. Such guidelines ought to be mandated through the funding mechanisms that support large-scale development projects or be required by financial insurers.

Wide gaps in public health, veterinary and medical infrastructure, and training exist between developed and developing countries. These gaps affect disease prevention, surveillance, and control. Furthermore, little integration of ecological approaches in zoonotic disease prevention and control efforts has occurred in most countries. These challenges need to be addressed urgently, and the One Health approach perhaps provides a wider, holistic view with which to achieve this aim. Although the causes and risks of zoonoses vary widely from one region or culture to the next, our global connectivity demands the attention and alertness of health professionals everywhere. That human activities are a driving force for where and how zoonoses occur not only means that improved healthcare systems are needed, but also that multisectoral, policy-level approaches should be instigated to decrease the burden of endemic zoonoses and prevent emergence of new ones.

Contributors

All authors contributed equally to the writing and revision of the report. WBK developed the outline of the report, compiled sections, and integrated reviewer and additional comments with CCM. AD and JOL-S wrote panel 1, JL wrote panel 2, MB wrote panel 3, CCM wrote the key messages, and PF provided illustrations from which the figure was adapted.

Conflicts of interest

We declare that we have no conflicts of interest.

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Evidence for henipavirus spillover into human populations in Africa

Olivier Pernet[1], Bradley S. Schneider[2,*], Shannon M. Beaty[1,3,*], Matthew LeBreton[2], Tatyana E. Yun[4], Arnold Park[1,3], Trevor T. Zachariah[5], Thomas A. Bowden[6], Peta Hitchens[7], Christina M. Ramirez[8], Peter Daszak[9], Jonna Mazet[7], Alexander N. Freiberg[4], Nathan D. Wolfe[2] & Benhur Lee[1,3]

Zoonotic transmission of lethal henipaviruses (HNVs) from their natural fruit bat reservoirs to humans has only been reported in Australia and South/Southeast Asia. However, a recent study discovered numerous HNV clades in African bat samples. To determine the potential for HNV spillover events among humans in Africa, here we examine well-curated sets of bat (*Eidolon helvum*, $n = 44$) and human ($n = 497$) serum samples from Cameroon for Nipah virus (NiV) cross-neutralizing antibodies (NiV-X-Nabs). Using a vesicular stomatitis virus (VSV)-based pseudoparticle seroneutralization assay, we detect NiV-X-Nabs in 48% and 3–4% of the bat and human samples, respectively. Seropositive human samples are found almost exclusively in individuals who reported butchering bats for bushmeat. Seropositive human sera also neutralize Hendra virus and Gh-M74a (an African HNV) pseudoparticles, as well as live NiV. Butchering bat meat and living in areas undergoing deforestation are the most significant risk factors associated with seropositivity. Evidence for HNV spillover events warrants increased surveillance efforts.

[1] Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, California 90095, USA. [2] Global Viral/Metabiota Laboratory Sciences, San Francisco, California 90104, USA. [3] Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. [4] Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555, USA. [5] Brevard Zoo Veterinary Services, Brevard Zoo, Melbourne, 32940 Florida, USA. [6] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN UK. [7] Department of Medicine and Epidemiology, School of Veterinary Medicine, UC Davis, Davis, California 95616, USA. [8] Department of Biostatistics, School of Public Health, UCLA, Los Angeles, California 90095, USA. [9] EcoHealth Alliance, New York, New York 10001, USA. * These authors contributed equally to this work. Correspondence and requests for materials should be addressed to B.L. (email: Benhur.Lee@mssm.edu).

Nipah (NiV) and Hendra viruses (HeV) are highly pathogenic paramyxoviruses of the henipavirus (HNV) genus that cause acute encephalitis and respiratory illness. Their mortality rate in humans can be greater than 90% (refs 1,2) and they are the only paramyxoviruses that are classified as biosafety level 4 (BSL-4) pathogens. Until recently, the *Henipavirus* genus contained only two species—HeV and NiV viruses—which are phylogenetically closely related and exhibit serological cross-reactivity[3]. Fruit bats within the suborder *Megachiroptera*, particularly those of the genus *Pteropus*, have been identified as the natural reservoir for HNVs[4–7]. The geographical distribution of these reservoir bats partially coincides with the distribution of HNV outbreaks and spillover events around the Indian Ocean, reaching from Australia (HeV) to Southeast Asia and the Indian subcontinent (NiV). Ecological studies[8] have revealed several characteristics common to all regions of HNV outbreaks: (i) they are the natural habitat of Pteropid bats (*Pteropus* spp.), (ii) bat habitats in the region have been dramatically altered by the introduction of domestic plant and animal species and concomitant deforestation of the natural landscape[9] and (iii) humans or domestic animals have direct contact with bats in the area. Although these characteristics can be observed in other locations around the world, to date, HNV outbreaks and spillovers into human populations have only been recognized in Australia and South Asia.

The geographic distribution of *Pteropus* and other Pteropodids (Old World fruit bats) extends well beyond areas with documented HeV and NiV outbreaks. In 2007, a survey of Pteropodid species in Madagascar[10] reported that 2.3% and 19.2% of serum samples from *Pteropus rufus* and *Eidolon dupreanum*, respectively, tested positive for cross-reactive anti-HNV antibodies. Malagasy fruit bats share ecological niches, either roosting in the same caves or feeding in the same fruit trees. As *Eidolon* species are extremely mobile (they can fly up to 2,500 km per year[11,12]) and are present all around sub-Saharan Africa, Iehlé *et al.* raised the possibility of lateral transfer of HNV from or to other *Eidolon* species on mainland Africa and hypothesized a much wider distribution of HNV[10]. Indeed, anti-HNV antibodies were soon found in *E. helvum* (the common straw-coloured African fruit bat) from Ghana[13] on the west coast of Africa, and more recently on Annobón island[14] in the Gulf of Guinea. Furthermore, HNV-like RNA sequences have been identified in faecal droppings of urban roosting bats in Ghana[15], and more ominously, in fruit bat bushmeat in the Republic of Congo[16].

Recently, sequence analysis of a larger sample set collected from western and southern Africa revealed a surprising diversity of paramyxoviruses in African bats, including 19 new species of HNV-like viruses distinct from the Nipah and Hendra viruses found in Southeast Asia and Australia[17]. However, only one almost complete African HNV-like genome sequence (Gh-M74a clone) has been published to date, and the corresponding viral isolate has not been reported. This sequence was derived from a bat specimen originating in Ghana. We will refer to this putative HNV-like virus as the Ghana virus (GhV), and GhV-F and GhV-G when referring to its fusion (F) and attachment (G) envelope glycoproteins, respectively. In contrast to the 80–90% sequence identity shared between the F and G envelope glycoproteins of NiV and HeV, GhV-F and GhV-G share only about 70 and 40% sequence homology and even lower sequence identity (56 and 26%) with their respective NiV and HeV counterparts. Given this poor overall sequence conservation, it is unclear whether humoral responses elicited against the F/G proteins from African clades of HNV-like viruses would cross-react with F/G from NiV or HeV. This sequence divergence highlights the limitations faced by current seroprevalence studies that rely mostly on ELISA- or Luminex-based assays using recombinant NiV-G or HeV-G proteins as the target antigen[10,13,14,18].

ELISA-based screening assays, although efficient, can yield high false positive and false negative rates compared with functional seroneutralization (SN) assays[19]. Thus, whenever possible, ELISA/Luminex-positive samples are confirmed by a SN assay. Although SN assays are considered a gold standard for seroprevalence studies[19–21], follow-up confirmation with live virus SN assays is limited by the amount of sample available, and the requirement to work with live HNV in a high-containment facility (BSL-4). Consequently, in many prior studies only ELISA/Luminex-positive samples, and often only a small subset such as those with the highest binding activity, were confirmed with a biological or surrogate SN assay (reviewed in LF Wang *et al.*[21]; for example, AJ Peel *et al.*[22]). The latter is based on serum antibody competition of soluble receptor (sEphrinB2-Fc) binding to recombinant NiV-G or HeV-G conjugated to Luminex beads[18]. Although these procedures can guard against false positives, they do not address the loss of potential false negatives[10,13,14].

Given the recent reports that a diversity of HNV-like viruses are present and may be widely distributed in the bat reservoir host population across Africa[17,22], in the present study, we sought to evaluate the seroprevalence of HNV-like infections in both the bat and proximate human populations in Cameroon, and to assess risk factors that might be associated with any putative zoonotic transmission of African HNV-like viruses. To avoid the specificity and sensitivity issues associated with ELISA-based assays, as well as the impracticalities of using a live virus SN assay in BSL-4 as a screening test, we developed a VSV-based HNV envelope pseudotype particle (VSV-HNVpp) infectious SN assay[20], which can be used at BSL-2 conditions as a primary screen for anti-NiV cross-neutralizing antibodies (anti-NiV-X-Nabs). Wang and Daniels raised the possibility that the high sensitivity and specificity of the pseudotyped particle platform may allow for the combination of screening and confirmatory tests in a single assay[21]. Thus, we screened serum samples from hunted bats (*E. helvum*) in an urban area in Yaoundé, Cameroon, and from almost 500 humans living in various villages across the south of Cameroon. The specificity, breath and potency of anti-NiV-X-Nabs were confirmed using numerous specificity controls unique to our infectious SN assay, including isogenic viruses pseudotyped with irrelevant (VSV-G) or related HNV envelopes (HeV and GhV), and follow-up confirmation with a recombinant replication-competent reporter NiV specifically engineered for high-sensitivity detection of anti-NiV-X-Nabs. Remarkably, the seropositive human samples were found almost exclusively in individuals who reported butchering bats for bushmeat. The geographical and temporal clustering of these seropositive cases provides evidence for recent HNV-like spillover events into the human population in this part of Africa.

Results

NiV cross-neutralizing activity in bat serum samples. Despite the overall low sequence homology between GhV-G and NiV-G/HeV-G, mapping of the GhV-G sequence onto the crystal structure of NiV-G complexed with ephrinB2 (ref. 23) indicated that the vast majority of the sequence conservation was located at the receptor-binding interface, suggesting that GhV-G may also use ephrinB2 (and likely ephrinB3) as receptors for cell entry (Fig. 1). The clustering of conserved sequences around the receptor-binding site raises the possibility of biologically significant anti-NiV *cross-neutralizing* antibodies (anti-NiV-X-Nabs) in African bats exposed to African clades of HNV-like viruses despite their overall low sequence identity with NiV.

To determine the prevalence of anti-NiV-X-Nabs in that geographically proximate part of Western Africa, we screened fruit bat (*E. helvum*) serum samples from Cameroon, collected and curated by Global Viral/Metabiota. To conserve the use of such sera, we further optimized a previously validated VSV-based (VSV-ΔG-rLuc) NiV envelope pseudotyped particle (NiVpp) SN assay for high-specificity screening, and established appropriate control sera as described in Methods (Supplementary Fig. 1).

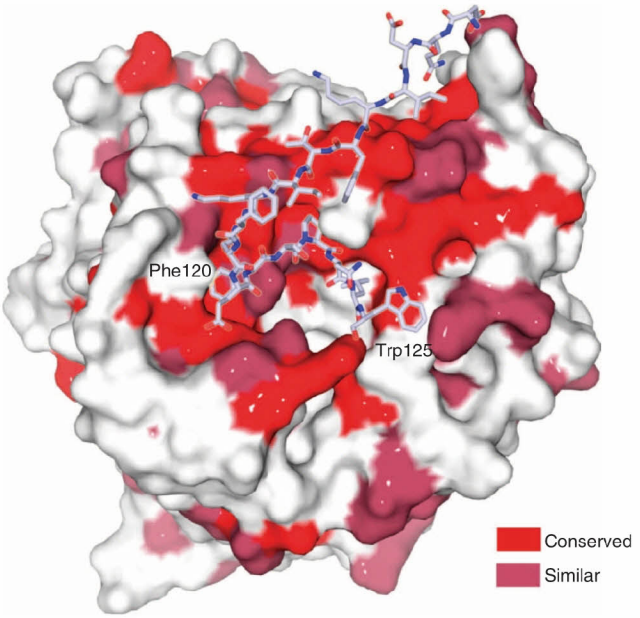

Figure 1 | Mapping sequence conservation of GH-M74a onto the surface of NiV-G. NiV-G surface is coloured according to residue conservation with GH-M74a: red, conserved; maroon, similar; white, no sequence conservation. EphrinB2 residues 107–127 are represented as sticks. Although NiV-G shares relatively low sequence conservation with GhV-G (approximately 25%), it shares greater sequence conservation (45%) in residues that make up the receptor-binding site (calculation performed with PISA EBI server).

In our screen of bat serum samples (Fig. 2), ~48% (21/44) were classified as being positive for anti-NiV cross-neutralizing activity (Fig. 2d; Supplementary Fig. 2a) when compared against the fetal calf serum (FCS)-negative control group (Fig. 2a) as determined by the Dunnett's test for multiple comparisons against a single control group. The specificity of our NiVpp SN assay is underscored by the lack of inhibition of the relevant serum samples against vesicular stomatitis virus-based pseudoparticles (VSVpp). Furthermore, 'normal bat sera' (NBS) from captive-bred bats in the United States did not show significant neutralization activity against NiVpp or VSVpp (Fig. 2b).

Anti-NiV X-Nabs in human serum samples from Cameroon. The relatively high prevalence of anti-NiV-X-Nabs in the bat populations surrounding Yaoundé in southern Cameroon prompted us to examine archival *human* sera collected from this region of Africa for the presence of similar anti-NiV-X-Nabs, which might indicate potential spillover event(s). Thus, we analysed almost 500 blood samples collected from healthy adults by Global Viral/Metabiota in southern Cameroon between February 2001 and January 2003 in 13 different locations. All samples were collected in rural areas, but represent different habitats (savanna, gallery forest and lowland forest) supporting wild game populations that provided a source for the bushmeat trade (Supplementary Table 1).

The careful curation of samples allowed us to segregate the sera into various dichotomous groups such as those who reported contacts with bats and those that did not (Table 1). Sera were analysed using the same SN assay and criteria as described for the bat serum analysis except that normal human sera (NHS) from blood donors were used as complementary negative controls to

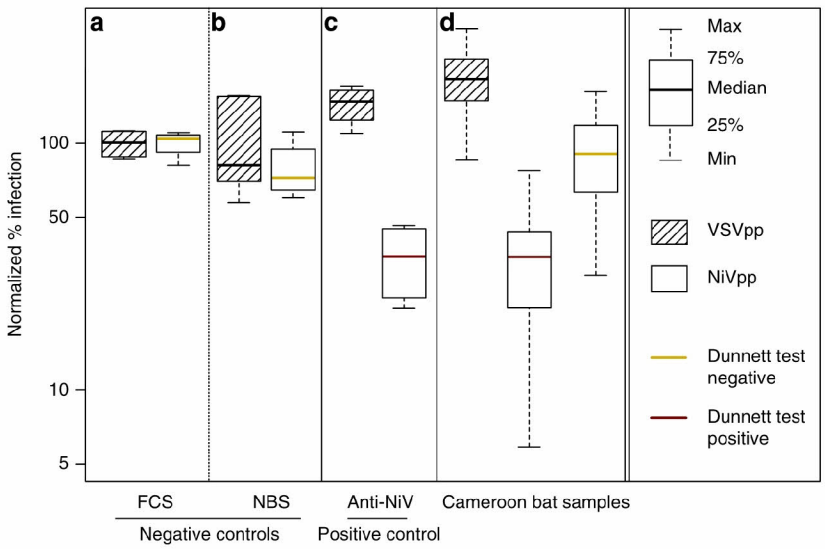

Figure 2 | Prevalence of anti-NiV cross-neutralizing antibodies in bat sera from Cameroon. Box-and-whisker plots showing the infection (normalized to the negative control fetal calf serum, FCS) of Vero cells by VSVpp (isogenic control, striped pattern box) and NiVpp (white box) in the presence of 100 × diluted sera from different groups: (**a**) FCS and (**b**) normal bat sera (NBS), negative control sera; (**c**) rabbit anti-NiV, positive control; (**d**) bat sera from Cameroon ($n = 44$). Seropositive ($n = 21$, median bar in red) and seronegative ($n = 23$, median bar in yellow) bat sera in **d** were segregated based on the Dunnett's test for significance using the FCS control group. The boxes represent the first and the third quartiles, and the solid horizontal lines within the box represent the median values. The whiskers represent the lowest and highest value. Each sample was tested in quadruplicate. The data for each serum sample are shown in Supplementary Fig. 2a.

Table 1 | Risk factor analysis.

Risk factor	Total	(*n*)	Seronegative (*n*)	Seropositive (*n*)	Seroprevalence (%)	*P* value*	Odds ratio† (95% CI)	*P* value
Contact with bats	Yes	227	220	7	3.1	**0.0045**	**17.72**	**0.0021**
	No	260	260	0	0			
	Total	487					(1.01–312.02)	
Butchering bats	Yes	171	164	7	4.1	**0.0006**	28.86	**0.0002**
	No	316	316	0	0			
	Total	487					(1.64–508.45)	
Hunting bats	Yes	99	96	3	3.0	0.1523	3.00	0.1357
	No	388	384	4	1.0			
	Total	487					(0.66–13.63)	
Deforestation	Yes	185	179	6	3.2	**0.0136**	10.09	**0.0088**
	No	302	301	1	0.33			
	Total	487					(1.20–84.48)	

CI, confidence interval.
*Two-tailed Fisher's Exact test.
†0.5 added to all cells where there is a zero cell count. see statistical methods.
Bold entries indicate significant statistical differences.

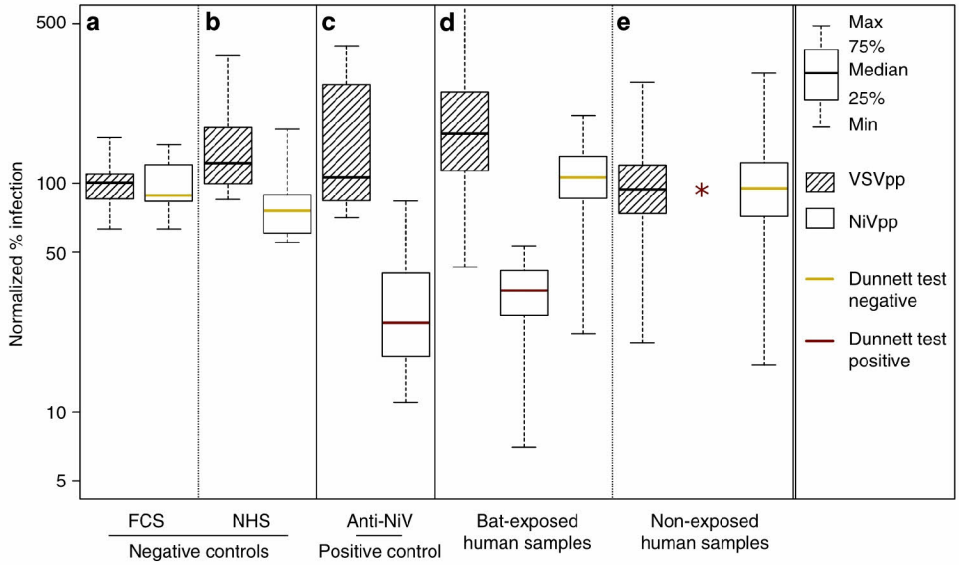

Figure 3 | Seroneutralization activity of NiV pseudoparticle infection by human sera collected from Cameroon villagers with documented differential exposure to bats. Box-and-whisker plots showing the normalized % infection of Vero cells by VSVpp (isogenic control, striped pattern box) and NiVpp (white box) in the presence of sera diluted 1:100 from the indicated sample groups (Panels 1–5). (**a**) All infections were normalized to the infectivity observed for NiVpp in the presence of the fetal calf serum (FCS) negative control (set at 100%); (**b**) normal human sera (NHS) from Los Angeles blood donors served as additional negative controls; (**c**) hyperimmune rabbit anti-NiV sera (positive control); (**d**,**e**) human sera from the bat-exposed ($n=227$) or non-exposed ($n=260$) cohort of Cameroon villagers, respectively. For the bat-exposed group, the Dunnett's test could stratify the NiVpp SN results into seropositive ($n=7$, median bar in red) and seronegative ($n=220$, median bar in yellow) subsets. In contrast, in the non-bat-exposed group, the Dunnett's test could not identify any serum sample as being significantly different from the negative control group (FCS; **e**), asterisk indicates no seropositive samples). Boxes encompass the first and the third quartiles, and the solid horizontal lines within the boxes represent the median values. The whiskers represent the lowest and highest values in each sample group. The data for individual serum samples ($n=487$), each tested in quadruplicates, are shown in Supplementary Fig. 2b and c.

FCS (Fig. 3). Using the Dunnett's test as a stringent measure of significance, 7 out of 227 samples (~3%) in the bat-exposed group were considered to have significant anti-NiVpp cross-neutralizing activity when compared against the FCS group (Fig. 3d, Table 1 and Supplementary Fig. 2c and f). On the other hand, none of the 260 samples in the non-bat exposed group were statistically different from the FCS control group (Fig. 3e, Table 1 and Supplementary Fig. 2d and f). Seropositive samples exhibited different neutralizing potencies. One seropositive sample gave an IC_{50} titre of ~1:1,000, whereas the other only achieved ~40% inhibition at 1:50 (the lowest serum dilution tested; Supplementary Fig. 2g). As expected, our hyperimmune anti-NiV control serum exhibited the highest IC_{50} titre of ~1:15,000. Interestingly, representative seronegative samples showed a slight enhancing effect as serum concentrations were increased, suggesting that we may even have underestimated the number of true positives.

Specificity of anti-NiV X-Nabs in human serum samples. To further confirm the specificity of the human sera positive for anti-NiV-X-Nabs, and to determine the potential breadth of

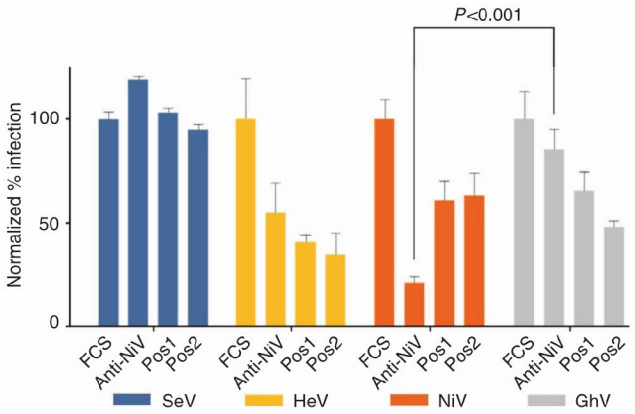

Figure 4 | Characterization of seropositive human sera for anti-henipavirus cross-neutralizing antibodies. Two seropositive human samples (*Pos1* and *Pos2*) from the bat-exposed group were tested for seroneutralization activity against different HNV pseudoparticles: Hendra virus (HeV, yellow), Nipah virus (NiV, orange) and Ghana virus (GhV, grey). Infection of Vero cells was performed in the presence of the indicated sera diluted 1:100 as in Fig. 3. As a specificity control for the virus, we used a recombinant GFP-expressing Sendai virus GFP (SeV, blue). Data are presented as normalized % infection (mean s.d. from three independent replicates) as in Fig. 3. FCS, fetal calf serum; anti-NiV, hyperimmune rabbit anti-NiV serum. Significant differences (asterisks) of inhibition was observed between the NiVpp and GhVpp for the anti-NiV serum (two-tailed Student's *t*-test, $P<0.001$).

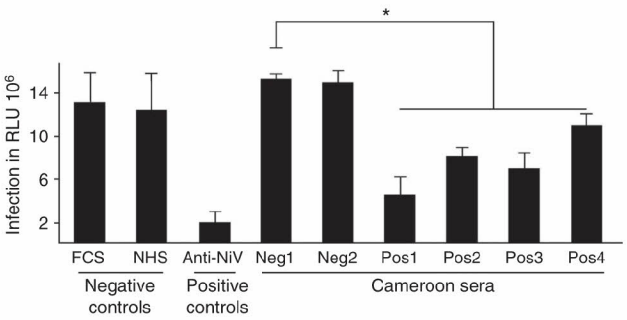

Figure 5 | Seropositive human samples neutralize live recombinant NiV infection. Infection of Vero cells by live recombinant NiV expressing secreted *Gaussia* luciferase (rNiV-GLuc, MOI = 1) in the presence of 1:100 dilution of the indicated sera: FCS (fetal calf serum, negative control), NHS (normal human serum, negative control), rabbit anti-NiV (positive control), two seronegative samples (picked randomly among the 480 seronegative samples) and four seropositive samples. 20 µl (out of 150 µl) of infected cell culture supernatant was collected and analysed for *Gaussia* luciferase activity at 24 h.p.i. Infectivity data are presented as mean relative light units (RLUs) s.d. from three independent replicates. Significant differences (asterisk) were observed between seropositive and seronegative sera ($P<0.05$; one-tailed Student's *t*-test followed by the Holm step-down procedure for multiple comparisons).

cross-reactivity of these antibodies, we chose two seropositive samples and tested them in our SN assay against three HNV pseudoparticles: NiVpp, HeVpp and GhVpp, the latter bearing F and G from the Gh-M74a clone reported by Drexler *et al.*[17], and an enhanced green fluorescent protein (eGFP)-expressing recombinant Sendai virus (rSeV-eGFP). SeV is a murine paramyxovirus (*Respirovirus* genus) not normally found in humans that enter cells via a pH-independent pathway using sialic acid-based receptors, and thus, should not be enhanced or inhibited by the seropositive human samples identified in Fig. 3. Indeed, neither the seropositive human Cameroon sera nor the hyperimmune rabbit anti-NiV serum inhibited the pH-independent entry of rSeV-eGFP (Fig. 4, blue bars). In contrast, seropositive human Cameroon samples inhibited NiVpp, HeVpp and GhVpp to varying degrees (Fig. 4, red, orange and grey bars, respectively). Interestingly, our hyperimmune anti-NiV sera showed good to moderate (50–80%) inhibition of NiVpp and HeVpp infection but only minimal inhibition (~10–15%) of GhVpp infection. The implications of these results will be discussed.

As a final confirmation regarding the specificity of the anti-NiV-X-Nabs that we detected in the seropositive human samples, we repeated the SN assay with live NiV under BSL-4 conditions. In order to conserve the use of these archival sera, which were only available in small amounts, we generated and rescued a recombinant NiV expressing secreted *Gaussia* Luciferase (rNiV-GLuc; see Methods and Supplementary Fig. 3a). *In vitro* growth kinetics of rNiV-GLuc was comparable to the parental NiV Malaysian strain and pathogenicity was demonstrated in the hamster model where bioluminescence in *ex vivo* harvested organs from the moribund animal correlated well with viral titres measured by traditional plaque assays (Supplementary Fig. 3b). Furthermore, when we infected Vero cells with rNiV-GLuc across a broad range of multiplicity of infection (MOI) (0.01–3), GLuc activity in cell culture supernatant at 24 h.p.i. was significantly and positively correlated with viral titres determined by traditional plaque assays (Supplementary Fig. 4A, $r^2=0.93$, $P=0.008$). Thus, rNiV-GLuc allowed us a highly sensitive and dynamic method to monitor NiV infection (or inhibition thereof) in cell culture by sampling infected cell culture supernatant for *Gaussia* luciferase activity. We then chose four seropositive and two seronegative human samples from our Cameroon cohort, and performed our SN assay with rNiV-GLuc along with the other appropriate negative and positive controls.

Figure 5 shows that only the seropositive samples significantly inhibited rNiV-GLuc infection, albeit with varying degrees of potency (15–65% reduction in GLuc activity). A relatively high inoculum was used, and raw luciferase activity values ($>10^7$ relative light units (RLUs)) are presented to demonstrate the robustness of our assay. Supplementary Fig. 4B and C shows how inhibition of GLuc activity correlated with decreases in viral titres across a range of MOI, and also indicates that we used a high MOI inoculum (≥ 3). Seronegative samples showed no significant inhibition of rNiV-GLuc infection, and the GLuc activity detected was not different from the FCS-negative or NHS-negative controls. As expected, the hyperimmune rabbit anti-NiV serum inhibited rNiV-GLuc infection by close to 90%.

Risk factor analysis. The questionnaire filled out by the participants before blood sample collection covered their contacts with some animals known to be HNV hosts, some of their 'at risk' activities and the location of the village with its associated environmental features. We analysed their answers to uncover any risk factors that might be associated with seropositivity. Table 1 shows that all of the seropositive samples came from the group that reported contact with bats in one form or another with those exposed to bats being 17 times more likely to be HNV seropositive (odds ratio = 17.72, $P=0.0021$, two-tailed Fisher's Exact test with zero-cell correction[24,25]). The highly statistically significant difference between seroprevalence rates in the bat-exposed (7/227, 3%) versus non-exposed groups (0/260; $P=0.0045$, Fisher's Exact test) supports the hypothesis that contact with bats increases one's risk of being infected by an antigenically related African HNV-like virus.

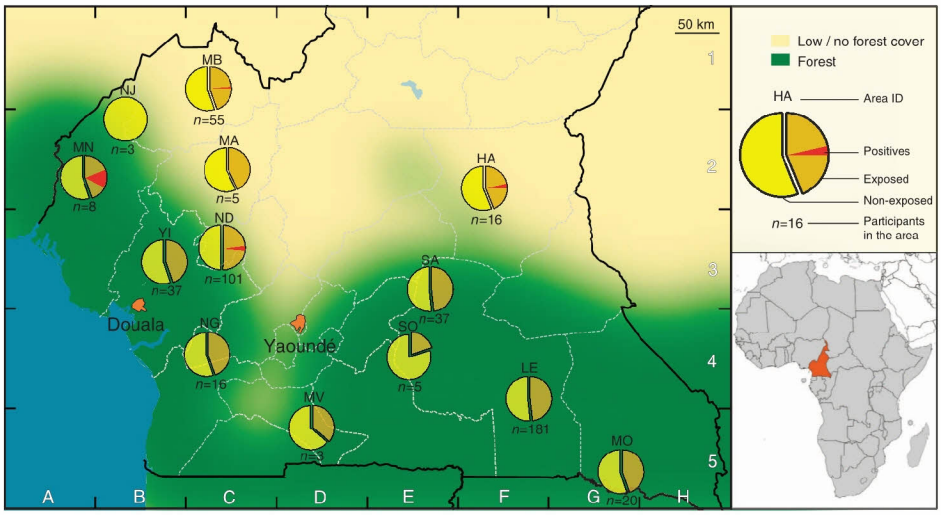

Figure 6 | Map of collection sites in Southern Cameroon. For each location, the proportion of participants with self-reported contacts with bats (exposed) or not (non-exposed) is indicated, respectively, by the brown or yellow segment of the accompanying pie-chart. The superimposed red segments in some pie-charts represent the seropositive samples identified in Fig. 4 (also Supplementary Fig. 2b). More detailed information on the samples collected from each region is presented in Supplementary Table 1. The number of participants from each location is shown below the corresponding pie chart. Green- or beige-shaded areas represent regions with high or low/no forest cover, respectively (see 'Geographical data' section in Methods for further details).

We next tried to determine whether a particular type of bat exposure was more significantly associated with seropositivity. The detailed questionnaire allowed us to segregate the tested serum samples into other dichotomous groups such as those who butchered bats (or not), those who hunted bats (or not) and those who remembered bites/scratches from bats (or not). Intriguingly, hunting bats alone was not a sufficient risk factor; however, those butchering bats were 29 times more likely to be seropositive than those not having contact with bats (7/164 (4%) versus 0/316, respectively; $P=0.0002$; Table 1). Although there was no statistically significant association with gender, it is interesting to note that with the exception of one 85-year-old case there were no seropositives above the age of 45 years (Supplementary Fig. 5).

Finally, we examined environmental and geographic parameters. Figure 6 shows a map of the indicated areas of our sample collection sites, and Supplementary Table 1 lists the map coordinates of the indicated areas shown in Fig. 6, for example, ND is in grid C3. The majority of the seropositive participants were from the ND area (4/7), whereas the others were from MB (C1), MN (A2) and HA (F2) areas. Ground reporting from Global Viral/Metabiota staff at the time of collection revealed that seropositive samples came almost invariably from low forest cover areas (Fig. 6). Indeed, Table 1 shows that seroprevalence for HNV-like viruses were significantly higher in samples originating from these four areas compared with those that did not (3.2% versus 0.3%, respectively, $P=0.0136$). In effect, those living in areas of putative deforestation were ten times more likely to be HNV-seropositive than those who were not (odds ratio = 10.1, $P=0.0088$). In addition to the seven 'true' seropositive samples classified by the Dunnett's test, there were three borderline-positive samples (~50% inhibition) in the bat-exposed group (Supplementary Fig. 2e, blue) that also came from areas associated with deforestation: ND, MN and HA (located in C3, A2 and F3, respectively, in Fig. 6 map). Within the ND area, 3 of the 4 seropositive samples were from the same village, and only 12 persons from this village with known bat contacts volunteered for this study, which would have resulted in a 25% seroprevalence rate among the bat-exposed group if this village were considered alone.

Discussion

In this study, we provide multiple lines of evidence that suggest HNV-like spillover events from its natural bat reservoir into the human population in southern Cameroon. Using our optimized pseudotyped virus SN assay as a primary screen for almost 500 human serum samples, we confidently identified at least seven HNV-seropositive samples based on stringent statistical criteria, internal specificity controls for both the virus and the sera, and follow-up confirmation with live recombinant NiV (rNiV-GLuc) or SeV, a paramyxovirus from a different genus. Together, these precautions enabled the risk factor analysis that revealed highly significant associations of seropositivity with the behavioural and environmental parameters that are known to facilitate zoonotic emergence[26–29].

By combining behavioural, geographic and serological data, we can provide more conclusive results than that which would be available from inclusion of one set of data alone. For example, that seropositivity was exclusively and most significantly associated with intimate bat exposure ($P=0.0006$), such as the slaughtering of bats for bushmeat (Table 1), is more informative than results from a SN assay with perfect sensitivity and specificity unlinked to an exposure risk. As for environmental factors, all seropositive samples come from villages located near open savannah lands or areas of deforestation as documented by our local field staff. *In toto*, we provide a strong body of evidence indicating spillover of HNV-like viruses into the human population in Africa.

In recent years, several groups have detected HNV-like sequences in African wildlife (bat) and domesticated pig populations[10,14–17]. A more recent study[22] provides evidence for continent-wide panmixia of the HNV reservoir host, the common straw-coloured African fruit bat (*E. helvum*), and reports an average seroprevalance of ~42% for cross-reactive NiV-G-binding antibodies using the Luminex assay. This is close to the 48% seroprevalance rate we found in our cohort of *E. helvum* serum samples using our SN assay. However, the former study reported a much lower seroprevalance rate (~5% in Tanzania and ~15% in Ghana) when using their Luminex-based serum antibody/sEphrinB2 competition assay as a surrogate viral

neutralization test. These contrasting results suggest that our infectious NiVpp SN assay has increased sensitivity, perhaps due to the ability of our SN assay to detect neutralizing anti-F antibodies as well as neutralizing anti-G antibodies that do not compete for sEphrinB2 binding[30].

The ability of seropositive bat sera to cross-neutralize NiVpp infection suggest a close antigenic relationship between the envelope glycoproteins of NiV and that of the putative 'Cameroon' HNV strain(s). Drexler *et al.* also showed that serum from the African bat that was infected with the parental GhV (Gh-M74a) exhibited cross-reactivity with antigens expressed on NiV-infected cells[17]. Mapping the sequence of GhV-G onto the NiV-G crystal structure indicated that despite the low overall sequence identity (~25%) between the two attachment glycoproteins, the ephrinB2 receptor-binding site was relatively conserved suggesting common receptor usage between these two divergent HNVs (Fig. 1). Indeed, sEphrinB2-Fc inhibits GhVpp infection, binds to cell surface-expressed GhV-G[31,32] and immunoprecipitates GhV-G[33], suggesting that GhV is a related African HNV that also uses ephrin B2 as an entry receptor. Conservation of receptor usage is strong biological evidence that GhV is a bona fide HNV, albeit distantly related to NiV and HeV. Indeed, the ability of a virus to use highly conserved receptors has predictive value when considering the likelihood of viral emergence and cross-species spillover[28,34].

The results of our SN assay comparing seropositive Cameroon human sera samples with our hyperimmune rabbit anti-NiV serum (Fig. 4) suggest that GhV, or at least the GhV-F and/or -G, is antigenically closer to the putative HNV common ancestor than NiV or HeV. Antibodies made against a more 'ancestral' virus such as the presumptive African HNV-like virus that infected the seropositive individuals, have a greater breadth of cross-neutralization than antibodies made against a more divergent lineage isolated by genetic drift (for example, anti-NiV), which does not neutralize GhVpp well, if at all. Note that the putative 'Cameroon' HNV strains that infected and elicited the anti-NiV-X-Nabs from the seropositive individuals were not even likely to be the same strain as GhV itself, and yet elicited antibodies that cross-neutralized NiV, HeV and GhV. These results have implications for a broad-coverage vaccine strategy: the use of envelope glycoproteins from a more 'ancestral' African HNV clade could induce more potent cross-neutralizing antibodies against emerging HNV-like viral strains. The increased breadth of cross-neutralization elicited by more ancestral viral envelopes have already been documented for HIV[35] and influenza virus[36], and forms the basis of vaccine strategies to elicit broadly neutralizing antibodies.

Given the relative ubiquity of HNVs or HNV-like viral agents in Africa, the commonality of the bushmeat trade in the resource-poor areas under study, and the presence of HNV-like sequences in up to one-third of *E. helvum* sold as bushmeat in neighbouring Brazzaville, Congo[16], we surmised that the conditions were alarmingly optimal for zoonotic transmission of African HNVs from a bat reservoir host to the human population group at highest risk for such zoonoses. If we classify all 487 serum samples by those that reported butchering bats ($n = 171$) and those that did not ($n = 316$), the seroprevalence rate for anti-NiV-X-Nabs among bat butchers is ~4% (7/171, Table 1). As a reference point, HIV-1 prevalence in Cameroon was estimated to be approximately 5% by UNAIDS in 2010.

Although no human HNV encephalitis cases have ever been documented in Africa, this does not preclude the existence of outbreaks or spillovers. Considering the shortage of physicians (1/10,400 according the WHO) and the endemicity of malaria, yellow fever, typhoid fever and meningococcal meningitis, it is not surprising that an emerging encephalitic virus would remain misdiagnosed and unreported. Indeed, HNV infections have a history of being misdiagnosed: NiV was initially incorrectly identified as Japanese encephalitis virus when it first appeared in 1998 (ref. 37) and the Siliguri outbreak of NiV was originally reported as 'aberrant measles'[38]. It is also possible that some of these African HNV-like viruses are non-pathogenic, not unlike Cedar Virus, a non-pathogenic strain of HNV isolated from Australian bats[39]. In any case, the virulence of African HNV-like viruses awaits experimental confirmation. In all likelihood, the high diversity of HNV-like viruses in Africa suggests a virulence spectrum that is equally diverse.

As humans and/or their domesticated animals encroach upon the ecological niche occupied by the reservoir hosts, increased opportunities for contact with the virus reservoir also increases the risk for cross-species infection[40,41]. The destruction of natural habitats can also lead wildlife to relocate, sometimes within greater proximity to human populations[27,28,41,42]. Our field data indicate that the vast majority of seropositive participants come from the western part of the country (ND, MN, MB), where a lack of forest cover because of natural or human causes was a noted feature (C3, A2 and C1 in Fig. 6). The clustering of seropositive participants is particularly striking in a village of the ND area where 3 out of 12 (25%) participants were positive. These three participants are all young adults (25–35 years old) with documented bat butchering activities. On the other hand, none of the >200 samples from the deep tropical forest locations such as LE and MO (Fig. 6, F4 and G5, respectively) were positive even though ~50% of these samples came from the bat-exposed group.

Bat hunting in Cameroon—typically by use of firearm, nets or catapults—do not involve physical contact between bats and hunters. Consistent with these observations, hunting bats *per se* was not associated with an increased risk for seroconversion. Furthermore, none of the pig owners or hunters (that do not also butcher their catch) enrolled in our study were seropositive. Thus, superficial contact with domestic animals does not appear to increase the risk of a HNV-like infection. In contrast, that butchering bats is the most significantly associated risk factor for HNV seropositivity suggests that close contact with bodily fluids (blood, saliva, excreta) is likely required for successful cross-species transmission.

Until recently, the range of the reservoir hosts was thought to confine HNV spillovers to Asia and Australia. However, there is increasing serological and molecular evidence documenting the widespread occurrence and diversity of HNVs in Africa, mainly in African fruit bat species. Our study now provides evidence for HNV spillover into human population groups in Africa (Cameroon) at high-risk for contracting zoonoses. In the various taxonomic schemes proposed for the transitional dynamics of zoonotic pathogens[27,43], features such as the (i) prevalence and diversity of HNVs in their African reservoir hosts, (ii) the increased opportunities for zoonotic transmission provided by the bushmeat trade, (iii) the unusually broad species tropism of HNVs facilitated by the use of highly conserved receptors and (iv) the documented human-to-human transmissibility of NiV, justifiably place HNV or HNV-like viruses at or close to the penultimate stage for sustained transmission in human outbreaks. Our data warrant increased surveillance efforts to determine the frequency of similar spillover events in Africa at large, and highlights the need for international collaborations and cross-disciplinary approaches to determine the virulence spectrum of African HNVs.

Methods

Mapping the putative GhV-G-ephrin-binding interface. Based upon sequence similarity with the NiV (24%) and HeV (25%) attachment glycoproteins, the

C-terminal 430 amino acids of GhV-G are predicted to comprise a globular six-bladed β-propeller domain[44]. To predict if the GhV-G β-propeller also shares receptor-binding specificity for ephrinB2 and ephrinB3, sequence conservation between GhV-G and NiV-G was mapped onto the crystal structure of NiV-G in complex with ephrinB2 (PDB accession code 2VSM)[23]. NiV-G residues involved in ephrin binding were identified with the PISA EBI server[45] and a structure-based sequence alignment of NiV-G, HeV-G and GhV-G was calculated with ClustalW[46] and plotted with ESPript[47]. Residue conservation mapping and image rendering was performed with the programme PyMOL (http://www.pymol.org).

Bat serum samples. Blood samples were obtained from dead, wild *E. helvum* fruit bats ($n=45$) hunted by local hunters in Yaoundé, Cameroon, between 8 May 2004 and 9 June 2007 in accordance with approvals from the Cameroon Government and Johns Hopkins University IACUC approvals (FS03M221 and FS06H205). No payments were made in relation to the collection of samples to ensure no increased hunting of bats occurred as a result of this research. Dead bats were bled by cardiac puncture shortly after death with a 3 ml syringe. The blood was transferred to EDTA (plasma) or CAT Plus (serum) vaccutainer and centrifuged at 300*g*/1,300 r.p.m. for 15 min. NBS samples were obtained from *Pteropus hypomelanus* that were born and raised in captivity in the United States (a kind gift from the Brevard Zoo, Melbourne, Florida, USA). Serum samples sent to UCLA were leftovers from a routine check-up of the animals in Fall 2011, and were considered as discarded material and exempt from IACUC approval by the veterinary team at Brevard Zoo. They were not collected prospectively or specifically for this project.

Human serum samples. Participation in the study was voluntary. Description of the study, informed consent procedures and questionnaire administration were done orally in either French or English, which are widely spoken as second languages in study villages. Participants were offered compensation approximately equivalent to 1 day of work, as participation precluded farm work on that day. The study protocol was approved by the Johns Hopkins Committee for Human Research, the Cameroon National Ethics Committee and the HIV Tri-Services Secondary Review Board. In addition, a single project assurance was obtained from the Cameroonian Ministry of Health and accepted by the National Institutes of Health Office for Protection from Research Risks. The UCLA Internal Research Board (IRB) confirmed that the use of these anonymized archival serum samples did not constitute 'human subjects' research and thus no independent IRB review was required. Human blood samples were collected on site by the Global Viral/Metabiota (previously known as GVFI) team from 497 participants between 2001 and 2003 in 13 different areas around southern Cameroon. Once drawn, the blood was transferred to EDTA (plasma) or CAT Plus (serum) vaccutainer and centrifuged at 300*g*/1,300 r.p.m. for 15 min. Sera were stored at −80 °C until processing for utilization in assays.

Serum sample handling and preparation. All bat and human serum samples were handled according to proposed WHO guidelines for working safely with diagnostic field specimens[19]. Sera were first heat-inactivated at 56 °C for 30 min, and then treated with Triton X-100 under BSL-2 conditions to ensure pathogen inactivation. All procedures were approved by the UCLA Institutional Biosafety Committee.

Vesicular stomatitis virus-based pseudoparticles. VSVpp were produced following established protocols[20]. Briefly, recombinant VSV with a *Renilla* luciferase reporter gene engineered in place of its native envelope glycoprotein (VSV-ΔG-rLuc) was pseudotyped with either its own G protein (**VSV-Gpp**), or the F and G envelope glycoproteins of NiV (**NiVpp**; Genbank accession codes NC_002728.1, GI:13559808), HeV (**HeVpp**; Genbank accession codes: NC_001906.3, GI:529283690), or the newly described African HNV from Ghana (**GhVpp**; clone Gh-M74a[17], Genbank accession codes: HQ660129.1, GI:384476032). Pseudotyping was accomplished by transfecting 293T cells with codon-optimized expression plasmids for the F and G envelope glycoproteins of NiV, HeV, and GhV, or for the VSV-G glycoprotein itself, and then infecting with VSV-ΔG-rLuc (complemented with VSV-G). Twenty-four hours after infection, pseudotype-containing media were clarified of cell debris by centrifugation at 1,500 r.p.m. for 5 min. Supernatants were then loaded on a 20% sucrose cushion and ultra-centrifugated for 2 h at 110,000*g*. The pellet of concentrated pseudoparticles was then resuspended in Opti-MEM (Life Technologies), aliquoted and stored at −80 °C.

GhV-F sequence rectification. Sequence inspection and bioinformatics analysis indicated that the GhV-F sequence in Genbank (accession code AFH96010.1) is likely incorrect due to a single-nucleotide deletion near the N-terminus, which resulted in an extra-long N-terminus with no predicted signal peptide. The details, rationale and functional evidence for sequence rectification of the GhV-F gene are provided elsewhere[32].

Recombinant Sendai virus and Nipah virus. The rSeV is a modified version of RGV0 (a kind gift of Nancy McQueen), a Fushimi strain construct with F1-R strain mutations in F and M as described by Hou *et al.*[48] We inserted an eGFP reporter between the N and P genes and made further modifications to increase rescue efficiency[49].

Recombinant NiV (reference Malaysian strain, Genbank accession codes NC_002728.1, GI:13559808), rNiV-GLuc, was engineered to express secreted *Gaussia* luciferase (descriptive name: NiV_{MAL} $T7_{P\text{-}3G}$ 3'Ribozyme A-(N_GLuc-p2A-eGFP_P) as described by Yun *et al.*[50]). The *Gaussia* luciferase (GLuc) open reading frame was modified with two mutations that provide greater signal and stability, M60L and M127L (refs 51,52); these residues are called M43 and M110 in refs 51,52 due to removal of the 17-aa secretion signal peptide. Rescue of rNiV-GLuc and SN assays were performed at the UTMB Galveston National Laboratory BSL-4 laboratory.

Optimization of VSVpp SN assay. Using a reference panel of human and pig sera, we and our collaborators at the United States Centers for Disease Control, Canadian Food Inspection Agency and Merial Sanofi (Lyon, France), previously validated our NiVpp SN assay to have a specificity of 94–100%, and an equivalent or lower sensitivity when measured against a standard live NiV plaque reduction neutralization test as the gold standard[20].

For our current study, we first determined the linear dynamic range of each pseudoparticle preparation, and a fixed amount of virus within the linear range (corresponding to the luciferase reporter output of ~20,000 RLU at 24 h.p.i.) was chosen for subsequent SN assays (Supplementary Fig. 1a). Next, the optimal serum dilution to be used was determined by comparing the SN activity of a well-characterized hyperimmune rabbit sera made against Nipah virus-like particles bearing both the NiV fusion (F) and attachment (G) envelope glycoproteins[53], and its pre-immune counterpart. Significant differences were observed for dilutions between 1:100 and 1:10,000 (Supplementary Fig. 1b). Based on these data, we diluted bat and human sera 1:100 for all our SN assays. Use of high serum dilutions (> 1:20) might also mitigate putative serum-induced cytotoxicity effects that often occur at high serum concentrations, which can confound SN results[54,55]. As an additional specificity control, we used an isogenic VSV-ΔG-rLuc pseudoparticle containing the envelope glycoprotein of VSV itself (Fig. 2 and Supplementary Fig. 2). VSV is endemic to the Americas, and has not been reported in Africa since 1900 (refs 56–59), so any serum samples that show inhibition of both VSVpp and NiVpp infection was considered nonspecific or cytotoxic, and was discarded from further analysis. In all, 1 out of 45 and 10 out of 497 bat and human samples, respectively, strongly inhibited both VSVpp and NiVpp infection and were discarded from analysis.

For the valid samples, SN assays were performed in DMEM (Invitrogen) containing 1:100 dilution of sera and an optimized amount of pseudotyped virus inoculum (VSVpp, NiVpp, HeVpp, or GhVpp) that will result in ~20,000 RLU of luciferase activity at 24 h.p.i. The medium containing infectious virus and serum was transferred to a monolayer of Vero cells and incubated at 37 °C for 2 h before removal and replacement with fresh DMEM containing 10% FCS. Cells were incubated at 37 °C for another 24 h before processing for detection of *Renilla* luciferase activity according to the manufacturer's directions (Promega). SN titres were performed using identical procedures except that the viral inoculum (NiVpp) was pre-mixed with serial fivefold dilution of sera from 1:50 to 1:31,250. All infections were performed in quadruplicates.

Live virus SN assay. The relevant bat and human serum samples were also tested for neutralizing antibodies using the replication-competent recombinant paramyxoviruses (rSeV-eGFP or rNiV-GLuc) generated as described above. SN of live rSeV-eGFP and rNiV-GLuc infection was performed in an identical manner as VSVpp (the latter under BSL-4 conditions), except that rSeV-eGFP infection was detected by FACS analysis and rNiV-GLuc infection was detected by quantifying GLuc activity (BioLux *Gaussia* Luciferase Assay, New England Biolabs) in 10% (v/v) of infected cell culture supernatant at 24 h.p.i.

FCS and hyperimmune rabbit anti-NiV serum[53] were used as negative and positive controls, respectively. Additional negative controls included NHS from Los Angeles blood donors. These anonymized and de-identified blood samples were obtained on a fee-for-service basis from the Virology Core at the UCLA AIDS Institute. Core services were approved by and consistent with all IRB policies at UCLA. NBS from captive-bred bats were generously provided by Brevard Zoo, Melbourne, Florida, USA. All SN assays were performed in quadruplicates.

Geographical data. Raw geographical data were extracted from http://www.openstreetmap.org (OpenStreetMap contributors) and are available under the Open Database License (http://www.openstreetmap.org/copyright). Maps were then built and modified with JOSM, Merkaartor and Inkscape software. Forest cover and deforestation were determined by onsite collaborators and documented in field reports.

Statistical methods. SN assay results for quadruplicates were grouped for statistical analysis. Tests between groups were done using Dunnett's test, a multiple

comparison procedure for testing groups against a single control. Categorical data were tested and confidence intervals were estimated using Fisher's Exact test. The strengths of association of seropositivity with bat contact and butchering were also estimated using two-tailed Fisher's exact test. Because no exposure was observed in the unexposed, a value of 0.5 was added to all cells to allow the odds ratios to be calculated[24,25]. Statistical tests were performed using R version 2.15.1 for Mac and version 3.0.1 for GNU/Linux with the *multcomp* package. A modified R script was written to allow for use of non-integers in the Fisher's Exact test.

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Author contributions

O.P. and B.L. designed the study. O.P., S.M.B., A.P. and T.E.Y. performed the experiments. M.L., B.S.S. and N.D.W. collected and provided the Cameroonian serum samples. TTZ collected and provided captive bred bat samples. TAB performed the sequence mapping. O.P., C.M.R., P.H. and J.M. analyzed the data and performed statistical analysis. B.L., O.P., P.D., N.D.W., A.N.F. and T.A.B. wrote the manuscript. The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

Additional information

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PREDICT Preliminary Test Results

This table displays PREDICT/Tanzania sequence confirmed viral findings that have been publicly approved for release by the Government of Tanzania. Please see “http://data.predict.global” for more information.

Display All results

Filter Results

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified evening bat within the Scotoecus genus	Tanzania	04-01-2012	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY22/2006, was found in 1 Unidentified evening bat within the Scotoecus genus (Scotoecus sp.). The samples were collected in Apr 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	04-01-2012	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY22/2006, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Apr 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Little wrinkle-lipped bat	Tanzania	04-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-88, was found in 1 Little wrinkle-lipped bat (Chaerephon pumila). The samples were collected in Apr 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Little wrinkle-lipped bat	Tanzania	04-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-89, was found in 2 Little wrinkle-lipped bat (Chaerephon pumila). The samples were collected in Apr 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Little wrinkle-lipped bat	Tanzania	04-01-2012	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY22/2006, was found in 9 Little wrinkle-lipped bat (Chaerephon pumila). The samples were collected in Apr 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Little wrinkle-lipped bat	Tanzania	04-01-2012	A new Paramyxovirus, PREDICT_PMV-15, was found in 3 Little wrinkle-lipped bat (Chaerephon pumila). The samples were collected in Apr 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Multimammate rat	Tanzania	09-01-2012	A strain of the known Arenavirus, Morogoro virus, was found in 5 Multimammate rat (Mastomys sp.). The samples were collected in Sep 2012.	This is a strain of the known Arenavirus Morogoro virus (GenBank Accession no. EU914103) found previously in rodents in Tanzania. Many Arenaviruses are significant human pathogens and can cause viral hemorrhagic fevers however Morogoro virus has not yet been linked to human illness. PREDICT investigators are currently further characterizing this virus.
Multimammate rat	Tanzania	09-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-85, was found in 1 Multimammate rat (Mastomys sp.). The samples were collected in Sep 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified member of the Rodentia order	Tanzania	09-01-2012	A strain of the known Arenavirus, Morogoro virus, was found in 1 Unidentified member of the Rodentia order (Rodentia). The samples were collected in Sep 2012.	This is a strain of the known Arenavirus Morogoro virus (GenBank Accession no. EU914103) found previously in rodents in Tanzania. Many Arenaviruses are significant human pathogens and can cause viral hemorrhagic fevers however Morogoro virus has not yet been linked to human illness. PREDICT investigators are currently further characterizing this virus.
Multimammate rat	Tanzania	06-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-86, was found in 1 Multimammate rat (Mastomys sp.). The samples were collected in Jun 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	06-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-86, was found in 1 Multimammate rat (Mastomys sp.). The samples were collected in Jun 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Multimammate rat	Tanzania	06-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-84, was found in 2 Multimammate rat (Mastomys sp.). The samples were collected in Jun 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	06-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-86, was found in 8 Multimammate rat (Mastomys sp.). The samples were collected in Jun 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified soft-furred mouse within the praomys genus	Tanzania	06-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-87, was found in 1 Unidentified soft-furred mouse within the praomys genus (Praomys sp.). The samples were collected in Jun 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	06-01-2012	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-86, was found in 3 Multimammate rat (Mastomys sp.). The samples were collected in Jun 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified field mouse in the Apodemus genus	Tanzania	09-01-2012	A new Arenavirus, PREDICT_ArenaV-1, was found in 1 Unidentified field mouse in the Apodemus genus (Apodemus sp.). The samples were collected in Sep 2012.	This is a new Arenavirus found in a rodent. It is unknown if this virus could be a threat to human health however many Arenaviruses are significant human pathogens and can cause viral hemorrhagic fevers. PREDICT investigators are currently further characterizing this virus.
Multimammate rat	Tanzania	09-01-2012	A new Coronavirus, PREDICT_CoV-64, was found in 1 Multimammate rat (Mastomys sp.). The samples were collected in Sep 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.
Little wrinkle-lipped bat	Tanzania	10-01-2012	A new Paramyxovirus, PREDICT_PMV-18, was found in 2 Little wrinkle-lipped bat (Chaerephon pumila). The samples were collected in Oct 2012.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	01-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-130, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jan 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	01-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 3 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jan 2013, Sep 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	04-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 4 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified bat within the Chiroptera order	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-132, was found in 1 Unidentified bat within the chiroptera order (Chiroptera). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-131, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-133, was found in 3 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-134, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-188, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	04-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 18 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	04-01-2013	The known Influenza virus, Influenza A, was found in 2 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Apr 2013.	This is the known virus Influenza A found in bats. Influenza viruses are important pathogens in humans and animals. PREDICT investigators are currently in the process of subtyping this virus and characterizing the full genome.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified white-toothed shrew within the Crocidura genus	Tanzania	04-01-2013	A new Coronavirus, PREDICT_CoV-46, was found in 1 Unidentified white-toothed shrew within the Crocidura genus (Crocidura sp.). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	04-01-2013	A strain of the known Arenavirus, Mopeia virus, was found in 1 Multimammate rat (Mastomys natalensis). The samples were collected in Apr 2013.	This is a strain of the known Arenavirus Mopeia virus (GenBank Accession no. M33879) found previously in rodents in Tanzania. Many Arenaviruses are significant human pathogens and can cause viral hemorrhagic fevers however Mopeia virus has not yet been linked to human illness. PREDICT investigators are currently further characterizing this virus.
Multimammate rat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-115, was found in 1 Multimammate rat (Mastomys natalensis). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified guano bat within the Tadarida genus	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-191, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	04-01-2013	A new Coronavirus, PREDICT_CoV-21, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	04-01-2013	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY22/2006, was found in 7 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified guano bat within the Tadarida genus	Tanzania	04-01-2013	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY41/2006, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	04-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-115, was found in 1 Multimammate rat (Mastomys natalensis). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Long-tailed field mouse	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-116, was found in 1 Long-tailed field mouse (Apodemus sylvaticus). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Long-tailed field mouse	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-129, was found in 1 Long-tailed field mouse (Apodemus sylvaticus). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	04-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-115, was found in 1 Multimammate rat (Mastomys natalensis). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Multimammate rat	Tanzania	04-01-2013	A new Paramyxovirus, PREDICT_PMV-64, was found in 1 Multimammate rat (Mastomys natalensis). The samples were collected in Apr 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	06-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 92 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	06-01-2013	A new Paramyxovirus, PREDICT_PMV-61, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jun 2013.	This is a new paramyxovirus in a bat that is related to the henipaviruses and warrants further investigation due its proximity to Hendra and Nipah. It is unclear whether this virus currently poses a threat to human health and PREDICT investigators are continuing to characterize it.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	06-01-2013	The known Influenza virus, Influenza A, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jun 2013.	This is the known virus Influenza A found in a bat sampled in and around a hospital. Influenza viruses are important pathogens in humans and animals. PREDICT investigators are currently in the process of subtyping this virus and characterizing the full genome.
Straw-colored fruit bat	Tanzania	06-01-2013	A new Paramyxovirus, PREDICT_PMV-56, was found in 4 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	06-01-2013	A strain of the known Paramyxovirus, Eidolon helvum Paramyxovirus/TZ13, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jun 2013.	This is a strain of the known Paramyxovirus Eidelon helvum Paramyxovirus/TZ13 (GenBank Accession no. JX870901) found in bats that is related to the henipaviruses and warrants further investigation due its proximity to Hendra and Nipah. It is unclear whether this virus currently poses a threat to human health and PREDICT investigators are continuing to characterize it.
Unidentified epauletted fruit bat within the Epomophorus genus	Tanzania	06-01-2013	A strain of the known Coronavirus, Kenya bat coronavirus BtKY56/BtKY55, was found in 1 Unidentified epauletted fruit bat within the Epomophorus genus (Epomophorus sp.). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified bat within the Chiroptera order	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-160, was found in 1 Unidentified bat within the Chiroptera order (Chiroptera). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified bat within the Chiroptera order	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-161, was found in 1 Unidentified bat within the Chiroptera order (Chiroptera). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
African sheath-tailed bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-139, was found in 2 African sheath-tailed bat (Coleura afra). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
African sheath-tailed bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-52, was found in 1 African sheath-tailed bat (Coleura afra). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-176, was found in 2 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-177, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-178, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-179, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-180, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-181, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-182, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-201, was found in 3 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-202, was found in 2 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-203, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-205, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-52, was found in 4 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-54, was found in 6 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	This is new Astrovirus in a bat within the genus Mamastrovirus. There is no evidence at this time to suggest this virus poses a threat to human health, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Coronavirus, PREDICT_CoV-21, was found in 14 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Persian trident bat	Tanzania	06-01-2013	A new Coronavirus, PREDICT_CoV-63, was found in 11 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	This is a new coronavirus found in bats belonging to the alphacoronavirus genus. While divergent enough to be considered a new virus it is genetically related to the human virus Human Coronavirus NL63. NL63 is known to cause various respiratory infections particularly in children the elderly and immunocompromized and thus PREDICT investigators are further characterizing this virus.
Persian trident bat	Tanzania	06-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 3 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Persian trident bat	Tanzania	06-01-2013	A new Paramyxovirus, PREDICT_PMV-57, was found in 1 Persian trident bat (Triaenops persicus). The samples were collected in Jun 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified bat within the Chiroptera order	Tanzania	08-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-200, was found in 1 Unidentified bat within the Chiroptera order (Chiroptera). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified bat within the Chiroptera order	Tanzania	08-01-2013	A new Coronavirus, PREDICT_CoV-62, was found in 1 Unidentified bat within the Chiroptera order (Chiroptera). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified bat within the Chiroptera order	Tanzania	08-01-2013	A new Paramyxovirus, PREDICT_PMV-60, was found in 1 Unidentified bat within the Chiroptera order (Chiroptera). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	08-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-191, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified guano bat within the Tadarida genus	Tanzania	08-01-2013	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY22/2006, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	08-01-2013	A new Paramyxovirus, PREDICT_PMV-15, was found in 2 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	08-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 31 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Aug 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	08-01-2013	The known Influenza virus, Influenza A, was found in 2 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Aug 2013.	This is the known virus Influenza A found in bats. Influenza viruses are important pathogens in humans and animals. PREDICT investigators are currently in the process of subtyping this virus and characterizing the full genome.
Straw-colored fruit bat	Tanzania	08-01-2013	A strain of the known Paramyxovirus, Eidolon helvum Paramyxovirus/U6B, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Aug 2013.	This is a strain of the known virus Eidolon helvum Paramyxovirus/U6B (GenBank Accession no. JN648086) found in a bat that is related to the henipaviruses and warrants further investigation due its proximity to Hendra and Nipah. It is unclear whether this virus currently poses a threat to human health and PREDICT investigators are continuing to characterize it.

Species	Collection Location	Date of Collection	Virus	Interpretation
Multimammate rat	Tanzania	09-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-115, was found in 1 Multimammate rat (Mastomys natalensis). The samples were collected in Sep 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	09-01-2013	A new Astrovirus within the genus Mamastrovirus, PREDICT_MAstV-187, was found in 5 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Sep 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Straw-colored fruit bat	Tanzania	09-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 9 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Jan 2013, Sep 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Straw-colored fruit bat	Tanzania	09-01-2013	A strain of the known Paramyxovirus, Eidolon helvum Paramyxovirus/U6B, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Sep 2013.	This is a strain of the known virus Eidolon helvum Paramyxovirus/U6B (GenBank Accession no. JN648086) found in a bat that is related to the henipaviruses and warrants further investigation due its proximity to Hendra and Nipah. It is unclear whether this virus currently poses a threat to human health and PREDICT investigators are continuing to characterize it.
Straw-colored fruit bat	Tanzania	09-01-2013	A strain of the known Paramyxovirus, Eidolon helvum Paramyxovirus/UG23/U67N, was found in 1 Straw-colored fruit bat (Eidolon helvum). The samples were collected in Sep 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Unidentified epauletted fruit bat within the Epomophorus genus	Tanzania	09-01-2013	A strain of the known Coronavirus, Kenya bat coronavirus BtKY56/BtKY55, was found in 1 Unidentified epauletted fruit bat within the Epomophorus genus (Epomophorus sp.). The samples were collected in Sep 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	11-01-2013	A strain of the known Coronavirus, Chaerephon bat coronavirus/Kenya/KY22/2006, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Nov 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.
Unidentified guano bat within the Tadarida genus	Tanzania	11-01-2013	A strain of the known Coronavirus, Eidolon bat coronavirus/Kenya/KY24/2006, was found in 1 Unidentified guano bat within the Tadarida genus (Tadarida sp.). The samples were collected in Nov 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

Species	Collection Location	Date of Collection	Virus	Interpretation
Mauritian tomb bat	Tanzania	11-01-2013	A new Paramyxovirus, PREDICT_PMV-59, was found in 1 Mauritian tomb bat (Taphozous mauritianus). The samples were collected in Nov 2013.	There is no evidence at this time to suggest this virus poses a threat to human health.

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Comparison of intervention methods for reducing human exposure to *Mycobacterium bovis* through milk in pastoralist households of Tanzania

Annette Roug[a,*], Andres Perez[b,1], Jonna A.K. Mazet[a,1], Deana L. Clifford[a,c], Elizabeth VanWormer[a], Goodluck Paul[d], Rudovick R. Kazwala[d], Woutrina A. Smith[a]

[a] *Wildlife Health Center & One Health Institute, School of Veterinary Medicine, University of California, Davis, 1089 Veterinary Medicine Drive, Davis, CA 95616, USA*
[b] *Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 335A Animal Science Veterinary Medicine Building, 1988 Fitch Avenue, St. Paul, MN 55108, USA*
[c] *California Department of Fish and Wildlife, 1701 Nimbus Road, Suite D, Rancho Cordova, CA 95670, USA*
[d] *Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania*

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ABSTRACT

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a disease of zoonotic concern, especially in countries with no control programs in livestock and where routine pasteurization of milk is not practiced. In Tanzania, bTB is widespread in livestock and has been diagnosed in humans; however, herd bTB testing is primarily carried out for bTB-free certification in commercial dairy herds at the expense of the dairy cattle owner. For rural livestock holders, such an expense is prohibitive, and consequently there is no control of bTB in most areas. Although effective long-term solutions to control bTB in livestock are desirable, there is a need to assess the effect of preventive measures on reducing human exposure to bTB in such settings. We utilized locally relevant cattle herd characteristics and management data from the Health for Animals and Livelihood Improvement (HALI) project in south-central Tanzania to build a Reed-Frost model that compared the efficacy of alternative methods aimed at reducing the exposure of humans to infectious milk from a typical pastoralist cattle herd. During a 10-year simulation period, the model showed that boiling milk 80% of the time is necessary to obtain a reduction in liters of infectious milk approximately equivalent to what would be obtained with a standard 2-year testing and removal regimen, and that boiling milk was more effective than animal test and removal early in the time period.

In addition, even with testing and removing infected cattle, a residual risk of exposure to infectious milk remained due to imperfect sensitivity of the skin test and a continuous risk of introduction of infectious animals from other herds. The model was sensitive to changes in initial bTB prevalence but not to changes in herd size. In conclusion, continuous complimentary treatment of milk may be an effective strategy to reduce human exposure to *M. bovis*-infected milk in settings where bTB is endemic and a comprehensive bTB control program is yet to be implemented.

* Corresponding author. Tel.: +1 530 752 4167; fax: +1 530 752 3318.
E-mail addresses: aroug@ucdavis.edu, wildlifehealth@ucdavis.edu (A. Roug).
[1] These authors contributed equally.

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1. Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a zoonotic disease of global concern due to its economic impact on livestock farming and trade, its threat to wildlife conservation, and its serious effects on human health, especially in developing countries (Cosivi et al., 1998; Michel et al., 2010). People with close contact with cattle such as livestock keepers, slaughterhouse workers, and people consuming raw animal products are especially at risk for bTB infection (Grange and Yates, 1994). The oral infectious dose in humans has not been established, but generally, children and immunocompromised individuals are considered to have the highest risk of contracting bTB through milk consumption (O'Reilly et al., 1995; Ayele et al., 2004; Mfinanga et al., 2004). Bovine tuberculosis, which mainly causes extra-pulmonary lesions in people (Grange and Collins, 1987) used to be fairly common in Europe, especially in children (Grange and Yates, 1994). With the beginning of bTB control programs in cattle and routine pasteurization of milk, *M. bovis* infection has become increasingly rare in humans in developed countries (Doran et al., 2009). Zoonotic bTB continues to be of concern in the developing world where infection in cattle is widespread, pasteurization of milk is not routinely practiced, and the HIV epidemic has caused higher susceptibility to secondary infections in the population (Cosivi et al., 1998). In Tanzania, bTB occurs in cattle (Jiwa et al., 1997; Durnez et al., 2009), wildlife (Cleaveland et al., 2005; Clifford et al., 2013), and humans (Kazwala et al., 2001; Cleaveland et al., 2007), with pastoralists considered a high-risk group for contracting bTB due to their close contact with livestock and consumption of raw animal products (Mfinanga et al., 2003; Kankya et al., 2010).

Although isolation of *M. bovis* in milk was found to be low near our study area in south-central Tanzania when using culture (Kazwala et al., 1998), low rates may be explained in part by the limited sensitivity of *M. bovis* culture in milk. A recent study detected *M. bovis* DNA in milk of skin test-negative cattle from a bTB infected herd in Brazil using culture and PCR (Zarden et al., 2013), a finding that could indicate that shedding in milk is likely higher than previously thought in infected herds.

Although regular herd testing and culling of infected animals, as well as slaughter house surveillance, remain the most widely applied and long-term effective control option for bTB in developed countries, developing nations typically do not have the resources for country-wide government funded bTB control programs. For example, in Tanzania, biannual testing of dairy cattle to obtain tuberculosis and brucellosis free certification status is carried out at the expense of the dairy cattle owners. For most non-commercial livestock holders, such expense is prohibitive.

Another factor that may contribute to low testing rates outside of the commercial dairy industry is a low level of community concern about the disease, since bTB progresses slowly in livestock and does not cause high mortality. Studies have shown that awareness of possible public health implications due to infected cattle may not be widespread (Ameni and Erkihun, 2007; Kang'ethe et al., 2007; Munyeme et al., 2010). Also, livestock are of high economic and cultural value for pastoralists (Homewood et al., 2009), and so traditional disease prevention methods, such as removal of infected animals, may therefore not be perceived as acceptable. Boiling milk or simply not using milk from infected animals, which may be a practice more acceptable than culling, may reduce human exposure to *M. bovis*, as well as other zoonotic diseases such as brucellosis and Q-fever (O'Leary et al., 2006; Guatteo et al., 2007) in pastoralist communities, although it does not address the fundamental problem of disease transmission within a herd. The relative value of such temporary measures in reducing risk of bTB transmission to humans has not been examined.

Here, we used a stochastic disease modeling approach to compare the efficacy of traditional and alternative bTB prevention and control options in reducing the mean amount of infectious milk that an "average" pastoralist household within our study area in south-central Tanzania would obtain from a bTB-infected cattle herd over time. The methods evaluated included testing and removing infected animals, avoiding the use of or boiling milk from infected animals, and boiling milk from all animals at different frequencies. The sensitivity of the model to varying initial infection prevalences and herd sizes was evaluated, and the pros and cons of the different methods are discussed.

2. Materials and methods

2.1. Study area and field data

The Health for Animals and Livelihood Improvement (HALI) project in Tanzania (Mazet et al., 2009) is an international collaborative "one health" project that has been working with pastoralist, agro-pastoralist, and agriculturalist households in the Pawaga and Idodi Divisions of the Iringa District in south central Tanzania (07°19′ S to 07°36′ S and from 35°05′ E to 35°29′ E) since 2006. From 2006 to 2010, bovine tuberculosis has been detected in 18% of 102 cattle herds tested with the single comparative intradermal tuberculin test (Monaghan et al., 1994), and within infected herds the average bTB prevalence was 7.3% (range 3–12%). On a regional basis, 2% of 1350 pastoralist cattle were bTB reactors, and *M. bovis* was also cultured from eight different wildlife species (Clifford et al., 2013). Over 90% (133/145) of pastoralist households in the HALI study reported consuming milk on a daily basis, and 32/82 (39%) reported always boiling the milk before drinking (HALI project, unpublished data).

2.2. Model and assumptions

The within herd spread of bTB infection was described using a stochastic modified Reed-Frost model (Fig. 1). Animals were assumed to be in one of three mutually exclusive states, S = susceptible, Clat = latently infected, not infectious but responsive to a skin test, and Cinf = infectious and responsive to a skin test (Fig. 1). The transition between the susceptible and latently infected state was determined by the probability of effective contact (p) and calculated based on the Reed-Frost equation $C_{t+1} = S_t(1 - q^{Ct})$, where C_t is the number of infectious cases at each time step, S_t

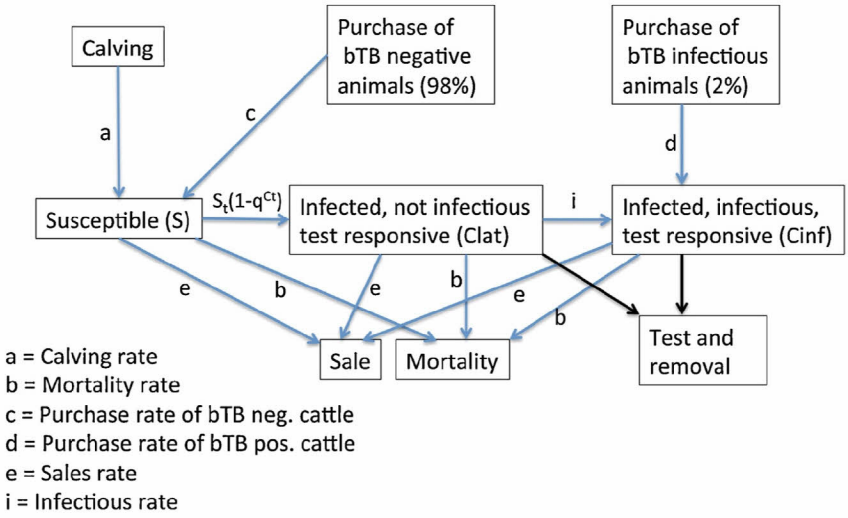

Fig. 1. Structure of the modified Reed-Frost model to evaluate different disease management approaches to reduce human exposure to bovine tuberculosis from infected cattle in rural Tanzanian pastoralist households.

is the number of susceptible animals at each time step, and $q = 1 - p$, or the probability of avoiding effective contact. The probability of effective contact (p) is dependent on the contact rate (k), or the average number of effective contacts (i.e. contacts that result in disease transmission) made by an individual in the population in a time period, calculated as $p = k/(N - 1)$, where N is herd size. A contact rate of 2–3 effective contacts/year has been found in other studies of bTB transmission in cattle in various settings (Barlow et al., 1997; Perez et al., 2002; Alvarez et al., 2012a). As area-specific contact rates were not available but presumed to be lower than in commercial systems, we therefore elected to let k follow a Pert distribution with a range between 1.5 and 2.5 and a most likely value of 2.0 contacts/year. This range produced an average within herd bTB prevalence of 6.7% and a maximum prevalence of 11%, which was consistent with field observations within infected herds. Studies have shown that cattle may remain non-infectious for 15–226 days after initial bTB infection (Neill et al., 1991, 1992; Barlow et al., 1997), though in Australian range cattle latent periods of up to 7 years were observed (Stoneham and Johnston, 1986 cited in Barlow et al., 1997). There are no Tanzania-specific data on the duration of the bTB latent period in pastoralists herds, thus for the model, we assumed that the duration of the latent period followed a Pert distribution with a most likely value of 180 days and a range of 15–360 days. The transition between the Clat and Cinf states was calculated as 1/duration of the state.

Calving rate, purchase rates, and the proportion of the herd in milk were estimated based on data collected from interviews with pastoralist households as well as field observations in the study area (Table 1). It was assumed that all calves were susceptible to bTB at birth, and that 2% of all purchased cattle were infectious, 98% were susceptible, and none of the purchased animals were latently infected. In order to keep the herd size constant, the mortality rate was set equal to the calving rate, and sales rates were set equal to the purchase rate, but sale was assumed to happen from all of the three states. Based on literature values (O'Reilly et al., 1995; Ben Kahla et al., 2011), 1–7% of infectious, lactating cows were assumed to shed *M. bovis* into milk, and shedding from infected cows were therefore assumed to follow a Pert distribution with a most likely value of 3.5% and a range 0 and 7%. The average milk production per cow per day was assumed to be approximately 1 L (Bekure et al., 1991). Local pastoralist households reported cattle herd sizes ranging from 2 to 800 cattle with a mean of 80 and a median of 40 animals.

Testing intervals were chosen based on the "standard" 2-year testing regimen applied in Tanzanian dairy herds for bTB free certification in order to understand how this "standard" regimen compared to other disease prevention methods. In addition, the effect of testing every 5 years was assessed.

De la Rua-Domenech et al. (2006) reported the sensitivity of the caudal fold and single cervical comparative skin test to vary between 63.2 and100% with a median value of 83.9%, and the sensitivity of the comparative cervical tuberculin test to vary between 52 and 100% with median values of 80% and 93.5% for standard and severe interpretations. A standard interpretation only classifies definite bTB reactors as positive, whereas a severe interpretation also classifies suspect reactors as positive. Alvarez et al. (2012b) estimated the sensitivity of the single intradermal tuberculin skin test to be 53–56.6% for the standard interpretation and 66.1–69.4% for the severe interpretation using Bayesian methods. Since bTB testing in livestock is not standardized in Tanzania, it can be expected that both the single and comparative skin test, as well as both the standard and severe interpretations, may be applied on different occasions. In order to account for this variation in the model, we assumed that the skin test sensitivity followed a Pert distribution with a range of 53–70% and a most likely value in the middle of this range at 61.5%. According to Kleeberg (1960), animals may be test non-responsive for 8–65 days after initial infection, and the OIE Manual of diagnostic tests and vaccines for terrestrial animals reports that the delayed hypersensitivity response may not develop for 3–6 weeks

Table 1
Parameters used in the modified Reed-Frost model to evaluate different approaches to reduce human exposure to bovine tuberculosis from infected cattle in rural Tanzanian households. Abbreviations used in Fig. 1 are indicated in parentheses.

Parameter	Used in model	References
Contact rate (k)	Pert distribution most likely k: 0.005479/d (range 0.004109–0.006849/d)	Barlow et al. (1997), Perez et al. (2002), Alvarez et al. (2012a)
Latent period (Clat)	Pert distribution most likely period: 180 d (range 15–360 d)	Neill et al. (1991, 1992), Barlow et al. (1997)
Infectious rate (i)	1/latent period	–
Proportion of herd in milk	20%	HALI field observations
Percent infectious shedding in milk	Pert distribution most likely proportion shedding: 3.5% (range 0–7%)	Ben Kahla et al. (2011), O'Reilly and Daborn (1995)
Average milk yield/day	1 L	Bekure et al. (1991)
Calving rate (a)	0.00234 calves per animal in the herd/d	HALI socioeconomic surveys, 2007–2009 (n = 160 households)
Mortality rate (b)	Equal calving rate to keep herd size constant	–
Purchase rate (c and d)	0.0007 cattle per animal in the herd/d	HALI socioeconomic surveys, 2007–2009 (n = 160 households)
Sales rate (e)	Equal purchase rate to keep herd size constant	–
Skin test sensitivity	Pert distribution most likely sensitivity: 61.5% (range 55–70%)	Alvarez et al. (2012b)
Proportion of purchased animals shedding *M. bovis*	2%	HALI livestock surveys, 2006–2009 (n = 102 households)

(OIE, 2009). We did not consider a period of reduced test sensitivity separately in the model and assumed that the distribution of the sensitivity parameter on average captured the uncertainty associated with the stage of the latent period. An overview of the parameters used in the model is given in Table 1. The model was coded in an Excel spread sheet (v. 14.3.6, Microsoft Corp, Redmond, WA, USA). Simulations were conducted using @Risk (v. 6, Palisade Corp., Ithaca, NY, USA) and run over 3650 days (10 years) with a time step of one day. The model outputs of means of daily proportion of infectious animals and liters of infected milk over the 10-year period were simulated using 999 iterations. This number of iterations was sufficient to achieve model convergence, which was evaluated graphically by repeating simulations with an increasing number of iterations in order to reach the point where no further changes could be detected in the output.

2.3. Scenarios modeled to reduce human exposure to M. bovis

Seven different disease prevention scenarios were evaluated using the model: (1) no control; (2) intradermal skin testing of cattle every 2 years over the 10-year period beginning on day 2 with removal of skin test reactors from the herd; (3) intradermal skin testing of cattle every 5 years over the 10-year period beginning on day 2 with removal of skin test reactors; (4) intradermal skin testing every 2 years beginning on day 2 without removal of reactor cattle from the herd, but with discontinued use of or boiling of the milk from infected animals; (5) no testing, but all milk boiled every third day (33%); (6) no testing, but all milk boiled every second day (50%); and (7) no testing, but all milk boiled 4 out of 5 days (80%). These frequencies of boiling were chosen in order to represent pastoralist households who boiled milk occasionally, approximately half the time, or most of the time. The outcome variable was the mean liters of infectious milk that were obtained from a herd on a daily basis. It was assumed that bringing the milk to a boiling point (100 °C) was sufficient to kill all infectious particles in the milk, since *Mycobacteria* are destroyed if milk is heated to 71.7 °C for 15 s (Holsinger et al., 1997).

2.4. Model equations

The model equations were formulated as follows:

2.4.1. No control applied

$$S_{t+1} = S_t - [S_t \times (1 - q^{Cinf})] + \text{herdsize}_t \times \text{calving rate} - S_t \times \text{mortality rate} + \text{herdsize}_t \times (0.98 \times \text{purchase rate}) - S_t \times \text{sales rate}$$

$$Clat_{t+1} = Clat_t + [S_t \times (1 - q^{Cinf})] - Clat_t \times \text{infectious rate} - Clat_t \times \text{mortality rate} - Clat_t \times \text{sales rate}$$

$$Cinf_{t+1} = Cinf_t + Clat_t \times \text{infectious rate} - Cinf_t \times \text{mortality rate} + \text{herdsize}_t \times (0.02 \times \text{purchase rate}) - Cinf_t \times \text{sales rate}$$

2.4.2. Testing with removal every 2 and 5 years

Two different test and removal scenarios were evaluated; test and removal every second year and every five years with the first testing on day 2 of the simulation period. All test responsive animals were assumed to leave the herd during a single time step. The equation for $Clat_{t+1}$ and $Cinf_{t+1}$ changed as follows for the time steps of testing and removal:

$$Clat_{t+1\text{-test \& remove}} = [Clat_t + [S_t \times (1 - q^{Cinf})] - Clat_t \times \text{infectious rate} - Clat_t \times \text{mortality rate} - Clat_t \times \text{Sales rate}] - \text{test sensitivity} \times [Clat_t + [S_t \times (1 - q^{Cinf})] - Clat_t \times \text{infectious rate} - Clat_t \times \text{mortality rate} - Clat_t \times \text{sales rate}]$$

$$Cinf_{t+1\text{-test \& remove}} = [Cinf_t + Clat_t \times \text{infectious rate} - Cinf_t \times \text{mortality rate} + \text{herdsize}_t \times (0.02 \times \text{purchase rate}) - Cinf_t \times \text{sales rate}] - \text{test sensitivity} \times [Cinf_t + Clat_t \times \times \text{infectious rate} - Cinf_t \times \text{mortality rate} + \text{herdsize}_t \times (0.02 \times \text{purchase rate}) - Cinf_t \times \text{sales rate}]$$

Table 2
Mean, initial (day 1), and final (day 3650) daily proportion of infectious cattle/day and liters of infected milk/day, as well as cumulative liters of infectious milk over a 10-year simulation period using 999 iterations/day of a modified Reed-Frost model, to evaluate different disease prevention options at a median herd size of 40 cattle and initial bovine tuberculosis prevalence of 2%.

	Mean	Initial	End	Cumulative
Mean daily proportion of infectious animals				
No testing	0.067	0.025	0.113	–
Test and remove positive reactors every 2 years	0.014	0.025	0.022	–
Test and remove positive reactors every 5 years	0.034	0.025	0.069	–
Mean amount of infected milk (L/day)				
No testing or boiling milk	0.019	0.007	0.031	68.030
Test and remove positive reactors every 2 years	0.004	0.007	0.006	14.719
Test and remove positive reactors every 5 years	0.009	0.007	0.019	34.451
Test every 2 years, positive reactors remain in herd but their milk is boiled or not used	0.009	0.007	0.015	31.554
No testing, but boil milk every third day (33%)	0.012	0.007	0.021	45.297
No testing, but boil milk every second day (50%)	0.009	0.007	0.016	34.316
No testing, but boil milk 4 out of 5 days (80%)	0.004	0.007	0.006	13.606

2.4.3. Daily yield of infectious milk (L/day)

Number of cattle shedding *M. bovis* in $milk_t$ = $Cinf_t$ × proportion of herd in milk × percent infectious shedding in $milk_t$

Liters of infectious $milk_t$ = average milk production per cow/day × number of cattle shedding in $milk_t$

2.4.4. Daily yield of infectious milk if testing was applied every 2 years as described above but the infectious animals not removed from the herd and the milk from the infectious animals not used or always boiled

Liters of infectious milk/day (days 3–730) = liters of infectious milk/day when no control is applied − (test sensitivity × number of cattle shedding in milk on day 2 × average milk production per cow/day)

Liters of infectious milk/day (days 731–1460) = (liters of infectious milk/day when no control is applied) − (test sensitivity × number of cattle shedding in milk on day 730 × average milk production per cow/day)

Liters of infectious milk/day (days 1461–2190) = (liters of infectious milk/day when no control is applied) − (test sensitivity × number of cattle shedding in milk on day 1460 × average milk production per cow/day)

Liters of infectious milk/day (days 2191–2920) = (liters of infectious milk/day when no control is applied) − (test sensitivity × number of cattle shedding in milk on day 2190 × average milk production per cow/day)

Liters of infectious milk/day (days 2921–3650) = (liters of infectious milk/day when no control is applied) − (test sensitivity × number of cattle shedding in milk on day 2920 × average milk production per cow/day)

2.4.5. Liters of infectious milk obtained a day on average when boiling milk every third day (33%), every second day (50%), or four out of five days (80%)

Boiling milk every third day (33%) = 0.67 × liters of infectious milk/day

Boiling milk every second day (50%) = 0.50 × liters of infectious milk/day

Boiling milk three out of four days (80%) = 0.20 × liters of infectious milk/day

Boiling milk every day (100%) = 0 × liters of infectious milk/day was not modeled since the results would always be zero, assuming that all infectious particles were killed

2.5. Model implementations

Using the local median pastoralist cattle herd size of 40 animals and regional average ~2% bTB prevalence (1 initially infectious animal), the mean, initial, and final daily proportion of infectious animals and mean daily liters of infectious milk was simulated over a 10-year period. Further, the cumulative liters of infectious milk over the 10-year period was calculated.

The linear relationship of the simulated mean daily liters of infectious milk and time were evaluated for changing initial prevalences and herd sizes. Significant differences between the mean daily liters of infectious milk with the various control options were compared for the different prevalences and herd sizes by using the Mann–Whitney *U* test. *P*-values ≤0.05 were considered significant. A sensitivity analysis was conducted evaluating the impact of variations in the input parameters on the simulated mean daily liters of infectious milk over the 10-year period for each of the 7 different control options.

3. Results

The number of infectious animals in the herd varied depending on the disease control method modeled (Table 2). If no control was applied, the number of infectious animals in a herd of 40 animals with 1 initially infectious animal increased to a maximum prevalence of 11.3% after the 10-year simulation period, with an average of 6.7%, which is consistent with the average prevalence of 7% and maximum prevalence of 12% observed in infected herds in the field. If testing and removal of animals every 2 years was applied to such a herd, the prevalence of infectious animals decreased by 12% in end of the testing period, with an mean prevalence of 1.4% over the 10 years. If testing every 5 years, the prevalence of infectious animals would increase 2.7 times by the end of the simulation period (Table 2).

Similarly, the daily liters of infectious milk varied depending on the control or treatment method. If no

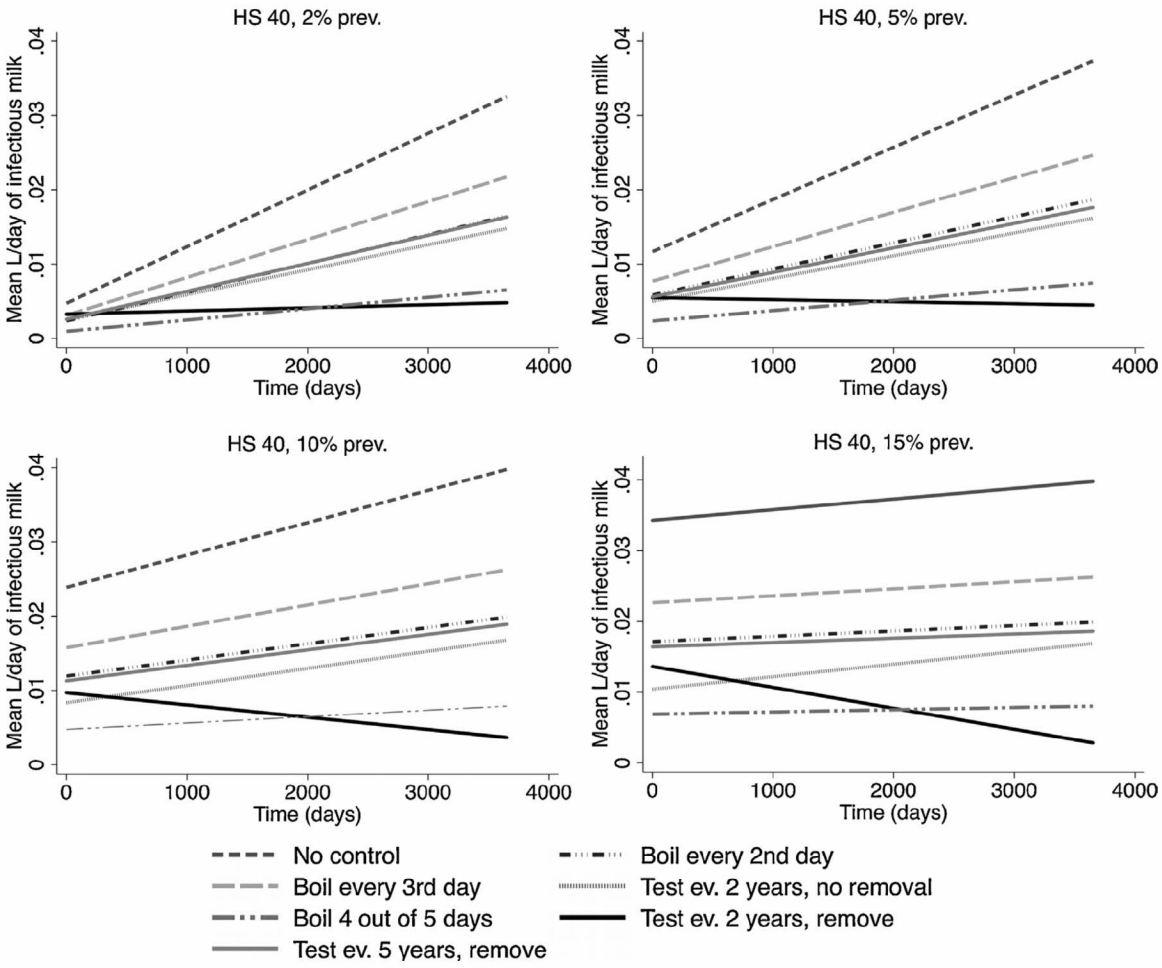

Fig. 2. Linear relationship between the mean simulated daily liters (L/d) of infectious milk obtained over a 10-year period using a modified Reed-Frost model to evaluate approaches to reduce human exposure to bovine tuberculosis from infected cattle in rural Tanzania when different prevention options are applied, and the herd size (HS) is kept constant at 40 animals while the initial herd prevalence (prev.) is varied between 2 and 15%.

control was applied, the mean daily liters of infectious milk increased 4.4 times by the end of the simulation period (Table 2). Testing every 2 years and removing animals, and boiling milk 4 out of 5 days resulted in equal mean and final daily amounts of infectious milk, and nearly equal cumulative liters of infectious milk over the 10-year period. Testing animals every 2 years without removing positive reactors but instead avoiding the use of milk from test positive animals or consistently boiling all milk from these animals resulted in a mean, and final daily amount of infectious milk and cumulative amount of infectious milk largely equivalent to what was achieved when boiling milk every second day on average, and testing and removing animals every 5 years (Table 2).

The model was sensitive to variation in the initial infection prevalence (Fig. 2). Boiling milk 4 out of 5 days resulted in a significantly lower mean amount of infectious milk/day than testing and removing infectious animals every 2 years up until day 2000 where after the test and removal regimen resulted in significantly lower daily liters of infectious milk ($P \leq 0.05$).

There was no significant difference between testing animals every 2 years but not removing them from the herd and not using the milk, boiling every second day, and testing and removing animals every 5 years at 2% initial prevalence. However, biannual testing and targeted non-use of milk became a more effective method than boiling every second day on average at 5% initial prevalence, and more effective than testing and removing animals every 5 years at 10% initial prevalence (Fig. 2). Testing and removing animals every 5 years became significantly more effective in reducing the mean daily amount of infectious milk than boiling every second day at 15% initial prevalence (Fig. 2). The model was not sensitive to changes in herd size, and the efficacy of the different disease prevention options tested in relation to each other did not differ with varying herd size.

The sensitivity analysis showed that variation in the duration of the latent period was the most influential factor in reducing the mean daily liters of infectious milk in the model, followed by the test sensitivity (Table 3). Variation in the proportion of infectious animals shedding *M. bovis*

Table 3
Sensitivity of a simulation model for *M. bovis* transmission in milk in Tanzania (as indicated by the value of the regression coefficient, β) to variation of the model input parameters (latent period, contact rate, % of infectious shedding, and test sensitivity) on the model outcome (mean daily liters of infectious milk), stratified per control scenarios, herd size (HS), and initial prevalence (%) over a 10-year simulation period.

	No control			Test every 2 years, remove infected			Test every 5 years, remove infected			Test every 2 years, no removal, treat milk		
	HS 40 2%	HS 200 2%	HS 40 15%	HS 40 2%	HS 200 2%	HS 40 15%	HS 40 2%	HS 200 2%	HS 40 15%	HS 40 2%	HS 200 2%	HS 40 15%
β, latent period	−0.65	−0.67	−0.58	−0.52	−0.52	−0.57	−0.63	−0.61	−0.61	−0.67	−0.69	−0.63
β, contact rate k	0.35	0.39	0.32	0.28	0.28	0.30	0.32	0.36	0.35	0.35	0.39	0.34
β, % infectious shedding	0.49	0.47	0.62	0.48	0.51	0.58	0.47	0.42	0.55	0.46	0.45	0.52
β, test sensitivity	–	–	–	−0.13	−0.13	−0.17	−0.10	−0.06	−0.08	−0.07	−0.07	−0.14

was the most influential factor in increasing the mean daily amount of infectious milk, followed by the contact rate. Variations in the proportion of infectious animals shedding in milk and in the test sensitivity became increasingly influential on the outcome when increasing the initial infectious animal prevalence (Table 3).

4. Discussion

Testing and removing infected animals from the herd was the only preventive measure modeled that reduced bTB prevalence in cattle over the simulation period, and consequently this method was also superior in achieving an overall reduction of the amount of infectious milk/day from the herd in the long term. Boiling milk may be an effective method for decreasing human exposure but does not address the underlying problem of continuous disease transmission among animals. Reduction of disease prevalence is a desirable goal, but it is unlikely that a test and removal regimen can be successfully implemented in resource-limited developing country settings like the study area. Livestock are highly valued by pastoralists, and, based on information from the HALI households surveyed, culling an animal due to a disease that does not cause acute symptoms is often culturally and economically unacceptable. In addition, the cost of bi-annual cattle testing may be financially unmanageable for most livestock keepers in Tanzania without any government support. For example, in 2013, the average government fee for bTB testing of cattle was approximately 7000 Tsh/animal (~4.30 USD) if including ear tags, and therefore, the estimated cost for testing a herd of 40 animals once is approximately 280,000 Tsh or 172 USD. In comparison, the average annual rural household income in Tanzania in 2007 was 28,418 Tsh (17.5 USD) (*Source*: 2007 Tanzanian National Household Budget Survey: http://www.nbs.go.tz/tnada/index.php/catalog/2), and although a household owning 40 cattle may have more resources than the average rural household, the expenditure for regular testing is clearly significant. Isolating infected cattle from the main herd could be an alternative way to prevent further transmission, but is likely not practical in most cases, as it would require multiple night enclosures (bomas) and additional labor to graze the cattle separately during the day.

Boiling milk at least 4 out of 5 days (80%) reduced the risk of human exposure to a higher level than that achieved by testing and removing positive cattle every 2 years in the first 2000 days of the 10-year period evaluated, whereas after that time animal test and removal was more effective, and the two approaches resulted in approximately equal amounts of infectious milk over the 10-year time period. Such a high level of compliance with boiling is only obtainable if the benefits are clearly understood, especially because boiled milk may be perceived as less palatable than raw milk to many pastoralists and other consumers (HALI project, unpublished data), and education about zoonotic diseases through outreach activities is therefore essential in order to create incentives to follow-through with preventive measures.

Regardless of testing regimen, we showed that preventive measures, such as boiling milk can be an important component in reducing human exposure to bTB. Even if conventional test and removal programs are employed, imperfect diagnostic tests, animal trade, herd mixing, and possibly sharing of the same pastures and water sources cause a low, but continuous risk of disease transmission within and between herds. Especially early in a control regimen, our model demonstrated that boiling substantially contributes to reducing human exposure; for example, 4 biannual test and removal events were necessary to reduce the level of exposure to an equivalent level to what could be obtained immediately by boiling milk 80% of the time.

Another fact to keep in mind when considering suitable preventive measures for bTB in the study area is the fairly low (2%) regional estimated cattle bTB prevalence, while the disease is fairly widespread (18% herd prevalence). Although different study designs make comparison to previous studies difficult, there is currently no indication that the lack of control is causing an overall increase in the cattle disease prevalence. Kazwala et al. (2001) tested cattle between 1994 and 1997 in nearby areas within the Mbeya and Iringa regions and found that 13.2% of animals were skin test reactors. Although our study area does not fully overlap with that of Kazwala et al., this data could indicate that the individual cattle bTB prevalence in the area at least has not increased, despite the lack of control programs. Given the absence of any large-scale government supported disease control programs that more effectively would reduce the bTB prevalence in livestock, preventive methods to reduce human exposure such as boiling milk, may be warranted as a primary measure of control. Further, bTB is present in multiple species of local wildlife (Clifford et al., 2013), which significantly reduces the prospect of completely controlling the disease in livestock. Given the apparently stable but endemic bTB infection levels in the study area, we recommend boiling milk to achieve

immediately effective exposure prevention in humans, and that prevention be coupled with zoonotic disease education programs at the household or community level to increase knowledge about bTB.

All control options have advantages and disadvantages, but their importance will vary depending on local conditions, and thus those conditions should be considered before specific prevention measure recommendations are made. In areas where fuel, such as firewood, is readily available, boiling milk may be incorporated into daily cooking activities without much additional effort or time investment. However, in areas where access to fuel is more limited, increased firewood use may have environmental consequences, such as deforestation, or may unacceptably increase household expenses. Further, exposure to smoke from cooking fires can increase the risk of respiratory diseases, and alternative energy sources, such as low fuel or solar powered stoves should be considered. Keeping an infected animal may have the advantage that it still can produce offspring, milk, and provide income when sold or slaughtered, however, with the great disadvantage of continuing to spread the disease within the herd, grazing area, and potentially to humans.

Limitations to the model include the lack of area-specific contact rates; that birth, mortality, sale and purchase factors were kept constant throughout the simulation; that bTB transmission from other sources, such as small ruminants and wildlife, were not specifically taken into consideration; and that other factors, such as variations in herd management between the different tribes, geographical differences, variations in breed susceptibility, seasonal forage availability, water quality, and stocking density in the night boma were not considered in the model. Further, the number of bacteria in milk could not be modeled, as the bacterial count highly depends on the severity and site of infection in an animal.

The model was sensitive to changes in input parameters, which can be explained by the wide range of the parameters. Based on comparing the output (daily liters of infectious milk) at the 5% and 95% confidence limits, the model conclusions remained the same despite the variations, except in the case of a very short latency period and high (15%) initial infection prevalence, where testing and removing animals every 2 years became significantly more effective in reducing the daily liters of infectious milk than boiling 80% of the time. However, a latency period in the extreme low end of the range can be considered exceptional and not representative of the most likely scenario in the field. The model was not sensitive to changes in herd size, likely because most parameters were already adjusted for this factor. This observation is consistent with results from bTB screening of 102 herds in the HALI study where no relationship between bTB prevalence and herd size was detected, but other studies of bTB in cattle in various production systems in Tanzania, Zambia, and Eritrea have found an association between larger herd size and higher bTB prevalence (Cook et al., 1996; Omer et al., 2001; Cleaveland et al., 2007). Future work should include quantifying the economic burden that preventive measures would impose on a household, evaluating effective approaches to zoonotic disease education, conducting sequential testing of local herds to establish area specific contact rates, and investigating the frequency and quantity of *M. bovis* shedding in milk.

5. Conclusion

Widespread control of bTB in cattle may not be achievable in Tanzania in the near future. Consequently, it is a reality that bTB-infected milk may be consumed or sold, and preventive measures for reduction of human exposure are necessary. Within our study area, the model showed that high compliance of boiling milk is required in order to achieve a reduction in infectious milk/day equivalent to what would be obtained with a standard 2-year testing regimen, but also demonstrated that significant human exposure reduction through boiling is possible without a test and slaughter regimen. In addition, even if a standard testing regimen were applied, low sensitivity of the skin test and possible disease introduction from other herds or wildlife would result in residual exposure to infectious milk. Consequently, treatment of milk to reduce zoonotic disease risk from bTB must be promoted.

Conflict of interest

None.

Acknowledgements

This research is part of the Health for Animals and Livelihood Improvement (HALI) Project, a One Health project addressing zoonotic diseases in Tanzania (http://haliproject.org). A special thanks to Michel Masozera, Mwokozi Mwanzalila, and Asha Makweta for information on HALI and Tanzania specific socioeconomic factors, and to Matt MacLachlan for reviewing the model. Funding was provided by the Global Livestock Collaborative Research Support Program by the Office of Agriculture, Bureau for Economic Growth, Agriculture and Trade, United States Agency for International Development under the terms of Grant no. PCE-G-00-98-00036-00, and the National Institutes of Health International Collaborations for Infectious Diseases Research Grant no. U01AI088679.

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From: Tracey Goldstein <tgoldstein@ucdavis.edu>
Sent: Thu, 9 Mar 2017 08:49:20 -0800
Subject: Re: SL results -- follow up
To: Andrew Clements <aclements@usaid.gov>
Cc: Jonna Mazet <jkmazet@ucdavis.edu>, David J Wolking [REDACTED], Alisa Pereira <apereira@usaid.gov>, Shana Gillette <sgillette@usaid.gov>

Hi Andrew,
Thanks for the update. Yes we also understand the president will be briefed today. We will let you know once we get confirmation on that.
Tracey

On Thu, Mar 9, 2017 at 4:19 AM, Andrew Clements <aclements@usaid.gov> wrote:

> Just heard from the mission. Embassy and USAID senior leadership are supportive of ensuring that the Ministries are in the lead in informing the President of the findings. The mission believes that the meeting with the Minister of Health and the President may happen as soon as today.

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

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Tracey Goldstein, PhD
One Health Institute
School of Veterinary Medicine
University of California
Davis, CA 95616
Phone: (530) 752-0412
Fax: (530) 752-3318
E-mail: tgoldstein@ucdavis.edu

From: "William B. Karesh" <karesh@ecohealthalliance.org>
To: Ilaria Capua <icapua@ufl.edu>
Cc: Dennis Carroll <dcarroll@usaid.gov>, Peter Daszak <daszak@ecohealthalliance.org>, "Morzaria, Subhash (TCE) ([redacted])" <[redacted]>, Jonna Mazet <jkmazet@ucdavis.edu>, Cara Chrisman <cchrisman@usaid.gov>
Subject: Re: G7
Sent: Thu, 16 Mar 2017 17:59:51 +0000

Hi Ilaria,
Dennis is really the USG Administration PoC for now. Ariel Pablo Mendez was very engaged last year, but he stepped down from his post in December. Dennis might also have a few more suggestions for people within USG.

BK

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

+1.212.380.4463 (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

On Mar 15, 2017, at 4:45 PM, Capua,Ilaria <icapua@ufl.edu> wrote:

Hello Everyone,

I am in touch with the G7 sherpa (Ambassador Raffaele Trombetta), and will be speaking to him over the next few days.
Any names of people in the US administration who can present /represent GVP are welcome.

Thanks

Ilaria

From: Jon Epstein <epstein@ecohealthalliance.org>
To: David J Wolking <djwolking@ucdavis.edu>
CC: Jonna Mazet <jkmazet@ucdavis.edu>
Sent: 3/20/2017 8:05:40 AM
Subject: Re: Animal Care and Use: Re-Training Notice

Done!

(Sorry - I thought I had done it already)
-Jon

On Fri, Mar 17, 2017 at 8:12 AM, David J Wolking <djwolking@ucdavis.edu> wrote:
Jon,

I'm renewing the IACUC this week so we need you to update your ACU 101 training, they are sure to blast us on that one :-)

Thanks!

On Thu, Dec 8, 2016 at 11:02 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

---------- Forwarded message ----------
From: <iacuc-staff@ucdavis.edu>
Date: Thu, Dec 8, 2016 at 9:35 AM
Subject: Animal Care and Use: Re-Training Notice
To: jepstein@ucdavis.edu, jkmazet@ucdavis.edu
Cc: iacuc-staff@ucdavis.edu

Dear Jon Epstein,

As you know, keeping the knowledge of investigators current regarding all aspects of the UC Davis Animal Care and Use Program is important to maintaining our AAALAC accreditation.

The UC Davis, Institutional Animal Care and Use Committee (IACUC) has decided that all personnel involved with the use of live vertebrate animals must be formally retrained about the animal care and use program at least every three years.

The course can be renewed by either taking a short, 15 question on-line exam, or taking the Animal Care and Use 101 on-line course. The following links can be used to take the on-line exam, or on-line course.

To take the Three-Year On-line Retraining Exam:

http://iacuc.ucdavis.edu/training/update_exam.cfm?ocvid=8201

If you would prefer to take the full ACU 101 course, this can now be done on-line at:

http://safetyservices.ucdavis.edu/tr/animalCareAndUseTraining

The IACUC has several policies that are designed to assist investigators in complying with the various

regulations, and in ensuring the health and welfare of animals at the University. The IACUC continues to develop and revise these policies, which may be accessed at http://safetyservices.ucdavis.edu/ps/a/IACUC/po. Investigators must be familiar with the policies that pertain to their work.

If you have questions, please contact us at (530) 752-2364.

Thanks in advance for your cooperation.

IACUC Staff

--

Jonathan H. Epstein DVM, MPH, PhD

Vice President for Science and Outreach

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4467 (direct)
1.917.385.5315 (mobile)

web: ecohealthalliance.org

Twitter:

-

From: David J Wolking <djwolking@ucdavis.edu>
To: Patrick Dawson <dawson@ecohealthalliance.org>
CC: David J Wolking <djwolking@ucdavis.edu>;predict@ucdavis.edu <predict@ucdavis.edu>;William B. Karesh <karesh@ecohealthalliance.org>;Amanda Andre <amanda.andre@ecohealthalliance.org>;Emily Hagan <hagan@ecohealthalliance.org>;Leilani Francisco <francisco@ecohealthalliance.org>
Sent: 4/10/2017 8:09:18 AM
Subject: [predict] Re: Jordan IRB Pre-Submission Materials

Patrick,

Apologies for the delay. Everything looks good, a few comments in the main protocol document for you to review (e.g., translations of docs?, use of modules from the human questionnaire?, mentions of results sharing in the protocol itself, etc....).

If you do plan to include the UCD approved master IRB with the packet like we did with Egypt then I don't think this will raise any eyebrows here.

Let me know if you have any questions,

David

On Tue, Mar 28, 2017 at 9:06 PM, Patrick Dawson <dawson@ecohealthalliance.org> wrote:
Hi David,

Thank you for your reply -- We would like to get started as soon as possible so we can fulfill Jordan's Y3 work plan of 200 individuals by September. We expect the local IRB to take approximately 3 weeks once we submit, and we would like to get started by late May/early June if at all possible. We have tentative plans to conduct trainings in Jordan later this month or during May.

Thank you so much,
Patrick

On Tue, Mar 28, 2017 at 7:14 PM, David J Wolking <djwolking@ucdavis.edu> wrote:
Thanks Patrick much appreciated. I'm buried with reporting right now. What's the timeline for Jordan submission (optimal date to get this in locally, expected review/turnaround time, and your best bet for when you'd like to launch activities?). That info will help me prioritize review.

Cheers,

D

On Thu, Mar 23, 2017 at 4:27 PM, Patrick Dawson <dawson@ecohealthalliance.org> wrote:
Dear David,

I am writing to submit materials for IRB pre-submission approval for Jordan. Adhering to the pre-submission checklist available on EIDITH, the following items are included:

Submission Checklist:

- [ ] 1. Submitted a bulleted list of changes made to all global document(s) to predict@ucdavis.edu.

- [ ] 2. Shared the country plan developed on the PREDICT global protocol template (using Track Changes as descried in the instructions) with predict@ucdavis.edu.

- [ ] 3. Used the most recent version of the Master Protocol documents (available at http://eidith.org/Resources/PREDICTIRBProtocols.aspx) to develop in-country materials.

- [ ] 4. Submitted English language versions of the country protocol, written consent form, verbal consent form, introductory script, and human questionnaire (Word.doc preferred) to predict@ucdavis.edu. Provided English language versions of any printed advertising materials to predict@ucdavis.edu.

- [ ] 5. Provided in-country IRB submission requirements (in copy or by web link, or if not in English via a document explaining the requirements) to predict@ucdavis.edu.

- [ ] 6. Clearly listed all Country Coordinators, Human Surveillance Coordinators, Global Leads, and key US-based staff involved in the study in the country protocol personnel list. These individuals will require CITI training.

Please let me know if anything else is needed. Thank you!

Best regards,
Patrick

--

Patrick Dawson, MPH
Research Scientist and PREDICT Country Liaison

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.646.868.4712 (direct)
1.848.888.2402 (mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

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Patrick Dawson, MPH
Research Scientist and PREDICT Country Liaison

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.646.868.4712 (direct)
1.848.888.2402 (mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

Title:

العنوان:

PREDICT Survey for Behavioral Risk Characterization and Community Surveillance

Introduction:

Emerging infectious diseases (EIDs) pose substantial threats to the health of animals, people and economies globally (Smolinski et al. 2003; Daszak et al. 2004). Zoonotic pathogens shared with wild or domestic animals account for the majority of EIDs, and viruses comprise 25-44% of these emerging and re-emerging pathogens (Jones et al. 2008; Taylor et al. 2001). Over the past decade, attempts to control deadly zoonotic viruses, like Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS) coronaviruses and highly pathogenic avian influenza viruses, have been, out of necessity, almost entirely reactionary, however, the world is poised to move beyond this costly approach.

Building on the surveillance activities to date, the PREDICT project, which is implemented in 31 countries globally, focuses surveillance activities in locations where environments and market systems are changing in ways that are conducive to the spillover of viruses from animals to people. Despite greater recognition of emerging and re-emerging zoonoses, the exact mechanisms of viral spillover and transmission from animals to humans are poorly understood (Murray and Daszak 2013). This proposed research aims to provide a better understanding of the drivers and host-pathogen dynamics, including human risk behaviors and practices, and the circumstances under which risk behaviors facilitate spillover of zoonotic viruses. This research will contribute to a better understanding of MERS-CoV illnesses experienced by people with exposure to animals.

Since the first documented cased of Middle East Respiratory Syndrome coronavirus (MERS-CoV) in 2012 (Zaki et. al. 2012) there have been more than 1,900 laboratory confirmed documented cases and more than 680 related deaths to the virus in 27 different countries according to the World Health Organization (http://www.who.int/emergencies/mers-cov/en/). The virus has ability to spread from person to person resulting in secondary cases among close contacts of those infected, including those without travel history to the Middle East. MERS-CoV and related viruses have been linked to animal hosts in recent studies. Bat species in Africa have tested positive for related MER-CoV virus (Ithete et. al. 2013) and dromedary camels in Egypt have been positive with MERS-CoV in multiple studies (Chu et. al. 2014 and Nowotny & Kolodziejek 2014). Direct transmission links from camels to humans have also been documented (Azher et.al. 2014) but lack clear understanding of transmission, epidemiological information critically needed (Kayali & Peiris, 2015). The next step is to examine the human behaviors that increase the risk of further disease transmission.

مقدمة:

Aim of work:

- To detect and characterize MERS-CoV in potential in high-risk communities;
- Identify biological, behavioral, and ecological factors influencing the risk of viral spillover, amplification, and spread;

- Determine potential targets for intervention based on high-risk human behaviors and practices that amplify disease transmission in hotspots for viral evolution, spillover, amplification, and spread.

Along with the objectives stated above, a goal is to conduct concurrent MERS-CoV surveillance in animals that is temporally and spatially aligned with the sampling of people in high-risk communities.

Plan of work:

- ***Type of study:***
  It is a cross sectional study to be conducted through September 2019.

- ***Patients and methods:***
  People living in, working in, or visiting targeted high-risk communities who have close contact with camels. High risk community members include camel market workers, traders, abattoir workers and butchers, camel farmers and quarantine workers. In high-risk community settings, we anticipate interviewing and collecting biological samples from individuals with a range of exposure to camels and bats.

  Enrolled research subjects will provide biological samples and complete a brief behavioral survey that is designed to obtain demographic information, as well as medical history and quantitative data on interactions with and exposures to animals. It is anticipated that up to 1,000 individuals will participate in the community sampling and surveillance component of the study over the course of the project.

- ***Inclusion criteria:***
  - People in high-risk communities who have close contact with camels and bats.
  - Adults (18 years of age or greater) who provide informed consent
  - Children (12 -17 years of age) with an accompanying parent or guardian who is able to provide informed consent, with assent of children 12 years or older also required
  - Pregnant women will be considered eligible

- ***Exclusion criteria:***
  - Individuals over the age of 12 years who refuse to provide informed consent
  - Adults unable to provide informed consent, including individuals with physiologically or medically induced cognitive impairments
  - Children without an accompanying parent or guardian who is able to provide informed consent, or a child 12 years or older unable or unwilling to provide assent
  - Children < 12 years of age
  - Prisoners

- ***Methods:***
  - The study will be explained to participants and written informed consent will be obtained from all subjects.

- All participants will be administered the human survey (Appendix B) to determine participant demographics and animal exposure and contact. Estimated duration of this procedure is 15-20 minutes.
- Following consent, biological samples will be collected for detection of MERS-CoV and other viruses from other viral families and can include:
  - Blood sample – maximum of 12mL in EDTA vacutainer tube
  - 2x Oral swab
  - 2x Nasal swab

Ethical aspects:

Participation in the study will be strictly voluntary and will require signed, informed consent (Appendix A). Participants will be given study information and a consent form prior to being asked to participate in this study. They will review the consent form with the research staff and will be given time to ask questions. After reviewing the consent form, study staff will explain details of the study including: why they were selected, potential risks due to their participation, how their participation is beneficial, that their participation is completely voluntary, and that they can withdraw their participation at any time. Responses will be kept strictly confidential. Measures will be taken to assure the respect, dignity and freedom of each participant. During training of research staff, we will emphasize the importance of avoiding coercion and protecting the privacy of participants.

Statistical Analysis:

Serology to characterize exposure in humans and to detect spillover:
ELISA will be used to test samples from high-risk interfaces to determine whether pathogen sharing has occurred. Serology will be used to characterize the frequency of MERS-CoV spillover within specific amplification zones, such as on farms and in markets.

Human quantitative behavioral survey:
Survey data will be complied and data will be entered into a secured database and analyzed with R Statistical Software. Risk factor analyses will be conducted.

Viral detection and discovery:
Virus detection in human samples may be performed using a combination of consensus PCR (cPCR) and high throughput sequencing (HTS) to MERS-CoV and other viral families from different sample types.

Facilities to acclaim benefits:

- Jordan University of Science and Technology
- Jordan Ministry of Health
- Jordan Ministry of Agriculture
- EcoHealth Alliance

Risks and benefits:

- Risks: This is a minimal risk study.

- Benefits: The goal is to identify animal reservoir and amplification hosts for zoonotic viruses, especially MERS-CoV, to strengthen human and animal disease surveillance system capacities and to establish collaborative One Health platforms to reduce the risk of disease spillover, amplification, and spread.

Risk benefit ratio:

Potential benefits exceed the expected risks. Data for exposure history of patients are scarce. Survey data on human behaviors and animal exposures will fill in this gap. Previous studies suggest contact with livestock, including dromedary camels may be linked to MERS-CoV spillover. Therefore, serological studies to provide evidence of viral exposure are best suited for screening high risk populations to understand the epidemiology of MERS-CoV. Serosurveys are important to establish whether camels or their products are a potential source of human infections in order to break the chain of transmission. Serosurveys in combination with human survey data will help identify specific risk factors for the spillover of MERS-CoV from camels or bats to humans.

Privacy and confidentiality of subjects:

All survey data and biological samples will be labeled with a unique alphanumeric identification code, assigned to each enrolled participant. No personal identifying information will be recorded on the sample vials or on the surveys. Biological samples will be stored in locked liquid nitrogen dry shippers and later transported to the laboratories.

Protection during data transport: All data collected on paper forms will be transported from the study site to the research institute in locked containers. Password-protected laptops or tablets will be used to collect coded data only. Personal identifying information will not be stored in electronic form. Only dedicated project password-protected laptops or tablets will be used for data collection and storage (i.e. no personal computers or tablets will be used). All data transmitted electronically will be 128-bit encrypted. Access will be restricted to trained personnel.

As described above, no identifying information will be stored with or paired with questionnaire data or biological specimens. As the data samples will be coded within the database for the lifetime of the study and the on-site data log will be stored in a secure manner, the risk of a loss of confidentiality is minimized for the study volunteers. In the case of positive results for relevant viral families, a summary report with interpreted results will be provided to Ministry of Health and local health officials as appropriate. When questionnaires are moved to the country headquarters, records will only contain coded data to ensure the safety and confidentiality of participants and will be maintained in a secure database. The only document that will link the participant with a unique ID number is the consent form, which will be stored in a locked file separately from participant data in the offices of the Country Coordinator.

References:

Daszak, P., G. Tabor, A. Kilpatrick, J. Epstein, and R. Plowright. 2004. Conservation medicine and a new agenda for emerging diseases. Annals of the New York Academy of Sciences 1-11.

Jones, K.E., N.G. Patel, M.A. Levy, A. Storeygard, D. Balk, J.L. Gittleman, and P. Daszak. 2008.

Global trends in emerging infectious diseases. Nature 451: 990–994.

Murray, K.A., and P. Daszak. 2013. Human ecology in pathogenic landscapes: two hypotheses on how land use change drives viral emergence. Current Opinion in Virology 3:79-83.

Smolinski, M.S., M.A. Hamburg, J. Lederberg (editors), and Committee on emerging microbial threats to health in the 21st Century. 2003. Microbial threats to health: emergence, detection, and response. Washington, DC: The National Academies Press.

Taylor, L.H., S.M. Latham, and M.E.J. Woolhouse. 2001. Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London B 356: 983-989.
Zaki, Ali M., et al. "Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia." New England Journal of Medicine 367.19 (2012): 1814-1820.

Ithete, Ndapewa Laudika, et al. "Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa." Emerg Infect Dis19.10 (2013): 1697-9.

Chu, D. K., et al. "MERS coronaviruses in dromedary camels, Egypt." Emerg Infect Dis 20.6 (2014): 1049-53.

Nowotny, N., and J. Kolodziejek. "Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013." Euro Surveill19.16 (2014): 20781.

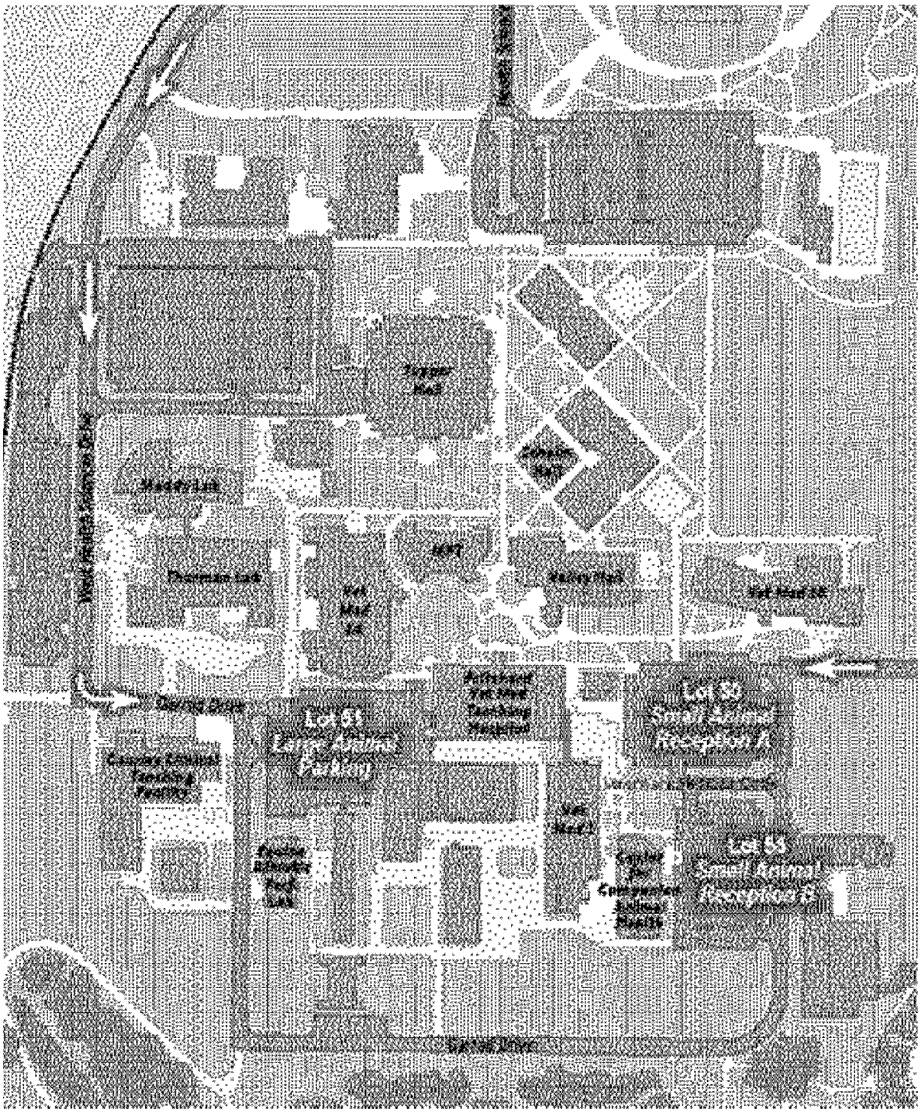
Azhar, Esam I., et al. "Evidence for camel-to-human transmission of MERS coronavirus." New England Journal of Medicine 370.26 (2014): 2499-2505.

Kayali, Ghazi, and Malik Peiris. "A more detailed picture of the epidemiology of Middle East respiratory syndrome coronavirus." The Lancet Infectious Diseases 15.5 (2015): 495-497.

From:	Elizabeth Leasure <ealeasure@ucdavis.edu>
To:	Christine Kreuder Johnson <ckjohnson@UCDAVIS.EDU>;Jonna Mazet <jkmazet@UCDAVIS.EDU>;Tracey Goldstein <tgoldstein@UCDAVIS.EDU>;David John Wolking <djwolking@UCDAVIS.EDU>;Peter Daszak <daszak@ecohealthalliance.org>;Molly Turner <turner@ecohealthalliance.org>;Kevin Olival <olival@ecohealthalliance.org>;Jon Epstein <epstein@ecohealthalliance.org>;Leilani Francisco <francisco@ecohealthalliance.org>;William B. Karesh" <karesh@ecohealthalliance.org>;Dr. Melinda Rostal" <rostal@ecohealthalliance.org>
CC:	Katherine Leasure <kaleasure@UCDAVIS.EDU>
Sent:	5/16/2017 5:00:23 PM
Subject:	EB and EHA country review meetings in rm 1023 in Valley Hall

Hi everyone. For EB and the EHA country by country review meeting tomorrow, we will be in room 1023 in Valley Hall. EB will run from 9-10 am, and the country by country meeting will begin at 10 am.

For the out-of-towners, Valley Hall is located next to the OHI offices on the western side (see map below). See you soon.



Elizabeth Leasure
One Health Institute
University of California, Davis
530-754-9034 (office)
530-304-1403 (cell)

From: Leilani Francisco <francisco@ecohealthalliance.org>
Sent: Wed, 26 Jul 2017 14:35:14 -0400
Subject: RE: behavioural surveillance
To: Jonna Mazet <jkmazet@ucdavis.edu>, "William B. Karesh" <karesh@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>
Attachment

Hi everyone,
I had a call with Amanda from the Red Cross this AM.
Please find her request attached.
It is not urgent so I can bring it up on the next EB call.
Best,
Leilani

From: Leilani Francisco [mailto:francisco@ecohealthalliance.org]
Sent: Tuesday, July 18, 2017 11:01 AM
To: 'Jonna Mazet' <jkmazet@ucdavis.edu>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>
Subject: RE: behavioural surveillance

Will do.
Best,
Leilani

From: j[redacted] **On Behalf Of** Jonna Mazet
Sent: Tuesday, July 18, 2017 10:36 AM
To: Leilani Francisco <francisco@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>
Subject: Re: behavioural surveillance

Yes, please -- see what she wants and bring questions or opportunities to EB if not urgent.
Thanks,
Jonna

On Mon, Jul 17, 2017 at 11:34 AM, Leilani Francisco <francisco@ecohealthalliance.org> wrote:
Hi Jonna,
Would you like me to reach out to Amanda?
Happy to go with your preference.
Best,
Leilani

From: Andrew Clements [mailto:aclements@usaid.gov]
Sent: Saturday, July 15, 2017 9:23 AM
To: Amanda MCCLELLAND <amanda.mcclelland@ifrc.org>
Cc: Jonna Mazet <jkmazet@ucdavis.edu>; William Karesh <Karesh@ecohealthalliance.org>; francisco@ecohealthalliance.org
Subject: Re: behavioural surveillance

Hi Amanda,

I'm copying Jonna Mazet (Predict COP), Billy Karesh (Predict liaison to other EPT partners), and Leilani Francisco (Predict behavioral surveillance lead) on your request so one of them can follow up with you.

Andrew

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health

U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov
On Jul 15, 2017, at 2:28 PM, Amanda MCCLELLAND <amanda.mcclelland@ifrc.org> wrote:

Hi Andrew
Hope you are enjoying the Geneva summer
I wanted to follow up something you mentioned a few weeks ago n the EP3 kick off meeting. You mentioned that under the Protect project there was a behavioural surveillance component. I am interested to see how we could use behavioural surveillance and join in with our mapping team to help visualise behavioural risk. Do you have any more information about the project or a contact I could discuss with?

Amanda

Amanda McClelland
Health Secuirty and Risk Management
Community and Emergency Health Unit
Health Department

International Federation of Red Cross and Red Crescent Societies
Route de Pré-Bois, 1 | 1214 Vernier | Geneva | Switzerland
Mailing address : PO Box 303 | 1211 Geneva 19 | Switzerland
Tel. +41 (0)22 730 4635| **Fax** +41 (0)22 733 0395 | **Mob.** +REDACTED
Email: amanda.mcclelland@ifrc.org| **Skype** eh_amanda.mcclelland

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From: Amanda MCCLELLAND <amanda.mcclelland@ifrc.org>
To: Leilani Francisco <francisco@ecohealthalliance.org>
Sent: 7/26/2017 11:25:46 AM
Subject: IFRC epidemic preparedness follow up

Dear Leilani,

Thank you again for the discussion today. As i mentioned we are looking to create a roster o consultants (individual or company) to support the roll out of a community and civil society focused epidemic preparedness program.

We are specifically looking for technical support to explore the idea of spatial anthropology and human geography, combined with risk mapping to inform community engagement and prevention interventions in an outbreak.

Please see the link to the current advertisement for the roster
http://reliefweb.int/job/2135774/consultantcy-epidemic-and-pandemic-preparedness-external-experts

We also plan to host a meeting to discuss the potential for this type of activity and how we would roll it out. I will send more details shortly on when this would occur.

Thank you again
amanda

From: Andrew Clements <aclements@usaid.gov>
Sent: Thu, 21 Sep 2017 10:45:05 +0200
Subject: Producer Incentives in Livestock Disease Management | OECD Free Preview | Powered by Keepeek Digital Asset Management Solution | www.keepeek.com
To: Dennis Carroll <dcarroll@usaid.gov>, Lindsay Parish <lparish@usaid.gov>, Shana Gillette <sgillette@usaid.gov>, William Karesh <Karesh@ecohealthalliance.org>, "Juan (AGAH) [redacted] Lubroth" <[redacted]>, "Subhash Morzaria (FAORAP)" <[redacted], Jonna Mazet <jkmazet@ucdavis.edu>, "Wantanee (FAORAP) Kalpravidh" <[redacted]>, Peter Black [redacted], "Daniel Schar (RDMA/OPH)" <dSchar@usaid.gov>, "Sudarat Damrongwatanapokin (RDMA/OPH)" <sDamrongwatanapokin@usaid.gov>, ajatapai@usaid.gov, daszak@ecohealthalliance.org

FYI

http://www.keepeek.com/Digital-Asset-Management/oecd/agriculture-and-food/producer-incentives-in-livestock-disease-management_9789264279483-en#.WcIUrYpx0Xo#page7

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

From: David De Pooter <d.depooter@onehealthplatform.com>
Subject: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th
Sent: Tue, 3 Oct 2017 15:23:35 +0200
Cc: Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>, John MacKenzie <J.MacKenzie@curtin.edu.au>, Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>
To: Jonna Mazet <jkmazet@ucdavis.edu>, Martyn Jeggo [REDACTED], Amadou Sall <asall@pasteur.sn>, MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>, Wang Linfa <linfa.wang@duke-nus.edu.sg>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Dr. Ottorino Cosivi" <cosivio@paho.org>, "Andrew P. Dobson" <dobber@princeton.edu>, Casey Barton Behravesh <dlx9@cdc.gov>, malik <malik@hku.hk>, Baljit Singh <baljit.singh1@ucalgary.ca>, "Gerdts, Volker" <volker.gerdts@usask.ca>, Marietjie Venter <marietjie.venter@up.ac.za>, Penina Munyua <ikg2@cdc.gov>, Lorne Babiuk <lbabiuk@ualberta.ca>, Susan Kutz <skutz@ucalgary.ca>, Patrick Leighton <patrick.a.leighton@umontreal.ca>, samuel.iverson@canada.ca, Craig Stephen <cstephen@cwhc-rcsf.ca>
0-agenda OHS 6-10-17.pdf
1-summary OHS 20-7-17.pdf
4-SPI topics and schedule.pdf
5-fellowshipfund flyer.pdf
3-OHS co-chairs.pdf
2-IOHC18 schedule.pdf
Teleconference AccessNumbers.pdf

Dear Scientific Programme Committee members,

Many thanks for responding to last week's invitation to participate in a TC on Friday 6 October 2017. Kindly find the agenda and discussion documents for this teleconference attached to this e-mail. Since not all committee members will be available to participate, I will circulate the outcome of the call to all committee members for a final round of feedback.

The call will start on Friday 6 October at 16:00 CET (10am EDT - 10pm AWST/SGT). To join the call, select the appropriate number from the attached list and enter the participant's code: 47450406#

Kindest regards,

David De Pooter
management
ONE HEALTH PLATFORM
It's all connected
d.depooter@onehealthplatform.com <mailto:d.depooter@onehealthplatform.com>
mobile: +32 479 45 74 46
www.onehealthplatform.com <https://onehealthplatform.com/>

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Kindest regards,

David De Pooter
management
ONE HEALTH PLATFORM
It's all connected
d.depooter@onehealthplatform.com
mobile: +32 479 45 74 46
www.onehealthplatform.com

One Health Science Programme Committee

Telephone conference

6 October (16:00 CET)

agenda

1. Summary OHS programme committee 20 July 2017 (enclosure 1)
2. One Health Science programme (enclosure 2)
    - Keynote lectures and confirmation of session chairs
    - Description of topics (preparation call for abstracts)
    - Involvement early career co-chairs (enclosure 3)
    - Involvement underserved communities
    - Ethics of One Health/Philosophy of One Health
3. Science Policy Interface programme on One Health Science and satellite symposia (enclosure 4)
4. Fellowship Fund
    - Promotion (enclosure 5)
    - Input Fellowship Fund Committee
5. Any other business

Suggested co-chairs One Health Science

Dr. **Peng Zhou**, Chinese Academy of Sciences (Linfa Wang)

Julie Wittrock, grad student University of Saskatchewan (Craig Stephen)
months from completing a PhD on adapting cumulative effects and population health models to redefine how we assess and define fish and wildlife health

Janine Seetahal, ministry of Agriculture Trinidad&Tobago (Craig Stephen)
Is in the final stages of her PhD and has done some very nice work related to Rabies virus in bats, population genetics of the bats themselves and also virus discovery through full genome sequencing in different bat tissues

Dr. **Nistara Randhawa** (DVM, MPVM & PhD candidate)
She is a veterinarian from India who is about to complete her PhD in epidemiology looking at ecological influence on coronavirus movements in bats and people, as well as modeling for disease spread at a fine scale using road networks and geospatial data (Jonna Mazet)

Teresia Maina, University of Saskatchewan
Senior graduate student in her last year. She comes from Kenya, her research is aimed at developing a vaccine for Contagious Bovine Pleuropneumoniae in cattle. The project is an international project with partners in Kenya, South Africa and Canada. The project in particular is addressing small farm-holders in Sub-Saharan Africa, which clearly are part of the "Underserved Communities". (Babiuk/Gerdts)

Jeff Chen, University of Saskatchewan
A young investigator with an outstanding track record in tuberculosis. He was trained in Stewart Cole's lab in Switzerland and is now in Canada. His work is focused on human and bovine tuberculosis, and very much in line with the One Health theme of the Conference. Some of his research addresses the similarities of the human and the animal form of the disease, but he also works on improving the BCG vaccines and the disease pathogenesis of tuberculosis. For example, he recently developed a novel model for tuberculosis in pigs, which provides several advantages over the currently existing rodent models and in many ways is comparable to the NHP model (e.g. reactivation of disease, latency, etc.) (Babiuk/Gerdts)

One Health Science Programme Committee

Telephone conference

20 July (17:00 CET)

Summary

- The role of the Local Organizing Committee (as opposed to the Organizing Executive Board) is to secure participation of key Canadian players (First Nations, Inuit, Métis, universities, government...) in the organization and planning of the 5th International One Health Congress. The Local Organizing Committee members will identify key players first and then suggest concrete individuals to represent their organizations in the various congress committees.

- Dr. Sam Iverson, Environment and Climate Change Canada, has joined the OHS programme committee. An updated list of committee members is attached.

- We have reached a consensus on the seven session topics for the OHS programme track. These will be advertised in a call for abstract submission in early September 2017. Abstract submission opens on 2 November 2017.

- OHS programme committee members are asked to review the proposed names of session chairs for the OHS sessions (see schedule attached). The choice of session chairs is of crucial importance as they will be asked to review submitted

abstracts (after 15 Feb 2018) and make a selection of oral and poster presentations, with special attention for submissions from resource-challenged countries.

- OHS programme committee members are also asked to propose the names of early career scientists who could act as co-chairs of the sessions. These should be promising researchers, either senior PhD students (expected to complete their dissertations within a year of the Congress) or researchers who hold a PhD or a clinical qualification for no longer than 3 years.

- It is agreed that the current concern for chronic wasting disease in elk and the uncertainty as to whether the prion is transmissible to humans will be the topic of two satellite symposia (one on the science of CWD and one associated with policy development).

- The Open Call for Congress Fellowship Applications will be launched on 1 August 2017. Promising scientists will be given a chance to apply for funding that will cover the registration fee, accommodation for the duration of the conference, and travel (economy class). Deadline for submission of applications is 15 October 2017 and as of that date, all applications will be judged by an International Evaluation Committee of One Health experts. OHS programme committee members are asked to propose the names of possible Evaluation Committee members.

- **End of document -**

SCIENCE POLICY INTERFACE
DRAFT PROGRAMME

1. **Emerging diseases in a changing world: pathogens and interface / Understanding the dynamics of emerging and re-emerging infectious diseases – *Ab Osterhaus***

    a. The socio-economic impact of emerging and re-emerging infectious diseases – *Jonathon Rushton*

2. **Expect the unexpected: understanding the drivers of emergence, and can we predict and prepare for future pandemics? - *John Mackenzie***

3. **Policy implications and development of zoonotic diseases in underserved communities – Mark Rweyemamu**

4. **Emerging and re-emerging infectious diseases: challenges for policy makers and scientists / International funding implications for emerging zoonotic and pandemic diseases.**

    a. Preventing Emerging Infectious Diseases
    b. Creation of virus Platforms (WHO Blueprints)
    c. CEPI
    d. Bill & Melinda Gates Foundation
    e. DTRA / American Defence
    f. Welcome Trust

5. **MAJOR FACTS ON AMR / all you need to know about AMR – *Surbhi Malhotra***

    a. What is AMR?
    b. Drug resistance is a global problem
    c. What causes drug resistance – what accelerates the emergence and spread of antimicrobial resistance?
    d. Inappropriate use of medicines worsens drug resistance
    e. Lack of quality medicines contributes to drug resistance
    f. Animal husbandry is a source of resistance of antibiotics
    g. Poor infection prevention and control amplifies drug resistance
    h. Weak surveillance systems contribute to the spread of drug resistance
    i. The pipeline for new tools to combat drug resistance is almost dry
    j. Environmental concerns / concerns on the environmental impact of antibiotics

6. **AMR: challenges for policy makers and scientists – *David Heymann***

a. Strengthen the knowledge : identification of knowledge gaps / development of a strategic research agenda (Joint Programming Initiative on AMR, The Hague, JPIAMR, 2013)
b. Rapid sharing of information: towards a global forum (WHO Global Report on surveillance of antimicrobial resistance, 2014)
c. Develop the economic case for sustainable investment that takes into account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other interventions
d. Adapting the regulatory framework to enable efficient pathways for drug development in the AMR area.
e. Innovation and investment in research / development of new antimicrobial medicines, vaccines and diagnostic tools
f. AMR as a core competent of professional education, training, certification in the health and veterinary sectors and agricultural practice

7. **A call to stakeholders to combat drug resistance – *Gerard Wright***

a. Tripartite collaboration FAO/WHO/OIE
b. Need for a coordinated action: National action plans on AMR
c. G20
d. Optimize the use of antimicrobial medicines in human and animal health
e. Reduction in global human consumption of antibiotics (with allowance for the need of improved access in some settings) and reduction in the volume of antibiotic use in food production and the use of medical and veterinary antimicrobial agents for applications other than human and animal health.
f. Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures
g. Alternative approaches to tackling resistant infections

Separate satellites (to be included in the main programme):

1. **Potential impact of vaccination on antibiotic usage and antibiotic resistance – "the influenza case"**
2. **National and International Disease Monitoring and Risk Assessment: Impact on Policy Development**
3. **Neglected Zoonoses with high risk impact**

Fifth International One Health Congress
22 - 25 June 2018
Saskatoon, Canada

DRAFT CONFERENCE SCHEDULE

FRIDAY 22 JUNE 2018

13:15 -14:45	SATELLITE SYMPOSIUM SLOT 1
15:00 -16:30	SATELLITE SYMPOSIUM SLOT 2
16:45 - 18:15	SATELLITE SYMPOSIUM SLOT 3 - PREMIUM
18:30 - 20:00	**OPENING CEREMONY** Welcome (Conference Chairs/UoS) WHO/OIE/FAO statement Health Minister Canada/Public Health Agency Canada Keynote lecture (invited speaker) Stanley Prusiner, University of California
20:00 - 22:00	WELCOME RECEPTION

SATURDAY 23 JUNE 2018

<table>
<tr><td>7:30 - 9:00</td><td>SATELLITE SYMPOSIUM SLOT 4</td></tr>
<tr><td>9:15 -10:15</td><td>PLENARY SESSION
“From zoonoses to pandemics: lessons learned from HIV” – Mark Feinberg, IAVI

“” - Mike Ryan, WHO</td></tr>
<tr><td>10:15 -10:45</td><td>COFFEE BREAK</td></tr>
<tr><td>10:45 -12:30</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

One Health Science</td><td>Track 3

Antimicrobial agents and resistance</td><td>Track 4

Science Policy Interface</td></tr>
<tr><td>PATHOGEN DISCOVERY

Chair: Linfa Wang
Co-chair: Peng ZHOU, Chinese Academy of Sciences</td><td>DIAGNOSTICS

(switch with surveillance & early detection?)

Chair: Martyn Jeggo</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>12:30 -14:00</td><td>LUNCH/ SATELLITE SYMPOSIUM SLOT 5 - PREMIUM</td></tr>
<tr><td>14:00 -15:45</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

One Health Science</td><td>Track 3

Antimicrobial agents and resistance</td><td>Track 4

Science Policy Interface</td></tr>
<tr><td>SURVEILLANCE AND EARLY DETECTION

Chair: Ab Osterhaus</td><td>INTERVENTION STRATEGIES

Chair: Sarah Cleaveland</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>15:45 -16:15</td><td>COFFEE BREAK</td></tr>
<tr><td>16:15 - 18:00</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

One Health Science</td><td>Track 3

Antimicrobial agents and resistance</td><td>Track 4

Science Policy Interface</td></tr>
<tr><td>SOCIAL SCIENCE AND POLITICS

Chair: David Heymann</td><td>PATHOGENESIS

Chair: Marietjie Venter</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>18:00 -19:30</td><td>SATELLITE SYMPOSIUM SLOT 6</td></tr>
</table>

SUNDAY 24 JUNE 2018

<table>
<tr><td>7:30 - 9:00</td><td>SATELLITE SYMPOSIUM SLOT 7 - COMPANY LECTURES</td></tr>
<tr><td>9:15 -10:15</td><td>PLENARY SESSION
keynote lectures (invited speakers):

"New vaccines/antimicrobials"
David Heymann, Public Health England, UK</td></tr>
<tr><td>10:15 -10:45</td><td>COFFEE BREAK</td></tr>
<tr><td>10:45 -12:30</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

One Health Science</td><td>Track 3

Antimicrobial agents and resistance</td><td>Track 4

Science Policy Interface</td></tr>
<tr><td>DRIVERS FOR EMERGING DISEASES - 1

Chair: Andy Dobson</td><td>ONE HEALTH IN UNDERPRIVILEGED COMMUNITIES

Chair: Mark Rweyemamu</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>12:30 -14:00</td><td>LUNCH/ SATELLITE SYMPOSIUM SLOT 8 - PREMIUM</td></tr>
<tr><td>14:00 -15:45</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

One Health Science</td><td>Track 3

Antimicrobial agents and resistance</td><td>Track 4

Science Policy Interface</td></tr>
<tr><td>DRIVERS FOR EMERGING DISEASES - 2

Chair: Peter Daszak</td><td>PATHOGENESIS

Chair: Scott Weaver</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>15:45 -16:15</td><td>COFFEE BREAK</td></tr>
<tr><td>16:15 -18:00</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

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Science Policy Interface</td></tr>
<tr><td>VACCINES

Chair: George Gao
Co-chair: Volker Gerdts</td><td>Infectious diseases from an ecohealth perspective

chair: Craig Stephen</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>18:00 -19:30</td><td>SATELLITE SYMPOSIUM SLOT 9</td></tr>
<tr><td>20:00 - 23:00</td><td>FAREWELL DINNER</td></tr>
</table>

MONDAY 25 JUNE 2018

<table>
<tr><td>7:30 - 9:00</td><td>SATELLITE SYMPOSIUM SLOT 10</td></tr>
<tr><td>9:00 - 10:00</td><td>PLENARY SESSION
YOUNG ONE HEALTH session (invited speakers)</td></tr>
<tr><td>10:00 -10:30</td><td>COFFEE BREAK</td></tr>
<tr><td>10:30 -12:15</td><td>PARALLEL SESSIONS
<table>
<tr><td>Track 1

One Health Science</td><td>Track 2

One Health Science</td><td>Track 3

Antimicrobial agents and resistance</td><td>Track 4

Science Policy Interface</td></tr>
<tr><td>VACCINES

chair: Lorne Babiuk</td><td>Infectious diseases from an ecohealth perspective

chair: John Mackenzie
co-chair: Jonna Mazet</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>12:15 - 13:45</td><td>PLENARY SESSION LATE BREAKERS AND CLOSING CEREMONY</td></tr>
<tr><td>13:45 -14:30</td><td>LUNCH BUFFET</td></tr>
<tr><td>14:30 - ...</td><td>GUIDED EXCURSION</td></tr>
</table>

Country	Type	PhoneNumber
Argentina	Toll	+54(0)1152184097
Argentina	Toll Free	08004443006
Australia	Toll	+61(0)290371688
Australia	Toll Free	1800180972
Austria	Toll	+43(0)19282212
Austria	Toll Free	0800006427
Bahamas	Toll Free	18003890667
Bahrain	Toll	+973(0)16199617
Belarus	Toll Free	882000110234
Belgium	Toll	+32(0)27460055 EN
Belgium	Toll	+32(0)27460033 FR
Belgium	Toll	+32(0)27460022 NL
Belgium	Toll Free	080055746 EN
Belgium	Toll Free	080033746 FR
Belgium	Toll Free	080022746 NL
Botswana	Toll Free	002698003001319
Brazil	Toll	+55(0)1131725516
Brazil	Toll Free	08007621105
Brunei Darussalam	Toll Free	8014080
Bulgaria	Toll	+359(0)24917749
Canada	Toll	+1(0)4168493425
Canada	Toll Free	8557226093
Chile	Toll	+56(0)25994947
Chile	Toll Free	12300200313
China	Toll	+86(0)4006815471
China	Toll Free	108007123190
China	Toll Free	10800120190
Colombia	Toll Free	018009157501
Croatia	Toll	+385(0)18848014
Cyprus	Toll	+357(0)22007765
Cyprus	Toll Free	80096477
Czech Republic	Toll	+420(0)228882633
Denmark	Toll	+45(0)32727718
Denmark	Toll Free	80885642
Dominican Republic	Toll	+1829(0)9467500
Dominican Republic	Toll Free	18007514907
El Salvador	Toll Free	8006841
Estonia	Toll	+372(0)6226438
Estonia	Toll Free	8000100318
Finland	Toll	+358(0)931582705
France	Toll	+33(0)170721535
France	Toll Free	0805631583
Germany	Toll	+49(0)6922221004
Germany	Toll Free	08005892946
Greece	Toll	+30(0)2111981808
Greece	Toll Free	00800128773
Hong Kong	Toll	+852(0)58081350
Hong Kong	Toll Free	800905190
Hungary	Toll	+36(0)14088390

Country	Type	PhoneNumber
Italy	Toll Free	800145650
Japan	Toll	+81(0)350501254
Japan	Toll Free	00531122503
Kazakhstan	Toll Free	88003334129
Korea, Republic of	Toll Free	00798142031006
Latvia	Toll	+371(0)67782579
Latvia	Toll Free	880031384
Lithuania	Toll	+370(0)52055222
Lithuania	Toll Free	880031384
Luxembourg	Toll	+352 278 601 22
Luxembourg	Toll Free	80023803
Malaysia	Toll Free	1800815674
Mexico	Toll	+52(0)5547772235
Mexico	Toll Free	0018553477185
Moldova, Republic of	Toll Free	080061412
Monaco	Toll Free	80093801
Netherlands	Toll	+31(0)207168275
Netherlands	Toll Free	08000201227
New Zealand	Toll	+64(0)99291745
New Zealand	Toll Free	0800452528
Norway	Toll	+47(0)21030766
Norway	Toll Free	80017157
Panama	Toll	+507(0)8366127
Panama	Toll Free	008002260006
Philippines	Toll Free	180011101230
Poland	Toll	+48(0)222120691
Poland	Toll Free	008001214291
Portugal	Toll Free	800855558
Puerto Rico	Toll Free	18557461006
Romania	Toll	+40(0)215293913
Romania	Toll Free	0800895789
Russian Federation	Toll	+7(0)4957059771
Russian Federation	Toll Free	81080028603011
Saint Kitts and Nevis	Toll Free	18003001810
Singapore	Toll	+65(0)31580344
Singapore	Toll Free	8001012581
Slovakia	Toll	+421(0)233057799
Slovakia	Toll Free	0800606534
Slovenia	Toll	+386(0)18888205
Slovenia	Toll Free	0800800911
South Africa	Toll	+27(0)110197053
South Africa	Toll Free	0800984043
Spain	Toll	+34(0)911143444
Spain	Toll Free	800600599
Sweden	Toll	+46(0)850336485
Sweden	Toll Free	0200883166
Switzerland	Toll	+41(0)445804661
Switzerland	Toll Free	0800800883
Taiwan	Toll Free	00801136320

Hungary	Toll Free	0680019506
Iceland	Toll Free	8009374
India	Toll	+91(0)2233019439
India	Toll Free	18002001127
Indonesia	Toll Free	078030110478
Ireland	Toll	+353(0)14475800
Ireland	Toll Free	1800937230
Israel	Toll	+972(0)37631152
Israel	Toll Free	1809213064
Italy	Toll	+39(0)236009806

Thailand	Toll Free	001800120667099
Trinidad and Tobago	Toll Free	18002031006
Turkey	Toll Free	00800142031006
Ukraine	Toll Free	0800500273
United Arab Emirates	Toll Free	800119000
United Kingdom	Toll	+44(0)2033645302
United Kingdom	Toll Free	08002794098
United States	Toll	+1(0)6315614572
United States	Toll Free	8553477184
Uruguay	Toll Free	00040190512
Uzbekistan	Toll Free	8108001201084
Venezuela	Toll Free	08001029643
Vietnam	Toll	+84(0)444583283
Virgin Islands, U.S.	Toll Free	18557461006

THE 5TH INTERNATIONAL

ONE HEALTH PLATFORM one health CONGRESS

Saskatoon
CANADA 22-25 June
2018 www.onehealthcongress.com

Open Call for Congress Fellowship Applications

The 5th International One Health Congress offers early career scientists a unique opportunity to present their latest work, and to meet and foster collaborations with the international One Health community in a four days high-level congress.

The Open Call for Congress Fellowship Applications has been launched on 1 August 2017. The competition is open to participants from all over the globe. One Health Fellows will receive financial support to cover the registration fee, accommodation for the duration of the conference, and travel (economy class).

Deadline for submitting your application is 15 October 2017.

If you're interested in applying for the One Health Fellowships, check the eligibility and application guidelines and download the application form from **www.onehealthcongress.com**.

The 5th International One Health Congress will be held in Saskatoon, Canada, from 22 to 25 June 2018. To capture the multifaceted One Health paradigm, the Congress will have distinct program tracks on One Health Science (zoonoses, climate change, food and water safety), antimicrobial resistance and translational science. The organizers recognize the critical role the next generation of scientists, care providers and policy makers will have in developing and implementing measures to mitigate the effects of current and future challenges to public, animal and environmental health. They have therefore created a special fund to ensure that these decision makers of tomorrow from all parts of the world can attend the Congress.

UNIVERSITY OF SASKATCHEWAN

The 5th International One Health Congress is organized by the **One Health Platform** and the **University of Saskatchewan**, in close collaboration with the **Southern African Centre for Infectious Disease Surveillance (SACIDS)**, **CDC Kenya** and **One Health Eastern & Central Africa (OHCEA)**.

OHS ONE HEALTH SCIENCE · AMR ANTIMICROBIAL AGENTS AND RESISTANCE · SPI SCIENCE POLICY INTERFACE

From: Wang Linfa <linfa.wang@duke-nus.edu.sg>
To: malik <malik@hku.hk>, David De Pooter <d.depooter@onehealthplatform.com>, Jonna Mazet <jkmazet@ucdavis.edu>, Martyn Jeggo [redacted], Amadou Sall <asall@pasteur.sn>, MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Dr. Ottorino Cosivi" <cosivio@paho.org>, "Andrew P. Dobson" <dobber@princeton.edu>, Casey Barton Behravesh <dlx9@cdc.gov>, Baljit Singh <baljit.singh1@ucalgary.ca>, "Gerdts, Volker" <volker.gerdts@usask.ca>, Marietjie Venter <marietjie.venter@up.ac.za>, Penina Munyua <ikg2@cdc.gov>, Lorne Babiuk <lbabiuk@ualberta.ca>, Susan Kutz <skutz@ucalgary.ca>, Patrick Leighton <patrick.a.leighton@umontreal.ca>, "samuel.iverson@canada.ca" <samuel.iverson@canada.ca>, Craig Stephen <cstephen@cwhc-rcsf.ca>
Cc: Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>, John MacKenzie <J.MacKenzie@curtin.edu.au>, Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>
Subject: RE: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th
Sent: Tue, 3 Oct 2017 20:26:14 +0000

Dear David,

I am traveling during that time. So apologies from me as well

Linfa

Linfa (Lin-Fa) WANG, PhD FTSE
Professor & Director
Programme in Emerging Infectious Diseases
Duke-NUS Medical School,
8 College Road, Singapore 169857
E-mail:linfa.wang@duke-nus.edu.sg
Tel: +65 6516 8397
Fax: +65 6221 2529

From: malik [mailto:malik@hku.hk]
Sent: Wednesday, 4 October, 2017 4:23 AM
To: David De Pooter; Jonna Mazet; Martyn Jeggo; Amadou Sall; MARK RWEYEMAMU; Wang Linfa; William B. Karesh; Dr. Ottorino Cosivi; Andrew P. Dobson; Casey Barton Behravesh; Baljit Singh; Gerdts, Volker; Marietjie Venter; Penina Munyua; Lorne Babiuk; Susan Kutz; Patrick Leighton; samuel.iverson@canada.ca; Craig Stephen
Cc: Ab Osterhaus; John MacKenzie; Chris Vanlangendonck
Subject: Re: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th

Dear David,

I will be on a flight at the time of the telecon. My apologies.

Malik

From: David De Pooter <d.depooter@onehealthplatform.com>
Sent: Tuesday, October 3, 2017 21:23
To: Jonna Mazet; Martyn Jeggo; Amadou Sall; MARK RWEYEMAMU; Wang Linfa; William B. Karesh; Dr. Ottorino Cosivi; Andrew P. Dobson; Casey Barton Behravesh; malik; Baljit Singh; Gerdts, Volker; Marietjie Venter; Penina Munyua; Lorne Babiuk; Susan Kutz; Patrick Leighton; samuel.iverson@canada.ca; Craig Stephen
Cc: Ab Osterhaus; John MacKenzie; Chris Vanlangendonck
Subject: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th

Dear Scientific Programme Committee members,

Many thanks for responding to last week's invitation to participate in a TC on Friday 6 October 2017. Kindly find the agenda and discussion documents for this teleconference attached to this e-mail. Since not all committee members will be available to participate, I will circulate the outcome of the call to all committee members for a final round of feedback.

The call will start on Friday 6 October at 16:00 CET (10am EDT - 10pm AWST/SGT). To join the call, select the appropriate

number from the attached list and enter the participant’s code: 47450406#

Kindest regards,

David De Pooter
management
ONE HEALTH PLATFORM
It's all connected
d.depooter@onehealthplatform.com
mobile: +32 479 45 74 46
www.onehealthplatform.com

From: Professor Martyn Jeggo <[redacted]>
Subject: Re: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th
Sent: Sat, 7 Oct 2017 07:28:10 +1000
Cc: David De Pooter <d.depooter@onehealthplatform.com>, Jonna Mazet <jkmazet@ucdavis.edu>, Amadou Sall <asall@pasteur.sn>, MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>, Wang Linfa <linfa.wang@duke-nus.edu.sg>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Dr. Ottorino Cosivi" <cosivio@paho.org>, "Andrew P. Dobson" <dobber@princeton.edu>, Casey Barton Behravesh <dlx9@cdc.gov>, malik <malik@hku.hk>, Baljit Singh <baljit.singh1@ucalgary.ca>, Marietjie Venter <marietjie.venter@up.ac.za>, Penina Munyua <ikg2@cdc.gov>, Lorne Babiuk <lbabiuk@ualberta.ca>, Susan Kutz <skutz@ucalgary.ca>, Patrick Leighton <patrick.a.leighton@umontreal.ca>, "samuel.iverson@canada.ca" <samuel.iverson@canada.ca>, Craig Stephen <cstephen@cwhc-rcsf.ca>, Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>, John MacKenzie <J.MacKenzie@curtin.edu.au>, Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>, "Misra, Vikram" <vikram.misra@usask.ca>
To: "Gerdts, Volker" <volker.gerdts@usask.ca>

Hi Volker,
I would certainly see this as an important and valuable session. Key would be to invite papers and see what level and the quality of the response. If it is good then I reckon a session is merited,

Best wishes,

Martyn

Sent from my iPhone
Professor Martyn Jeggo, GCEID
[redacted]
E mail [redacted]
Mobile [redacted]

On 6 Oct 2017, at 10:58 pm, Gerdts, Volker <volker.gerdts@usask.ca> wrote:

> Hello Scientific Programme committee,
>
> I am wondering what your thoughts are on organizing a session on "One health approaches towards control of tuberculosis". Northern Canada is seeing some of the world's highest TB rates, which in some communities are > 150/100,000. Interestingly, none of them are multidrug-resistant TB. It is a one health issue here (and around the world), with many complicating factors such as housing, nutrition, environment and underlying co-infections, as well as animal reservoirs etc. While some communities have found ways to reduce the rates to national average, other have not. I think it would be interesting to have a comparative session in which we could bring community leaders and experts from around the world to compare some of the one health approaches and challenges they are dealing with in their regions around the world. For example, how they are dealing with TB in refugees or MDR.
>
> Speakers could include individuals involved in the national or regional control programs, for example we have some excellent Medical Health Officers here working with the Northern Inter-Tribal Authority (NITHA) and local health regions to control the issue, who I have heard giving excellent talks. Other speakers could include experts from South Africa, Asia, Russia, and Europe to compare the different approaches to attacking this very important issue. Since TB remains a huge problem in underserved communities, I think such a session would fit well with the overall theme of the conference and would create interest from health care and policy providers, scientists and community representatives.
>
> I am looking forward to discussing this further in an hour at our TC.
> Thank you
>
> Volker
>
> Volker Gerdts

Associate Director Research
Vaccine and Infectious Disease Organization-InterVac
120 Veterinary Rd
Saskatoon, SK, S7N5E3 Canada
www.vido.org

From: David De Pooter <d.depooter@onehealthplatform.com>
Date: Tuesday, October 3, 2017 at 7:23 AM
To: Jonna Mazet <jkmazet@ucdavis.edu>, Martyn Jeggo <[redacted] Amadou Sall <asall@pasteur.sn>, MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>, Wang Linfa <linfa.wang@duke-nus.edu.sg>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Dr. Ottorino Cosivi" <cosivio@paho.org>, "Andrew P. Dobson" <dobber@princeton.edu>, Casey Barton Behravesh <dlx9@cdc.gov>, malik <malik@hku.hk>, Baljit Singh <baljit.singh1@ucalgary.ca>, Gerdts Volker <volker.gerdts@usask.ca>, Marietjie Venter <marietjie.venter@up.ac.za>, Penina Munyua <ikg2@cdc.gov>, Lorne Babiuk <lbabiuk@ualberta.ca>, Susan Kutz <skutz@ucalgary.ca>, Patrick Leighton <patrick.a.leighton@umontreal.ca>, "samuel.iverson@canada.ca" <samuel.iverson@canada.ca>, Craig Stephen <cstephen@cwhc-rcsf.ca>
Cc: Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>, John MacKenzie <J.MacKenzie@curtin.edu.au>, Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>
Subject: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th

Dear Scientific Programme Committee members,

Many thanks for responding to last week's invitation to participate in a TC on Friday 6 October 2017. Kindly find the agenda and discussion documents for this teleconference attached to this e-mail. Since not all committee members will be available to participate, I will circulate the outcome of the call to all committee members for a final round of feedback.

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Kindest regards,

David De Pooter
management
ONE HEALTH PLATFORM
It's all connected
d.depooter@onehealthplatform.com
mobile: +32 479 45 74 46
www.onehealthplatform.com

Sent: Wed, 15 Nov 2017 14:42:17 -0800
Subject: Re: CBEP RFI for wildlife work in Cambodia, Laos, Vietnam
From: Jonna Mazet <jkmazet@ucdavis.edu>
To: Tracey Goldstein <tgoldstein@ucdavis.edu>
Cc: "William B. Karesh" <karesh@ecohealthalliance.org>, David Wolking <djwolking@ucdavis.edu>

Don't think he works there anymore ;)
J

On Wed, Nov 15, 2017 at 2:02 PM, Tracey Goldstein <tgoldstein@ucdavis.edu> wrote:

> Yes, I think this is the one that we are a sub under MRI Global. I can check in with Joseph.
> T

On Wed, Nov 15, 2017 at 12:26 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

> Thanks! In retrospect, I think we did agree to go as a sub with somebody on this one, but Tracey knows the details.
> Appreciate it very much,
> J

On Wed, Nov 15, 2017 at 11:00 AM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

--
Tracey Goldstein, PhD
One Health Institute
School of Veterinary Medicine
University of California
Davis, CA 95616
Phone: (530) 752-0412
Fax: (530) 752-3318
E-mail: tgoldstein@ucdavis.edu

From: Dennis Carroll <dcarroll@usaid.gov>
Sent: Thu, 16 Nov 2017 13:28:22 -0500
Subject: Re: Conflict with call today
To: Brooke Watson <watson@ecohealthalliance.org>
Cc: Cara Chrisman <cchrisman@usaid.gov>, Eddy Rubin <erubin@metabiota.com>, [redacted], Jonna Mazet <jkmazet@ucdavis.edu>, Nathan Wolfe <nwolfe@metabiota.com>, Peter Daszak <daszak@ecohealthalliance.org>

Sounds like 3 works. Lets call in then. I can give you the update on my discussion with Bill S.

d

On Thu, Nov 16, 2017 at 12:44 PM, Brooke Watson <watson@ecohealthalliance.org> wrote:

Peter and I are available at 2 or 3 - he has to leave at 4 PM for a board meeting and won't be available then.

Thanks,

Brooke

On Thu, Nov 16, 2017 at 12:26 PM [redacted] wrote:

Hi everyone,

Jonna will be available at 3pm ET today, but not later in the day.

As for me, I will be available for all the times Nathan proposed.

Best,

[redacted]

From: Nathan Wolfe [mailto:nwolfe@metabiota.com]
Sent: Thursday, November 16, 2017 9:16 AM
To: Dennis Carroll <dcarroll@usaid.gov>; Jonna Mazet <jkmazet@ucdavis.edu>; Brooke Watson <watson@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; Eddy Rubin <erubin@metabiota.com>; [redacted]; Cara Chrisman <cchrisman@usaid.gov>
Subject: Re: Conflict with call today

Hi All

I'm not available at 3pm ET today, but could do 2pm or any time after 4pm ET. I'm also free tomorrow after 2pm ET tomorrow if that's helpful. If 3pm ET today is best I can get an update from Eddy (assuming he can join).

Thanks

Nathan

From: Dennis Carroll <dcarroll@usaid.gov>
Date: Thursday, November 16, 2017 at 8:54 AM
To: Jonna Mazet <jkmazet@ucdavis.edu>, Brooke Watson <watson@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>, Eddy Rubin <erubin@metabiota.com>, Nathan Wolfe <nwolfe@metabiota.com>, REDACTED, Cara Chrisman <cchrisman@usaid.gov>
Subject: Conflict with call today

All, I have a meeting with Bill Steiger to discuss funding and funding strategies for GVP at the time of our call today. Can we reschedule for later today - after 3:00 ET?

d

--

Dr. Dennis Carroll

Director, Emerging Threats Program

Bureau for Global Health

U.S. Agency for International Development

Office: 202-712-5009

Mobile: REDACTED

--

Brooke Watson, MSc
Research Scientist

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4497 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
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Dr. Dennis Carroll

Director, Emerging Threats Program
Bureau for Global Health
U.S. Agency for International Development

Office: 202-712-5009
Mobile: REDACTED

From: Dennis Carroll <dcarroll@usaid.gov>
Sent: Wed, 29 Nov 2017 11:51:43 -0500
Subject: Fwd: Towards a genomics-informed real-time global pathogen surveillance sys.._ (2).pdf
To: Eddy Rubin <erubin@metabiota.com>, Jonna Mazet <jkmazet@ucdavis.edu>, Peter Daszak <daszak@ecohealthalliance.org>, Nathan Wolfe <nwolfe@metabiota.com>, Gian Luca Burci <gian-luca.burci@graduateinstitute.ch>
Towards a genomics-informed_real-time global pathogen_surveillance sys.._(2).pdf

Interesting Review Article. Predict gets a nice shout out. Are any of you familiar with "The Global Alliance for Genomics and Health" (GH4GH). Maybe a relevant platform for data sharing.

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Dr. Dennis Carroll
Director, Emerging Threats Program
Bureau for Global Health
U.S. Agency for International Development

Office: 202-712-5009
Mobile: REDACTED

APPLICATIONS OF NEXT-GENERATION SEQUENCING

Towards a genomics-informed, real-time, global pathogen surveillance system

Jennifer L. Gardy[1,2] *and Nicholas J. Loman*[3]

Abstract | The recent Ebola and Zika epidemics demonstrate the need for the continuous surveillance, rapid diagnosis and real-time tracking of emerging infectious diseases. Fast, affordable sequencing of pathogen genomes — now a staple of the public health microbiology laboratory in well-resourced settings — can affect each of these areas. Coupling genomic diagnostics and epidemiology to innovative digital disease detection platforms raises the possibility of an open, global, digital pathogen surveillance system. When informed by a One Health approach, in which human, animal and environmental health are considered together, such a genomics-based system has profound potential to improve public health in settings lacking robust laboratory capacity.

Public health surveillance
The systematic collection, analysis and dissemination of health-related data to support planning, implementation and evaluation of public health practices and response.

Outbreaks
Outbreaks and epidemics are both defined as increases in the number of cases of a particular disease beyond what is expected in a given setting. In outbreaks, the affected settings are smaller geographic regions; epidemics can span larger areas.

[1]*British Columbia Centre for Disease Control, Vancouver, British Columbia V5Z 4R4, Canada.*
[2]*School of Population and Public Health, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.*
[3]*Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, UK.*
Correspondence to J.L.G.
jennifer.gardy@bccdc.ca

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Everything that happens twice will surely happen a third time

Paulo Coelho — *The Alchemist*

In late 2013 and early 2014, a lethal haemorrhagic fever spread throughout forested Guinea (Guinée forestière), undiagnosed for months. By the time it was reported to be Ebola, the virus had spread to three countries[1] and was likely past the point at which case-level control measures, such as isolation and infection control, could have contained the nascent outbreak. In 2015, a new dengue-like illness was implicated in a dramatic increase in Brazil's microcephaly cases; one year later, analyses revealed that the Zika virus had been sweeping through the Americas, unnoticed by existing surveillance systems, since late 2013 (REFS 2–4).

Although public health surveillance systems have evolved to meet the changing needs of our global population, we continue to dramatically underestimate our vulnerability to pathogens, both old and new[5]. Indeed, the recent events in West Africa and Brazil highlight the gaps in existing infectious disease surveillance systems, particularly when dealing with novel pathogens or pathogens whose geographic range has extended into a new region. Despite the lessons learned from previous outbreaks[6], such as the severe acute respiratory syndrome (SARS) epidemic in 2002–2003 and the 2009 influenza pandemic — particularly the need for enhanced national surveillance and diagnostic capacity — infectious threats continue to surprise and sometimes overwhelm the global health response.

The cost of these epidemics demands that we take action: with fewer than 30,000 cases, the Ebola outbreak ultimately resulted in over 11,000 deaths, left nearly 10,000 children without parents[7] and caused cumulative gross domestic product losses of more than 10%[8]. As with prior crises, in the wake of Ebola, multiple commissions have offered suggestions for essential reforms[8,9]. Most focus on systems-level change, such as funding research and development or creating a centralized pandemic preparedness and response agency. However, they also call for enhanced molecular diagnostic and surveillance capacity coupled to data-sharing frameworks. This hints at an emerging paradigm for rapid outbreak response, one that employs new tools for pathogen genome sequencing and epidemiological analysis (FIG. 1) and that can be deployed anywhere. In this model, portable, in-country genomic diagnostics are targeted to key settings for routine human, animal and environmental surveillance or rapidly deployed to a setting with a nascent outbreak. Within our increasingly digital landscape, wherein a clinical sample can be transformed into a stream of data for rapid analysis and dissemination in a matter of hours, we face a tremendous opportunity to more proactively respond to disease events. However, the potential benefits of such a system are not guaranteed, and many obstacles remain.

Here, we review recent advances in genomics-informed outbreak response, including the role of real-time sequencing in both diagnostics and epidemiology. We outline the opportunities for integrating sequencing with the One Health and digital epidemiology fields, and we examine the ethical, legal

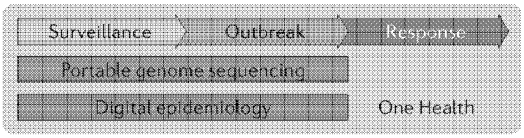

Figure 1 | **A genomics-informed surveillance and outbreak response model.** Portable genome sequencing technology and digital epidemiology platforms form the foundation for both real-time pathogen and disease surveillance systems and outbreak response efforts, all of which exist within the One Health context, in which surveillance, outbreak detection and response span the human, animal and environmental health domains.

and social issues that must be addressed if we are to move towards an era of genomics-informed pathogen surveillance.

Genomics in rapid-response diagnostics

Next-generation sequencing (NGS) platforms have recently moved from proof-of-concept studies to routine use in the clinical microbiology laboratory[10]. Most NGS services rely on bench-top instruments and sequencing from culture. However, when trying to proactively detect emerging infections or in many rapid outbreak responses, the aetiological agent behind a cluster is often unknown. Even if the agent is known, both the limited culture capacity in a field laboratory and the need for diagnostic turnaround times in hours, not days, preclude sequencing from culture. Sequencing directly from a sample using a portable sequencing platform is therefore more relevant in the field. Similarly, the need for a sequencer that can withstand being shipped and operated under rough field conditions, coupled with the need for rapid turnaround, make small, portable sequencers an attractive option.

Clinical metagenomics. With its untargeted approach to sequencing, clinical metagenomics can cross disciplines in a way that clinical microbiology struggles to — identifying viral, bacterial, fungal and other eukaryotic pathogens in a single assay[11] and coupling pathogen detection to pathogen discovery. Given the current high cost of the technique — conservatively estimated at several thousand dollars — it is most often used when dealing with potentially lethal infections that fail the conventional diagnostic paradigm, such as the recent diagnosis of an unusual case of meningoencephalitis caused by the amoeboid parasite *Balamuthia mandrillaris*[12] or the diagnosis and treatment of neuroleptospirosis in a critically unwell teenager[13]. In the latter case, despite a high index of suspicion for infection, *Leptospira santarosai* was not detected by culture or PCR, as the diagnostic primer sequences were eventually found to be a poor match to the genome of the pathogen. Intravenous antibiotic therapy resulted in rapid recovery. In such an example, the costs are easily justified, particularly when offset against the cost of a stay in an intensive treatment unit. However, routine diagnostic metagenomics is currently limited to a handful of clinical research laboratories worldwide; it is therefore regarded as a 'test of last resort' and kept in reserve for vexing diagnostic conundrums.

Substantial practical challenges hinder the adoption of metagenomics for diagnostics (FIG. 2) (reviewed in depth in REF. 11). Chief among these is analytic sensitivity, which depends on pathogen factors (for example, genome size, ease of lysis and life cycle); analytic factors (for example, the completeness of reference databases and the potential to mistake a target for a close genetic relative); and sample factors (for example, pathogen abundance within a sample and contaminating background DNA). As an example of a problematic sample, during Zika surveillance, attempts to perform untargeted metagenomics sequencing on blood yielded few, or in some cases zero, reads owing to low viral titres[14]. Target-enrichment technologies (reviewed in REF. 15) such as bait probes can be employed, but even these were unsuccessful at recovering whole Zika genomes, necessitating PCR enrichment[14]. In addition to sensitivity, universal pathogen detection through clinical metagenomics is complicated by specificity issues arising from misclassification or contaminated reagents, the challenge of reproducing results from a complex clinical workflow, nucleic acid stability under varying assay conditions, ever-changing bioinformatics workflows and cost.

Given these issues, could metagenomics replace conventional microbiological and molecular tests for infection? Recent studies have used metagenomics in common presentations, including sepsis[16], pneumonia[17], urinary tract infections[18] and eye infections[19]. These have generally yielded promising results, albeit typically at a lower sensitivity than conventional tests and at a much greater cost. Despite these problems, two factors will drive sequencing to eventually become routine clinical practice. First, the ever-decreasing cost of sequencing coupled with the potential for cost savings achieved by using a single diagnostic modality versus tens or hundreds of different diagnostic assays — each potentially requiring specific instrumentation, reagents, validation and labour — is attractive from a laboratory operations perspective. Second, and perhaps most compelling, is the additional information afforded by genomics, including the ability to predict virulence or drug resistance phenotypes, the ability to detect polymicrobial infections and phylogenetic reconstruction for outbreak analysis.

Novel technologies: portable sequencing. Given that outbreaks of emerging infectious diseases (EIDs) most often occur in settings with minimal laboratory capacity, where routine culture and bench-top sequencing are simply not feasible, the need for a portable diagnostic platform capable of *in situ* clinical metagenomics and outbreak surveillance is evident. A trend towards smaller and less expensive bench-top sequencing instruments was seen with the 454 Genome Sequencer Junior system (which has since been discontinued), the Ion Torrent Personal Genome Machine (PGM) system and the Illumina MiSeq system, which were released in close succession[20]. Each of these instruments costs <$150,000 and puts NGS capability into the hands of smaller laboratories, including clinical settings. In 2014, the MinION from Oxford Nanopore Technologies was released to early access users[21], heralding the potential

Pandemic
An epidemic that has grown to span multiple countries or continents, often with many affected individuals.

Cluster
A group of epidemiologically related cases defined by their relationship in space and time or via molecular methods.

Metagenomics
The sequencing of genetic material recovered directly from a sample, whether environmental or clinical, permitting the identification of all organisms represented in the sample.

Bait probes
Nucleic acid probes designed to recognize and capture specific DNA sequences, allowing for the enrichment of DNA from a specific organism of interest.

Emerging infectious diseases
(EIDs). Diseases that have recently appeared in a population or that have transitioned from a small number of isolated cases to many cases.

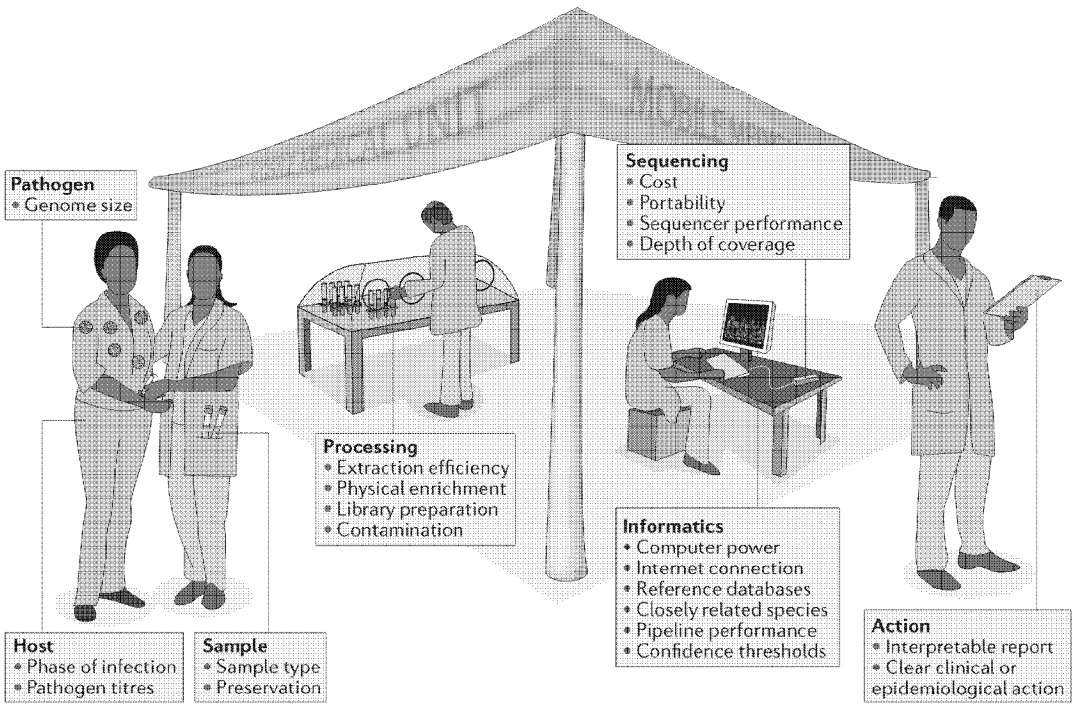

Figure 2 | **Challenges to in-field clinical metagenomics for rapid diagnosis and outbreak response.** A mobile medical unit deploying a portable clinical metagenomics platform has been established at the epicentre of an infectious disease outbreak, but the team faces challenges throughout the diagnostic process and epidemiological response. For example, in the case of Zika virus, samples, such as blood, with low viral titres, a small genome of <11 kb and transient viraemia[120] combine to complicate detection of viral nucleic acid by use of a strictly metagenomic approach. Furthermore, obtaining a sufficient amount of viral nucleic acids for genome sequencing beyond simple diagnostics requires a tiling PCR and amplicon sequencing approach[14]. Other challenges include, for example, access to a reliable Internet connection, the ability to collect sample metadata and translating genomic findings into real-time, actionable recommendations.

for highly portable 'lab-in-a-suitcase' sequencing. The MinION is pocket-sized and is controlled and powered through a laptop USB connection. It is provided under a model whereby the hardware is free but the consumer pays a premium for the reagent and flow cell consumables. Compared with bench-top instruments, the absence of a rolling service contract or regular engineer visits makes it theoretically possible to scale this platform out to potentially unlimited numbers of laboratories. Importantly, the MinION has been used in field situations, including in diagnostic tent laboratories during the Ebola epidemic[22,23] and in a roving bus-based mobile laboratory in Brazil as part of the ZiBRA project[3,24]. Others have taken the MinION to more extreme environments where even the smallest traditional bench-top sequencer could not go, including the Arctic[25] and Antarctic[26], a deep mine[27] and zero gravity aboard the reduced-gravity aircraft (nicknamed the 'Vomit Comet')[28] and the International Space Station[29].

However, this technology is not yet a panacea; remaining challenges include high DNA or RNA input requirements (currently hundreds of nanograms), which often necessitate PCR-based amplification approaches; a flow cell cost of $500, keeping the cost per sample high despite multiplexing approaches; and high error rates, which require that genomes are sequenced to high coverage for single nucleotide polymorphism-based analysis and analysed at the signal level. Moreover, although the long reads produced by the MinION overcome a number of challenges in assembling eukaryotic microbial pathogen genomes, such as the presence of discrete chromosomes or long repetitive regions, the upstream nucleic acid extraction steps required to obtain genomic DNA vary across microbial domains and might necessitate reagents and equipment far less portable than the MinION.

Genomic epidemiology

From transmission to epidemic dynamics. Genomics is capable of informing not just pathogen diagnostics but also epidemiology. Pathogen sequencing has been used for decades to understand transmission in viral outbreaks, from early studies of hantavirus in the United States of America[30] to human immunodeficiency virus (HIV) in the United Kingdom[31]; more recently, the approach has been successfully extended to include bacterial pathogens (reviewed in REF. 32) and has come to be known as genomic epidemiology, a term encompassing everything from population dynamics to the reconstruction of individual transmission events within outbreaks[32]. Most transmission-focused investigations to date have been retrospective, with only a subset unfolding in real time, as cases are diagnosed[33–37].

In transmission-focused investigations, genetic variants are used to identify person-to-person transmission

Transmission
The event through which a pathogen is transferred from one entity to another. Transmission can be person-to-person, as in the case of Ebola, vector-to-person, as with Zika, or environment-to-person via routes including food, water and contact with a contaminated object or surface.

Genomic epidemiology
The use of genome sequencing to understand infectious disease transmission and epidemiology. See FIG. 3.

events (FIG. 3), either through manual interpretation of the variants shared between outbreak cases[38] or via model-based approaches[39], with the result being a transmission network. Epidemic investigations are very different — only a subset of the epidemic cases are sequenced. Thus, the goal is to use the population structure of the pathogen to understand the overall dynamics of the epidemic. Here, phylodynamic approaches are used to infer epidemiological parameters of interest.

First conceptualized in 2004 by Grenfell *et al.* as a union of "immunodynamics, epidemiology, and evolutionary biology" (REF. 40), phylodynamics captures both epidemiological and evolutionary information from measurably evolving pathogens — those viruses and bacteria for which high mutation rates and/or a range of sampling dates contribute to a meaningful amount of genetic variation between sequences[41,42] — in other words, enough genetic diversity to be able to infer an evolutionary history for a pathogen of interest, even if that history is only over the short time frame of an outbreak or epidemic. This is possible for most pathogens, particularly single-stranded DNA viruses, RNA viruses and many bacterial species[42,43], but there are certain species for which the lack of a strict molecular clock and/or frequent recombination complicate both phylodynamics studies and attempts to infer transmission events[42].

Phylodynamics relies on tools such as Bayesian evolutionary analysis sampling trees (BEAST)[44], in which sequence data are used to build a time-labelled phylogenetic tree using a specific evolutionary process as a guide — often variations on a theme of coalescent theory[45]. From the tree, one can infer epidemiological parameters, including the basic reproductive number R_0 (REF. 46). While the insights that can be gained from genomic data alone are exciting, the utility of phylodynamic approaches is greatly extended when additional data are integrated into the models (reviewed in REF. 47).

Genomic epidemiology in action: Ebola. The many genomic epidemiology studies from the Ebola outbreak (reviewed in REF. 48) used bench-top and portable sequencing platforms to reveal outbreak-level events and epidemic-level trends. Real-time analyses published around the peak of the epidemic suggested the following: the outbreak probably arose from a single introduction into humans and not repeated zoonotic introductions[49,50]; sexual transmission had a previously unrecognized role in maintaining transmission chains[51]; and survivor transmission — another unrecognized phenomenon — contributed to disease flare-ups later in the outbreak[52]. The first sequencing efforts, all of which had an effect on the epidemiological response in real time, unfolded months into the epidemic. Had they been deployed earlier, we can only speculate as to their potential impact. Arguably, the most compelling use of early sequencing would have been to provide a definitive Ebola diagnosis in this previously unaffected region of West Africa. However, even after the outbreak was underway, sequencing could have benefited the public health response. For example, ruling out bush meat as a source of repeated viral introductions could have changed public health messaging campaigns from avoiding bush meat to the importance of hygiene and safe funeral practices[53], potentially averting some cases. Portable sequencing and phylodynamic approaches are currently being deployed in the ongoing Zika epidemic; whether the real-time reporting of genomic findings is able to alter the course of a vector-borne epidemic remains to be seen.

Retrospective phylodynamic investigations are also useful for pandemic preparedness planning. A recent analysis of 1,610 Ebola virus genomes — approximately 5% of all cases — reconstructs the movement of the virus across West Africa and reveals drivers for its spread[1]. The authors deduce that Ebola importation was more likely to occur between regions of a country than across international borders and that both population size and distance to a nearby large urban centre were associated with local expansion of the virus. These findings may affect decision-making around border closures in future Ebola outbreaks and point to the need to develop surveillance, diagnostic and treatment capacity in urban centres.

The role of the environment

In deploying genomics for surveillance, diagnostics and epidemiological investigation, a key question remains: where? Many regions lack the diagnostic laboratory capacity to carry out basic surveillance, but continuous genomic surveillance in all of these settings would be impossible. Numerous projects have attempted to describe the pool of geographic hot spots and candidate pathogens from which the next epidemic or pandemic will arise. Determining these factors is key to predicting and preventing spillover events (FIG. 4), but huge gaps in our understanding of disease ecology remain. Woolhouse *et al.* describe 1,399 human pathogens, of which 87 — mostly viral — have emerged since 1980 (REF. 54). Jones *et al.* extend this to include 335 new EIDs since 1940 (REF. 55). They report an increasing number of events each decade, generally located in hot spots defined by specific environmental, ecological and socio-economic characteristics.

Most EIDs are zoonotic in origin, with the highest risk of spillover in regions with high wildlife diversity that have experienced recent demographic change and/or recent increases in farming activity[55]. A global biogeographic analysis of human infectious disease further supports the use of biodiversity as a proxy for EID hot spots[56], and reviews focused on systems-level, rather than ecological, factors identify the breakdown of local public health systems as drivers of outbreaks, suggesting that surveillance ought to be targeted to settings where biodiversity and changing demographics meet inadequate sanitation and hygiene, lack of a public health infrastructure for delivering interventions and no or limited resources for control of zoonoses and vector-borne diseases[57].

These analyses provide a shortlist of regions, including parts of eastern and southeastern Asia, India and equatorial Africa, on which genomic and other surveillance activities should be focused[55,58]. Within these regions, sewer systems and wastewater treatment plants could be important foci for sample collection, providing a single

Basic reproductive number R_0
The average number of secondary cases of an infectious disease produced by a single infectious case, given a completely susceptible population.

Zoonotic
A term describing infectious diseases that typically exist in an animal reservoir but that can be transmitted to humans.

Survivor transmission
The transmission of an infectious disease, such as Ebola, from a survivor of that disease who has recovered from their symptoms.

Vector-borne
A term describing infectious diseases that are transmitted to humans through contact with a non-human species, particularly those diseases spread through insect bites. An example is the Zika virus, which is carried by mosquitos.

Hot spots
Geographical settings where a variety of factors converge to create the social and environmental conditions that promote disease transmission.

Spillover
The process by which an infectious disease changes from existing exclusively in animals to being able to infect, then transmit between, humans. See FIG. 4.

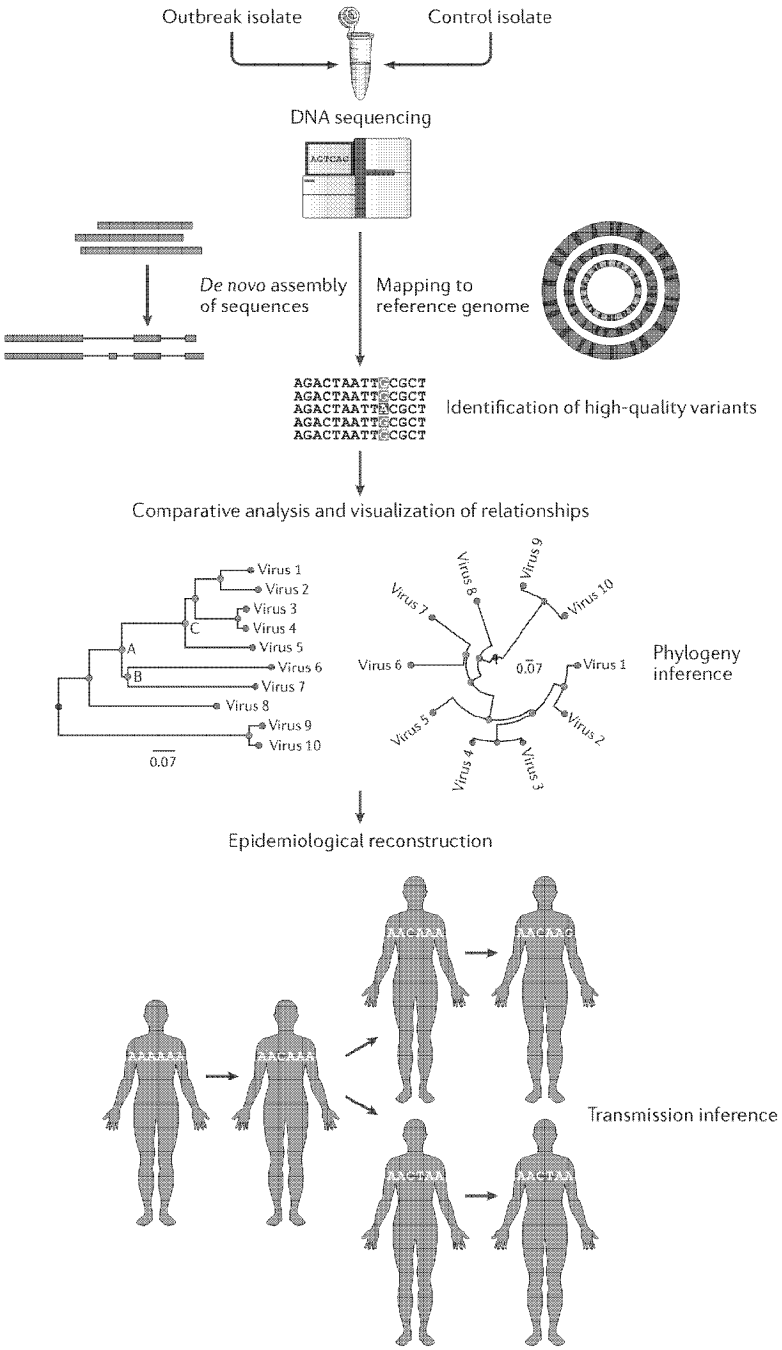

Figure 3 | **Inferring transmission events from genomic data.** Genomic approaches to identifying transmission events typically involve four steps. In the first step, outbreak isolates, and often non-outbreak control isolates, are sequenced and their genomes either assembled *de novo* or mapped against a reference genome. Next, the genomic differences between the sequences are identified — depending on the pathogen and the scale of the outbreak, these may include features such as genetic variants, insertions and deletions or the presence or absence of specific genes or mobile genetic elements. In the third step, these features are examined to infer the relationships between the isolates from whence they came — a variant common to a subset of isolates, for example, suggests that those cases are epidemiologically linked. Finally, the genomic evidence for epidemiological linkages is reviewed in the context of known epidemiological information, such as social contact between two cases or a common location or other exposure. Recently, automated methods for inferring potential epidemiological linkages from genomic data alone have been developed, greatly facilitating large-scale genomic epidemiological investigations[121].

point of entry to biological readouts from an entire community. Indeed, proof-of-concept metagenomics studies have revealed the presence of antibiotic resistance genes[59], human-specific viruses[60] and other pathogens of interest in this readily accessible sample type. Other recent surveys offer insight into what such systems might need to look for. In 2013, Rosenberg *et al.* reported that viruses dominate the list of agents newly recognized to cause disease in humans[61]. Most were zoonotic in origin, and over one-quarter had been detected in non-human species many years before being identified as human pathogens. A later review reiterates this observation, noting that recent agents of concern — Ebola, Zika and chikungunya — had been identified decades before they achieved pandemic magnitude[62]. As a result of NGS technology, the pace of novel virus discovery is accelerating, with recent large-scale studies revealing 184 new viruses sampled from macaque faeces in a single geographic location[63] and 1,445 new viruses discovered from RNA transcriptomic analyses of multiple invertebrate species[64]. However, understanding which of these new entities might pose a threat requires a new approach.

One Health. The emergence of a zoonotic pathogen proceeds in stages[65] (FIG. 4); in an effort to better anticipate these transitions and more proactively respond to emerging threats, the One Health movement was launched in 2004. Recognizing that human, domestic animal and wildlife health and disease are linked to each other and that changing land-use patterns contribute to disease spread, One Health aims to develop systems-minded, forward-thinking approaches to disease surveillance, control and prevention[66]. By investing in infrastructure for human and animal health surveillance, committing to timely information sharing and establishing collaborations across multiple sectors and disciplines, the goal of the One Health community is an integrated system incorporating human, animal and environmental surveillance — a goal in which genomics can have an important role.

The One Health approach has been implemented through the PREDICT project, which is part of the Emerging Pandemic Threats (EPT) programme of the US Agency for International Development (USAID). PREDICT explores the spillover of selected viral zoonoses from particular wildlife taxa[67], and early efforts have focused on developing non-invasive sampling techniques for wildlife[68], estimating the breadth of mammalian viral diversity across nine viral families and at least 320,000 undiscovered species[69] and demonstrating that viral community diversity is at least a partially deterministic process, suggesting that forecasting community changes, which potentially signal spillover, is a possibility[63]. Although the goal of using integrated surveillance information to predict an outbreak is still many years away, One Health studies are already leveraging the tools and techniques of genomic epidemiology to understand current outbreaks.

Combining genomic data with data streams from enhanced One Health surveillance platforms presents an opportunity to detect the population expansions

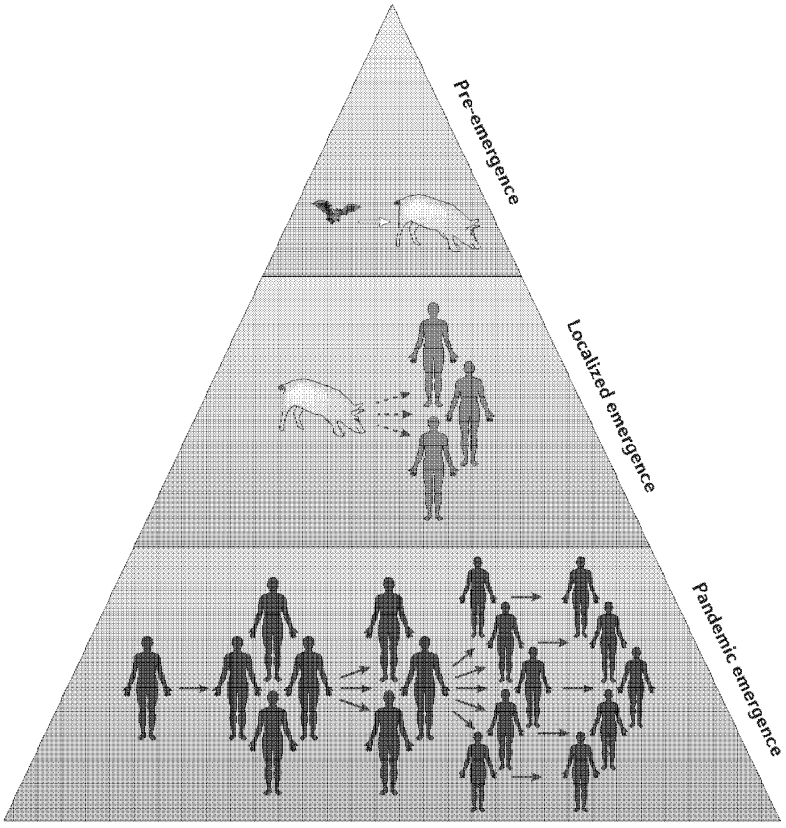

Figure 4 | **Emergence of infectious diseases.** In spillover, a pathogen previously restricted to animals gradually begins to move into the human population. During stage one (pre-emergence), as a result of changing demographics and/or land use, a pathogen undergoes a population expansion, extends its host range or moves into a new geographic region. During stage two (localized emergence), contact with animals or animal products results in spillover of the pathogen from its natural reservoir(s) into humans but with little to no onward person-to-person transmission. During stage three (pandemic emergence), the pathogen is able to sustain long transmission chains, that is, a series of disease transmission events, such as a sequential series of person-to-person transmissions, and its movement across borders is facilitated by human travel patterns[65].

and/or cross-species transmissions that may precede a human health event. For example, genome sequences from a raccoon-associated variant of rabies virus (RRV), when paired with fine-scale geographic information and data from Canadian and US wildlife rabies vaccination programmes, demonstrated that multiple cross-border incursions were responsible for the expansion of RRV into Canada and sustained outbreaks in several provinces[70]; this finding led to renewed concern about and action against rabies on the part of public health authorities[71]. One of the first studies coupling detailed wildlife and livestock movement data with phylodynamic analysis of a bacterial pathogen revealed that cross-species jumps from an elk reservoir were the source of increasing rates of *Brucella abortus* infections in nearby livestock[72]; as the most common zoonosis of humans, brucellosis control programmes will benefit substantially from this sort of One Health approach[73].

This model, in which diagnostic testing in reference laboratories triggers genomic follow-up, represents an effective near-term solution for integrating genomics into One Health surveillance efforts as the community explores solutions to the many challenges facing *in situ* clinical metagenomics surveillance of animal populations (reviewed in REF. 74). Initial forays into this area have been successful; for example, metagenomics analysis of human diarrhoeal specimens and stools from nearby pigs revealed potential zoonotic transmission of rotavirus[75]. However, metagenomic sequencing across a range of animal species and environments yields more questions than answers. What is an early signal of pathogen emergence versus background microbial noise[65]? Which emerging agents are capable of crossing the species barrier and causing human disease[74]? What degree of sampling is required to capture potential spillovers[67]? Ultimately, a more efficient use of metagenomics in a One Health surveillance strategy might be scanning for zoonotic 'jumps' in selected sentinel human populations rather than a sweeping animal surveillance strategy[62], with sentinels chosen according to EID hotspot maps and other factors[65] and interesting genomic signals triggering follow-up sequencing in the relevant animal reservoirs. By combining genomic data generated through these targeted surveillance efforts with phylodynamic approaches, it will be possible to take simple presence or absence signals and derive useful epidemiological insights: signals of population expansion; evidence of transmission within and between animal reservoirs and humans; and epidemiological analysis of a pathogen's early expansion.

Digital epidemiology

Most modern surveillance systems use human, animal, environmental and other data[76] to carry out disease-specific surveillance, in which a single disease is monitored through one or more data streams, such as positive laboratory test results or reportable communicable disease notifications. Despite marked advances over the preceding decades, testimony from multiple expert groups has repeatedly emphasized the need for improved surveillance capacity[8,77], including the use of syndromic surveillance, a more pathogen-agnostic approach aimed at early detection of emerging disease[78,79]. Syndromic surveillance systems might leverage unique data streams such as school or employee absenteeism, grocery store or pharmacy purchases of specific items or calls to a nursing hotline as signals of illness in a population. Increasingly, digital streams are being used as an input to these systems, be they participatory epidemiology projects such as Flu Near You[80], the automated analysis of trending words or phrases on social media sites, such as Twitter[81,82], or Internet search queries[83–85].

This new approach to surveillance is known as digital epidemiology and is also referred to as digital disease detection[86]. In digital epidemiology, information is first retrieved from a range of sources, including digital media, newswires, official reports and crowdsourcing; second, translated and processed, which includes extracting disease events and ensuring reports are not duplicated; third, analysed for trends; and fourth, disseminated to the community through media, including websites, email lists and mobile alerts[87]. At least 50 digital

epidemiology platforms are currently operating[88], and their flexible nature and cost-effective, real-time reporting make them effective tools for gathering epidemic intelligence, particularly in settings lacking traditional disease surveillance systems.

Modelling drivers of infectious-disease emergence. The fields of One Health and digital epidemiology are increasingly overlapping. In the PREDICT consortium, the HealthMap system[89] and local media surveillance were combined to identify 307 health events in five countries over a 16-week period[90]. PREDICT also suggested a role for digital epidemiology in not just event detection but also the identification of changing EID drivers. EIDs are driven by multiple factors, many of which have digital outputs and represent novel sources of surveillance data[91]. For example, human movement can be revealed by mobile phone data or by the patterns of lighted cities at night, hunting data collected by states can reveal interactions between humans and wildlife, and social media and digital news sources can reveal early signals of famine, war and other social unrest. A major challenge is that the number of digital data sets available for each driver varies substantially, from hundreds for surveying land use changes — many based on remote sensing data[92] — to mere handfuls around social inequalities and human susceptibility to infection, with most data biased towards North America and Europe.

The digital and genomic epidemiology domains are also starting to overlap. In the Ebola outbreak, digital epidemiology revealed that drivers of infection risk included settings where households lacked a radio, with high rainfall and with urban land cover[93], echoing the evidence from a genomic study suggesting that sites at which urban and rural populations mix contribute to disease[1]. During the Zika epidemic, Majumder *et al.* used HealthMap and Google Trends to estimate the basic reproductive number R_0 to be 1.42–3.83[94]; phylodynamic estimates from Brazilian genomic data gave similar ranges (1.29–3.85)[3], indicating that both types of data streams can be leveraged in calculating epidemiological parameters that help shape the public health response.

A digital pathogen surveillance era

Recent reports have called for the integration of genomic data with digital epidemiology streams[92,95]. When informed by a One Health approach, the epidemiological potential of this digital pathogen surveillance system is profound. Imagine parallel networks of portable pathogen sequencers deployed to laboratories and communities in EID hot spots — regions that are traditionally underserved with respect to laboratory and surveillance capacity — and processing samples collected from targeted sentinel wildlife species, insect vectors and humans (FIG. 5). Samples would be pooled for routine surveillance — either through targeted diagnostics or, if the issue of analytical sensitivity can be overcome, through metagenomics — with a full genomic work-up of individual samples should a pathogenic signal be detected. At the same time, existing Internet-based platforms such as HealthMap and new local participatory epidemiology efforts would be collecting data to both identify potential hotspot regions and detect EID events, enabling both prospective and rapid-response deployment of additional sequencers. Genome sequencing data coupled with rich metadata would then be released in real time to web-based platforms, such as Virological for collaborative analysis and Nextstrain for analysis and visualization[96]. These sites — already used in the Ebola and Zika responses — would act as the nexus for a global network of interested parties contributing to real-time phylodynamic and epidemiological analyses and looking for signals of spillover, pathogen population expansion and sustained human-to-human transmission. Results would be immediately shared with the One Health frontline — epidemiologists, veterinarians and community health workers — who would then implement evidence-based interventions to mitigate further spread.

The pathway to such a reality is not without its roadblocks. Apart from technical and implementation challenges, a series of larger concerns surrounds the rollout of genomics-based rapid outbreak response, ranging from the uptake of a new, disruptive technology to effecting systems-level change on a global scale.

Ethical, legal and social issues. Sequencing-based diagnostics, particularly clinical metagenomics approaches, are still straddling the boundary between research and clinical use. In this realm, uncertainty is a certainty, be it uncertainty inherent to the technology itself or informational uncertainty, such as how accurate, complete and reliable results actually are[97]. Early adopters of genomics in the academic domain are used to uncertainty, often acknowledging and appraising it, but routine clinical use requires meeting the evidentiary thresholds mandated by a range of stakeholders, from regulators to the laboratories implementing new sequencing-based tests. Decision criteria that influence whether a new genomic test is adopted include the ability of the assay to differentiate pathogens from commensals, the correlation of pathogen presence with disease, the sensitivity and specificity of the test, its reproducibility and robustness across sample types and settings and a cost comparable to that of existing platforms[98].

Validation — defining the conditions needed to obtain reliable results from an assay, evaluating the performance of the assay under said conditions and specifying how the results should be interpreted, including outlining limitations[99] — is also critical. Much can be learned from the domain of microbial forensics, where sequencing is playing a large part[100]. Budowle *et al.* review validation considerations for NGS[101], noting that this technology requires validating sample preparation protocols, including extraction, enrichment and library preparation steps, sequencing protocols, and downstream bioinformatics analyses, including alignment and assembly, variant calling, the underlying reference databases and software tools and the interpretation of the data. Complete validation of a sequencing assay may not always be possible, particularly for emerging

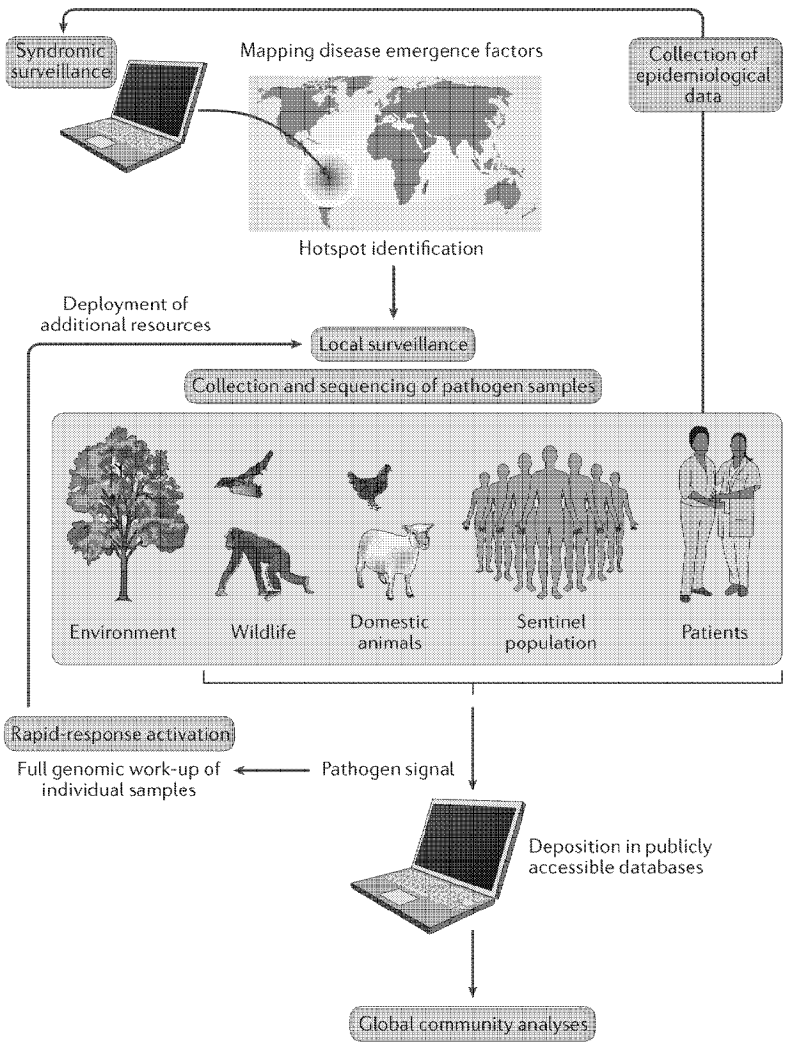

Figure 5 | **A future model for surveillance and early outbreak response.** It is 2027, and our planet's changing climate and land-use patterns have meant that new emerging infectious diseases (EIDs) are spilling over into humans from wildlife reservoirs with increasing frequency. Building off EID hotspot maps developed in 2008 (REF. 55), a global public health consortium has implemented an online surveillance tool that scans the digital output of citizens, news organizations and governments in those regions, including data from local retailers on key health-related products, such as tissues and over-the-counter cold remedies. In one such region, the syndromic surveillance system reports higher-than-average sales of a common medication used to relieve fever. Spatial analysis of the data from the pharmacies in the region suggests that the trend is unique to a particular district; a follow-up geographic information system (GIS) analysis using satellite data reveals that this area borders a forest and is increasingly being used for the commercial production of bat guano. An alert is triggered, and the field response team meets with citizens in the area. Nasopharyngeal swabs are taken from humans and livestock with fever as well as from guano and bat tissue collected in the area. The samples are immediately analysed using a portable DNA sequencer coupled to a smartphone. An app on the phone reports the clinical metagenomic results in real time, revealing that in many of the ill humans and animals, a novel coronavirus makes up the bulk of the microbial nucleic acid fraction. The sequencing data are immediately uploaded to a public repository as they are generated, tagged with metadata about the host, sample type and location and stored according to a pathogen surveillance ontology. The data release triggers an announcement via social media of a novel sequence, and within minutes, interested virologists have created a shared online workspace and open lab notebook to collect their analyses of the new pathogen.

pathogens. Therefore, just as the West African Ebola virus outbreak triggered a review of the ethical context for trialling new therapeutics and vaccines[102], the scale-up of NGS in emerging epidemics will engender similar conversations. Rather than wait for this to happen, an anticipatory approach is best, outlining the exceptional circumstances under which unvalidated approaches might be used, selecting the appropriate approach and examining the benefits of a potentially untested approach in light of individual and societal interests.

If the social landscape surrounding the introduction of a new technology is not considered, prior experience suggests that the road to implementation will be difficult, with hurdles ranging from public mistrust to moratoria on research[103]. The enthusiasm of the scientific community for new technology must not lead to inflated claims of clinical utility and poor downstream decisions around the deployment of that technology. Howard *et al.* outline several principles for successfully integrating genomics into the public health system, and as we pilot digital pathogen surveillance, the community would do well to keep many of them in mind: ensuring that the instruments and processes used are reliable and that reporting is standardized and readily interpretable by end users; that the technology is used to address important health problems; that the advantages of the approach outweigh the disadvantages; and that economic evaluation suggests savings to the health care system and society[104]. It is also important to reconsider the role of the diagnostic reference laboratory in the new genomic landscape. As their mandates expand to include enhanced surveillance and closer collaboration with field epidemiologists, laboratory directors will face new challenges, from managing exploratory work alongside routine clinical care to hiring a new sort of technologist, one with basic genomics and epidemiology training.

The ethical, social and legal implications of digital pathogen surveillance are an emerging area of research (reviewed in REF. 105). Chief among the issues that Geller *et al.* identify is the tension that exists when a new technology has the power to identify a problem but there is limited or no capacity to address the issue. Balancing the benefits and harms to both individuals and populations is challenging when the predictive insight offered by a genomic technology is variable — for example, using genomics to identify an individual as a 'super spreader' has important implications for quarantine and isolation, but that label may be predicated on a tenuous prediction. The problem is further compounded by the fact that many infectious disease diagnoses carry with them a certain amount of stigma and that an individual's right to privacy might be superseded by the need to protect the larger population[105].

Data sharing and integration. A critical need for successful digital pathogen surveillance is the capacity for rapid, barrier-free data sharing, and arguments for such sharing are frequently rehashed after outbreaks and epidemics. Genomic epidemiology was born largely in the academic sphere, with early papers coming from laboratories with

extensive histories in microbial genomics and bioinformatics. For this community, open access to genome sequences, software and, more recently, publications has tended to be the rule rather than the exception. Indeed, a 2004 National Research Council report described "the culture of genomics" as "unique in its evolution into a global web of tools and information" (REF. 106). The same report includes a series of recommendations on access to pathogen genome data, including the statement that "rapid, unrestricted public access to primary genome sequence data, annotations of genome data, genome databases, and Internet-based tools for genome analysis should be encouraged" (REF. 106).

As genomics has moved into the domain of clinical and public health practice, the notion of free and immediate access to genomic surveillance data has encountered several barriers: the siloing of critical metadata across multiple public health databases with no interoperability; balancing openness and transparency with patient privacy and safety; variable data quality, particularly in resource-limited settings; concerns over data reuse by third parties; a lack of standards and ontologies to capture metadata; and career advancement disincentives to releasing data[107–109]. Despite these challenges, the spirit of open access and open data remains strong in the community, with over 40 public health leaders from around the world recently signing a joint statement on data sharing for public health surveillance[110]. The Ebola and Zika responses in particular highlight the role of real-time sharing of data and samples, be it through the use of chat groups and a LabKey server to disseminate Zika data[111] or GitHub to share Ebola data[112].

In the wake of Ebola, Yozwiak *et al.*[113] and Chretien *et al.*[114] outline additional issues facing data sharing, from differing cultures and academic norms to complicated consent procedures and technical limitations. They note that we as a community must agree on standards and practices promoting cooperation — a conversation that could begin by examining how the Global Alliance for Genomics and Health (GA4GH) framework for responsible sharing of genomic and health-related data (BOX 1) could be adapted for the digital pathogen surveillance community.

The future: the sequencing singularity?

Transformative change to public and global health is profoundly difficult. Complicating the existence of a rapid, open, transparent response is the fact that no matter the setting, there are often conflicting interests at work. In an outbreak scenario, conflict may result from governments wishing to keep an outbreak quiet and/or from the tension between lower-income and middle-income countries with few resources for generating and using data and the researchers or response teams from better-resourced settings[115]. Indeed, the conflicting values in outbreak responses meet the definition of a 'wicked' problem, where issues resist simple resolution and span multiple jurisdictions and where each stakeholder has a different perspective on the solution. Even the International Health Regulations (IHR), which ostensibly provide a legal instrument for global health security, fail to effect a basic surveillance and outbreak response. As of the most recent self-reporting, only 30% of the 196 member countries of the IHR are in compliance, meeting the prescribed minimum public health core capacities[5]. In these settings, digital pathogen surveillance must be within the purview of the larger global health community and its diverse group of non-state actors rather than being solely the responsibility of nations themselves[116]. This raises an important issue: if nations are willing to cede a certain amount of surveillance and diagnostic control

Box 1 | The Global Alliance for Genomics and Health (GA4GH) framework for genomic data sharing

In the 1948 Universal Declaration of Human Rights, Article 27 outlines the right of every individual "to share in scientific advancement and its benefit". In this spirit, the Global Alliance for Genomics and Health (GA4GH) data-sharing framework[119], which covers data donors, producers and users, is guided by the principles of privacy, fairness and non-discrimination and has as its goal the promotion of health and well-being and the fair distribution of benefits arising from genomic research. The core elements of the framework include the following:

- Transparency: knowing how the data will be handled, accessed and exchanged
- Accountability: tracking of data access and mechanisms for addressing misuse
- Engagement: involving citizens and facilitating dialogue and deliberation around the societal implications of data sharing
- Quality and security: mitigating unauthorized access and implementing an unbiased approach to storing and processing data
- Privacy, data protection and confidentiality: complying with the relevant regulations at every stage
- Risk–benefit analysis: weighing benefits (including new knowledge, efficiencies and informed decision making) against risks (including invasion of privacy and breaches of confidentiality), minimizing harm and maximizing benefit at the individual and societal levels
- Recognition and attribution: ensuring recognition is meaningful to participants, providing due credit to all who shared data and ensuring credit is given for both primary and secondary data use
- Sustainability: implementing systems for archiving and retrieval
- Education and training: advancing data sharing, improving data quality, educating people on why data sharing matters, and building capacity
- Accessibility and dissemination: maximizing accessibility, promoting collaboration and using publication and digital dissemination to share results

to the global health community, the notion of reciprocity suggests that they should derive some corresponding local benefit. The 'trickle-down' effects of global genomic surveillance have yet to be fully articulated, but they are likely to be realized first in the zoonotic domain, where global surveillance efforts will feed back into improved animal health at a local level, in turn benefiting local farmers.

Outbreaks occur at the intersection of risk perception, governance, policy and economics[117], and outbreak response is often based on political instinct rather than data[5]. Building a resilient and responsive public health system is therefore more than just enhancing surveillance and coupling it to novel technology — it is about engagement, trust, cooperation and building local capacity[8], as well as a focus on pandemic prevention through development rather than pandemic response via disaster relief mechanisms[57]. Expert panels convened by Harvard and the London School of Hygiene and Tropical Medicine[9] and by the National Academy of Medicine[8] have called for a central pandemic preparedness and response agency and also underscored the need for deeper partnerships between formal and informal surveillance, epidemiology and academic and public health networks[5]. More recently, evolutionary biologist Michael Worobey wrote: "Systematic pathogen surveillance is within our grasp, but is still undervalued and underfunded relative to the magnitude of the threat" (REF. 118). If we are to achieve the sequencing singularity — the moment at which pathogen, environmental and digital data streams are integrated into a global surveillance system — we require a community united behind a vision in which public health and the attendant data belong to the public and behind the idea that we are a better, healthier society when the public is able to access and benefit from the data being collected about us and the pathogens we share the planet with.

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Author contributions
Both authors contributed equally to all aspects of the article.

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J.L.G. declares no competing interests. N.J.L. declares competing interests. See the Web version for full details.

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FURTHER INFORMATION
The PREDICT Project: http://www.vetmed.ucdavis.edu/ohi/predict/
USAID Emerging Pandemic Threats: https://www.usaid.gov/news-information/fact-sheets/emerging-pandemic-threats-program
HealthMap: http://www.healthmap.org/
Virological: http://www.virological.org
Nextstrain: http://www.nextstrain.org
Interactive WHO Zika timeline: http://www.who.int/emergencies/zika-virus/timeline/en
ZiBRA project: http://www.zibraproject.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

From: "Sacchetti, Ben" <Sacchetti.Ben@bcg.com>
To: [redacted], "watson@ecohealthalliance.org" <watson@ecohealthalliance.org>, "Linda Patterson" <linda.patterson@panoramaglobal.org>, Amy Lin <amylin@usaid.gov>, Dennis Carroll <dcarroll@usaid.gov>, "Kabay, Kendra" <Kabay.Kendra@bcg.com>, "Rodriguez, Andrew" <rodriguez.andrew@bcg.com>, Raelyn Campbell <raelyn.campbell@panoramaglobal.org>, Peter Daszak <daszak@ecohealthalliance.org>, Jennifer Fluder <jfluder@usaid.gov>, "Nathan Wolfe" <nwolfe@metabiota.com>, "erubin@metabiota.com" <erubin@metabiota.com>, Gabrielle Fitzgerald <gabrielle.fitzgerald@panoramaglobal.org>, "Harris, Samuel" <Harris.Samuel@bcg.com>, "Stroman, Trish" <Stroman.Trish@bcg.com>, "jkmazet@ucdavis.edu" <jkmazet@ucdavis.edu>
Cc: "Woods, Wendy" <Woods.Wendy@bcg.com>, "dstanton@usaid.gov" <dstanton@usaid.gov>, "pmahanna@usaid.gov" <pmahanna@usaid.gov>
Subject: GVP Core Team meeting
Sent: Thu, 4 Jan 2018 15:43:35 +0000

As discussed yesterday, we are extending future Core Team meetings to 90 minutes. We may not use the full time every week, but wanted to be safe and ensure we have time to cover all of the topics we need to. Thank you for your time!

JOIN WEBEX MEETING

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From: Leilani Francisco <francisco@ecohealthalliance.org>
Sent: Fri, 5 Jan 2018 18:19:17 -0500
Subject: Feedback requested by Tuesday COB: bat book
To: Brian Bird <bhbird@ucdavis.edu>, David John Wolking <djwolking@ucdavis.edu>, Christine Kreuder Johnson <ckjohnson@ucdavis.edu>, Tracey Goldstein <tgoldstein@ucdavis.edu>, Jonna Mazet <jkmazet@ucdavis.edu>, "William B. Karesh" <karesh@ecohealthalliance.org>, Jon Epstein <epstein@ecohealthalliance.org>, Manjunatha N Belaganahalli <mbelaganahalli@ucdavis.edu>, Emily Hagan <hagan@ecohealthalliance.org>, Corina Grigorescu Monagin <cgmonagin@ucdavis.edu>, Peter Daszak <daszak@ecohealthalliance.org>, Simon Anthony <sja2127@columbia.edu>, Emma Lane <lane@ecohealthalliance.org>
Cc: Katherine Leasure <kaleasure@ucdavis.edu>, Amanda Fuchs <fuchs@ecohealthalliance.org>, Brooke Genovese <bgenovese@ucdavis.edu>, Stephanie Martinez <martinez@ecohealthalliance.org>, Aleksei MacDurian <chmura@ecohealthalliance.org>, Alison Andre <andre@ecohealthalliance.org>
Bat Communication Campaign Picture Book_2018-1-5_v10.zip

Dear 'EHP' team,

We're pleased to share an updated version of the bat book.

Please find it attached in a zip file.

Revisions include:

- Incorporation of local level feedback
- Updated illustrations
- Lowered reading level
- Intro page with branding

Your feedback would be very appreciated.

We'd like to be able to share this on Thursday, so if you have any feedback, can you please send it to Stephanie Martinez before the end of the day on ***Tuesday (Jan 9)***?

Thanks in advance and we look forward to seeing you soon!

Leilani

--

Leilani Francisco, PhD, MA, PMP
Senior Scientist | PREDICT-2 Global Behavioral Risk Surveillance Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4493 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

Living Safely with Bats

Emerging Pandemic Threats Program 2 (EPT-2)

PREDICT-2 is part of USAID's Emerging Pandemic Threats (EPT) program and aims to strengthen global capacity for the detection, discovery, and prevention of viruses with pandemic potential, specifically those that can move between animals and people (zoonotic viruses). Implementing partners for PREDICT-2 are University of California-Davis, EcoHealth Alliance, Metabiota, Smithsonian Institution, and Wildlife Conservation Society.

A goal of the behavioral risk component of PREDICT-2 is to use scientific results to inform the development of intervention strategies that could reduce the spillover, amplification, and spread of novel viruses. Preliminary analyses identified an expressed need to provide behavior change strategies in West Africa as they relate to living safely with bats. The current resource was developed to address this need.

A moderated picture book format was recommended by local leaders. Subject matter experts from the PREDICT-2 consortium developed an initial draft which was updated based on feedback from local community members. If the picture book is used in other settings, it is recommended that additional local level feedback be incorporated to tailor the content to the specific context.

Moderation of the picture book is intended to be provided by a trusted community leader, and talking points are provided for each image that the storyteller can use to moderate the discussion. To facilitate this, the document should be printed out such that there is an image on one side with the talking points on the other side. The moderator can then hold up the images to show to the audience, and use the talking points on the back to guide the discussion.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. The contents of this document are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government.

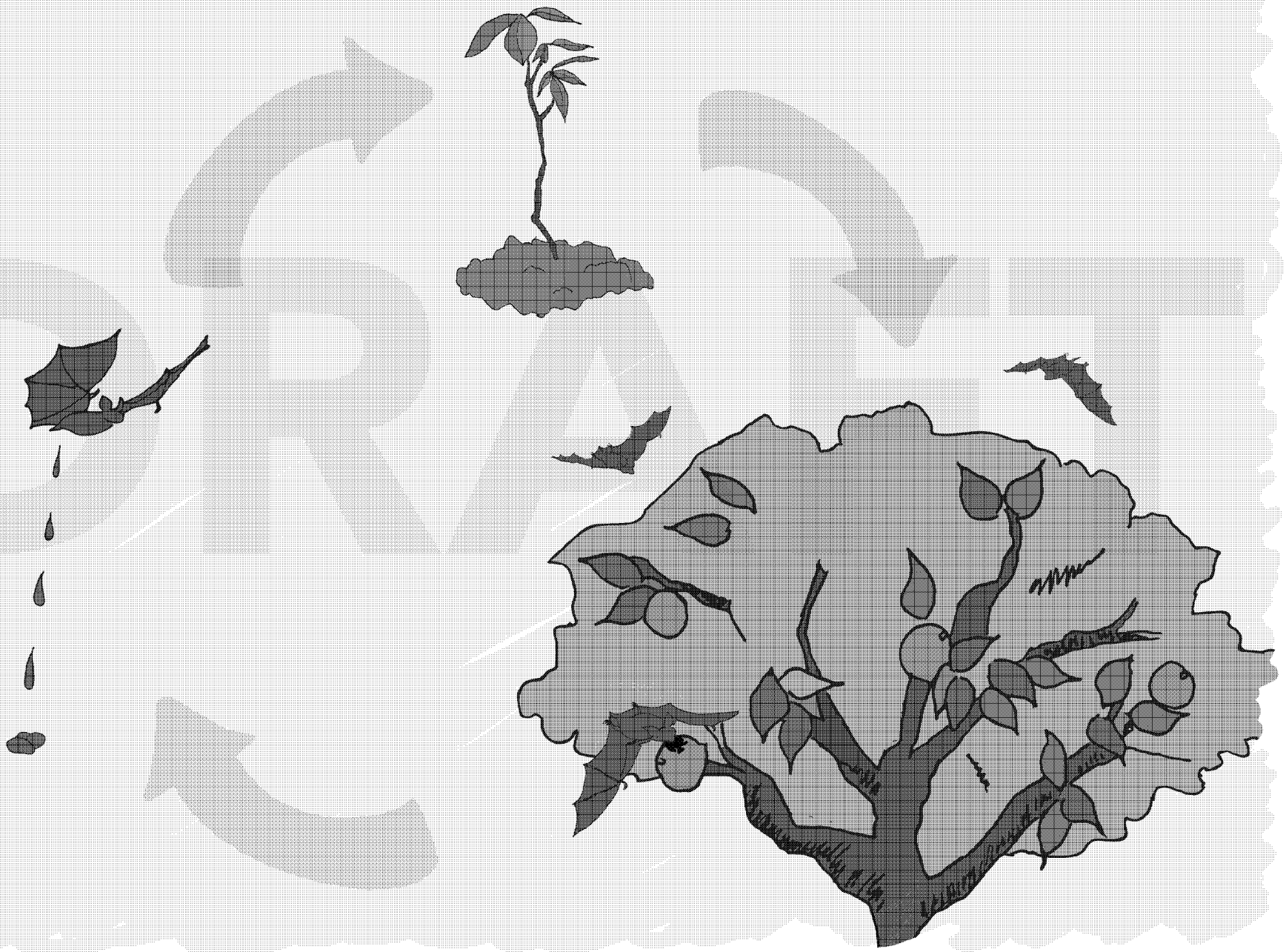
For more information about the contents of this resource, please contact predict@ucdavis.edu.

Prepared by:
Leilani Francisco[1], Ava Sullivan[1], Jonathan Goley[1], Stephanie Martinez[1]

With contributions from:
Karen Saylors [2], Jason Euren[2], Jon Epstein[1], Brian Bird[3], Chris Johnson[3], Tracey Goldstein[3], Billy Karesh[1], Peter Daszak[1], Jonna Mazet[3]

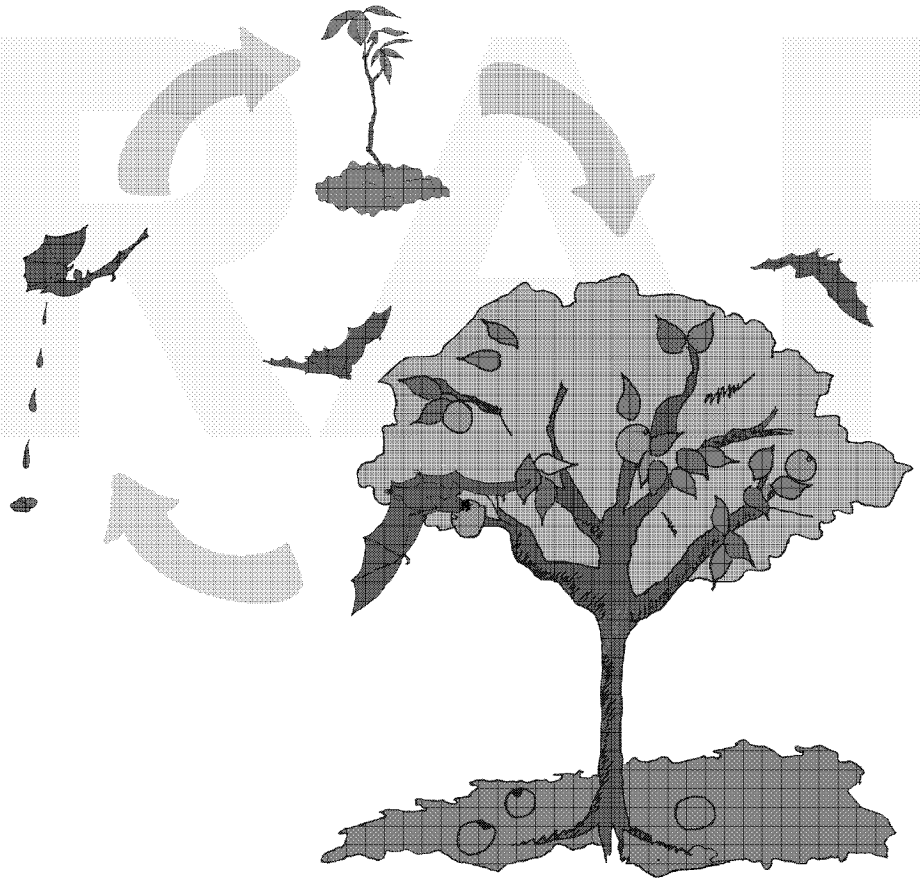
[1] EcoHealth Alliance
[2] Metabiota
[3] UC Davis

Bats are an Essential Part of our Ecosystem

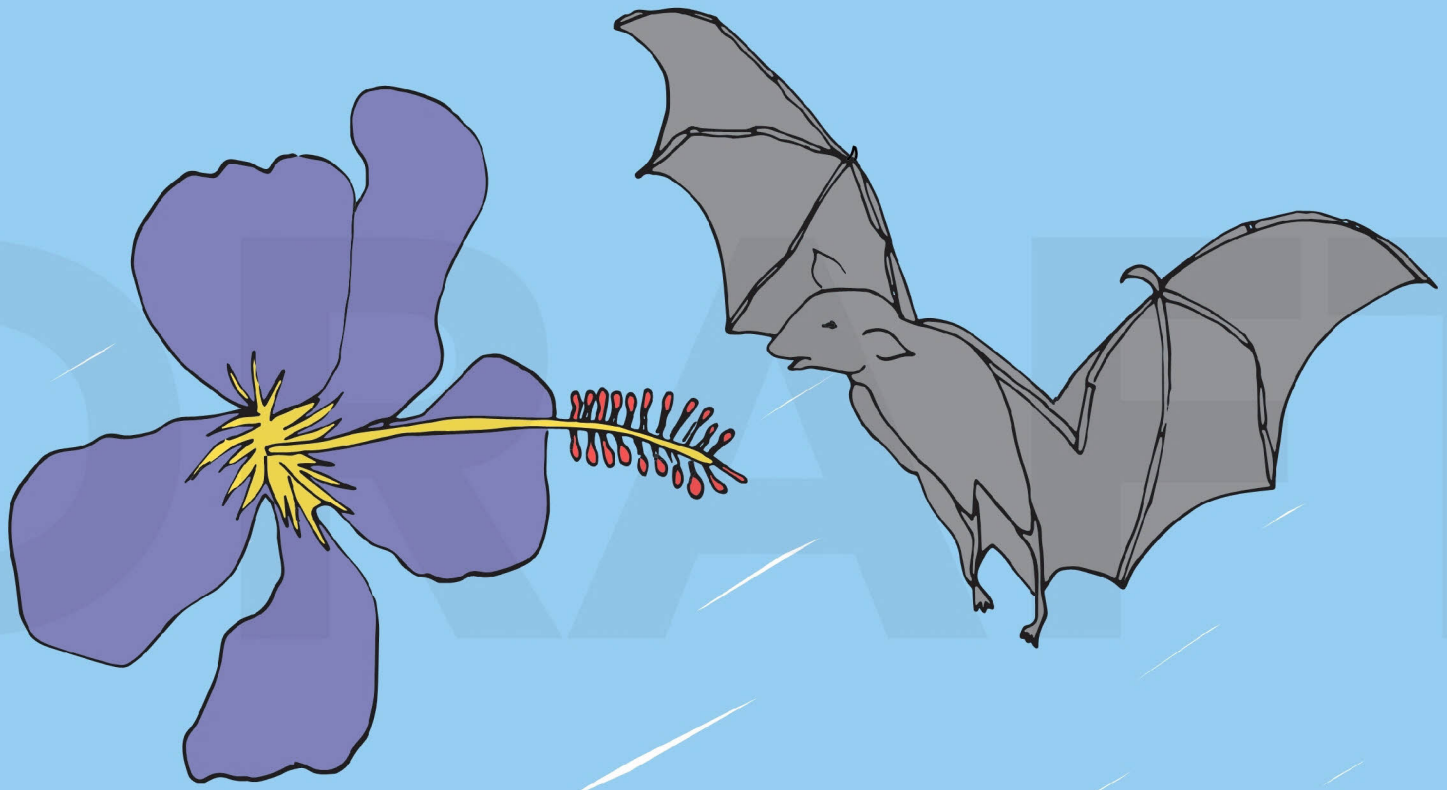


1a. Bats are an Essential Part of our Ecosystem

While some bats play a role in spreading disease, most bats play an important role in keeping us and our ecosystem healthy. For example, bats spread seeds from hardwood and fruit trees.

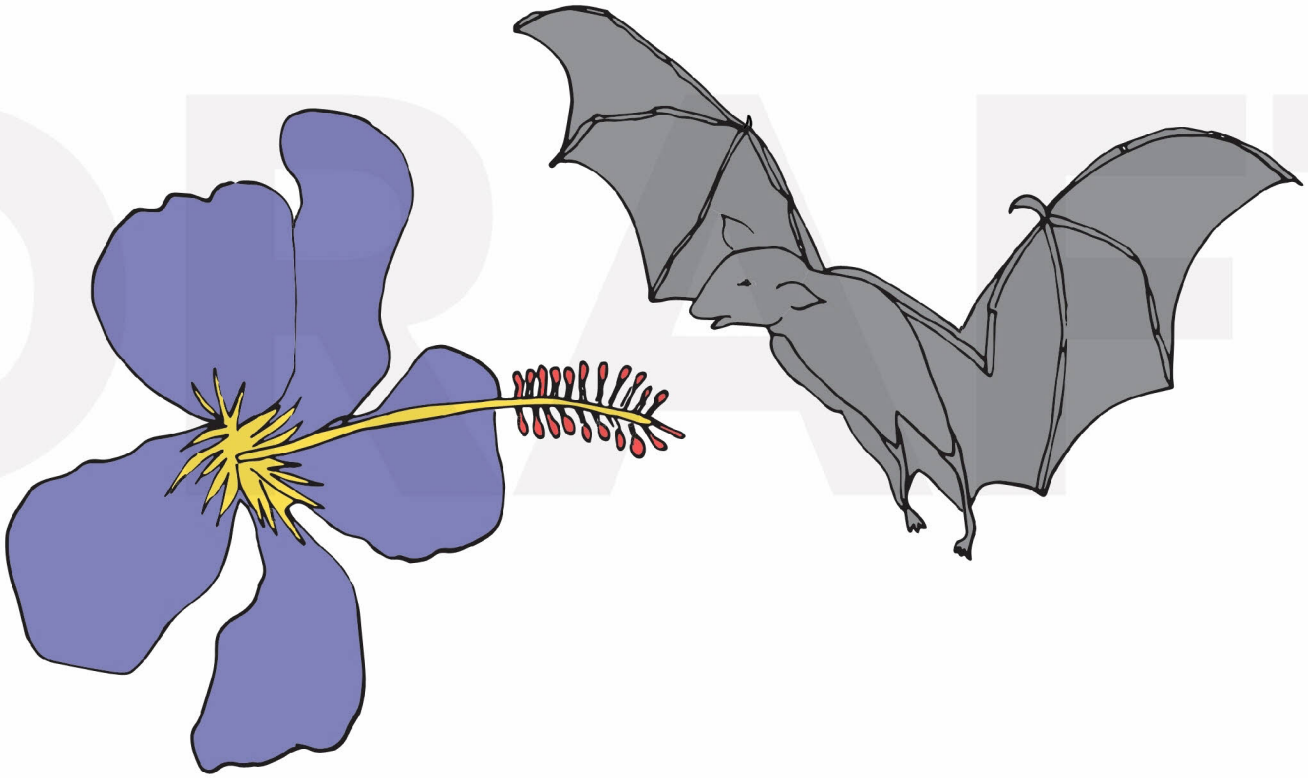


Bats are an Essential Part of our Ecosystem

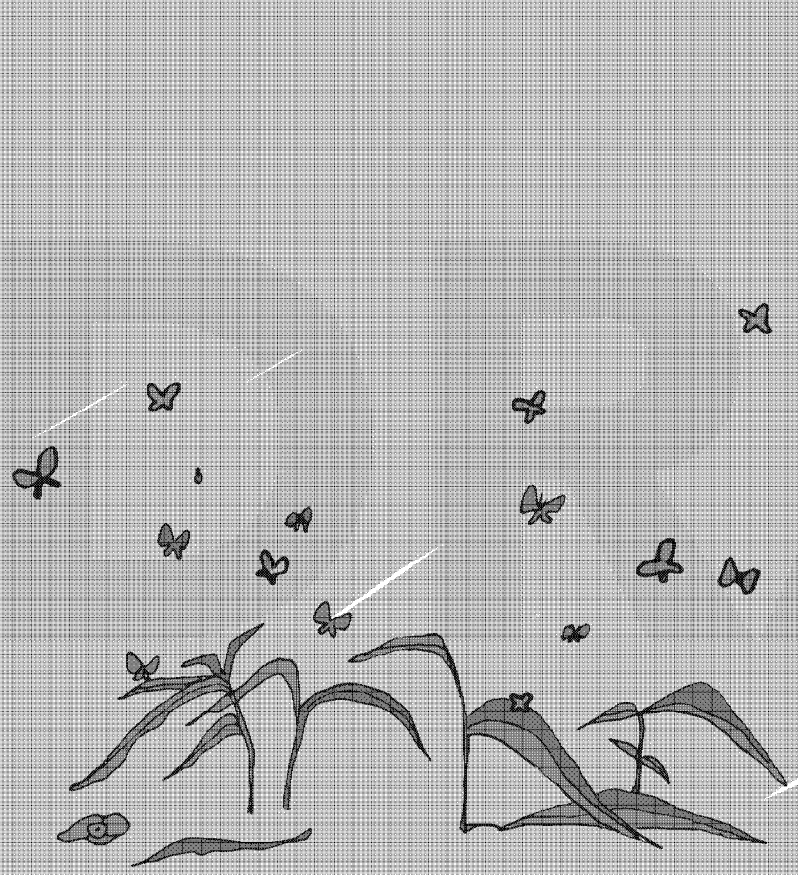


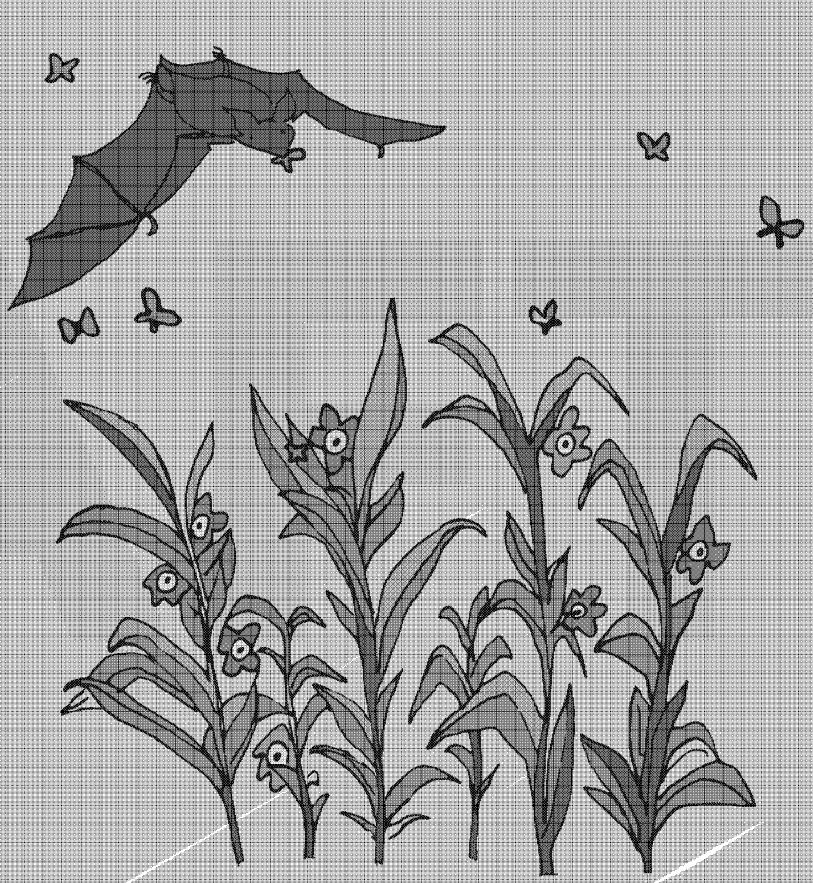
1b. Bats are an Essential Part of our Ecosystem

Bats also play an important role in keeping us and our ecosystem healthy by pollinating flowering plants.



Bats are an Essential Part of our Ecosystem





1c. Bats are an Essential Part of our Ecosystem

Bats also eat insects such as moths and beetles that damage crops.

Ways to Live Safely with Bats

2a. Ways to Live Safely with Bats

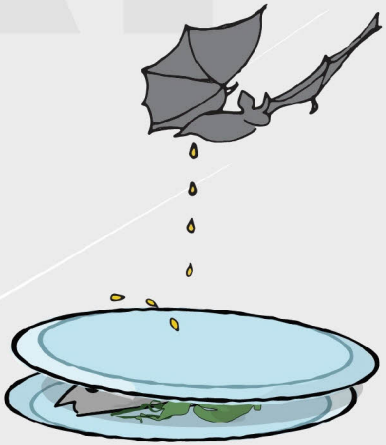
Bats have been connected to viruses like rabies and others that cause diseases in people. However, killing or disturbing the natural homes of bats can worsen the spread of disease, so it's best not to hunt, kill, or eat bats.

Ways to Live Safely with Bats

2a. Ways to Live Safely with Bats

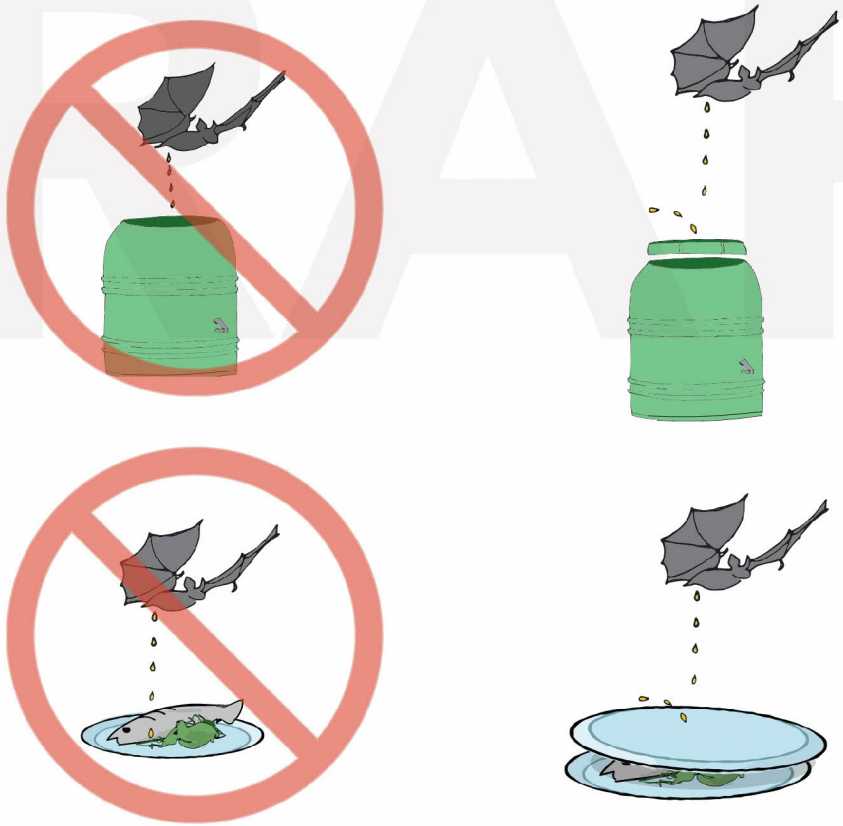
The best way to live safely with bats is to avoid all contact with both living and dead bats. It is especially important to avoid bodily fluids such as spit, blood, urine, or feces, and to prevent them from living inside homes and buildings (also called "roosting").

Ways to Live Safely with Bats



2b. Ways to Live Safely with Bats

One easy way to stay safe is to avoid eating food or drinking water that has come into contact with bat urine or feces. For example, you can cover your food and water. When you do, it is important to regularly clean these covers.



Ways to Live Safely with Bats

2c. Ways to Live Safely with Bats

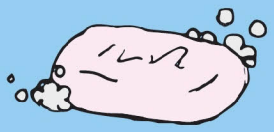
To reduce the risk of getting sick, avoid eating fruit that has been partially eaten by wild animals or livestock. You should never eat or drink something that you think bats have come in contact with. Do not try to save food that has been partially eaten by bats by removing the sections with obvious bite marks, and do not feed these partially eaten fruits to livestock. The entire item may have been contaminated.

How to Dispose of Dead Bats

3a. How to Dispose of Dead Bats

If a professional is not available to assist with handling dead bats, take steps to avoid directly touching the bat. If you do not have heavy gloves, plastic grocery bags can be used instead. Bats should be placed into a plastic bag or container, and then burned completely or buried 1 to 2 meters deep and covered by at least 60 centimeters of soil. Remember that it is still possible to be hurt by a dead bat. Bat claws are very sharp and can easily cut or scratch your skin.

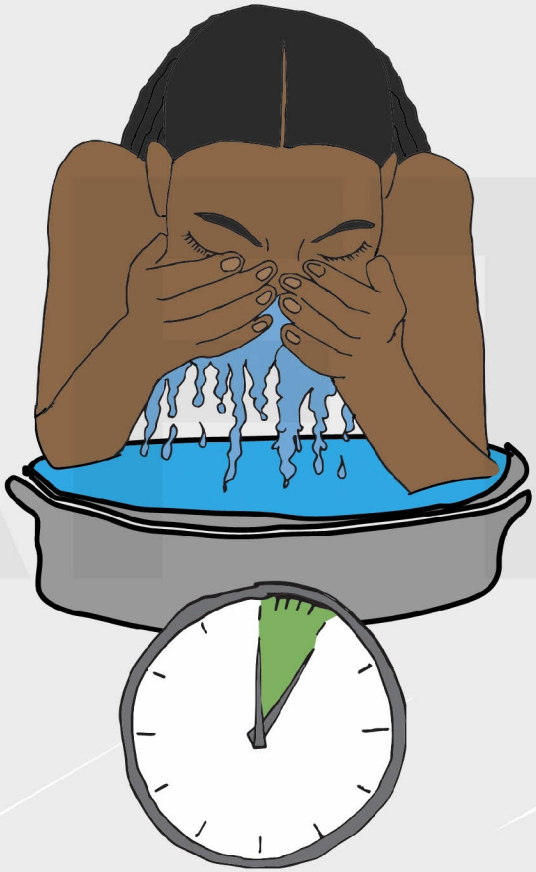
How to Dispose of Dead Bats



3b. How to Dispose of Dead Bats

After getting rid of the dead bat, wash your hands with soap and water.

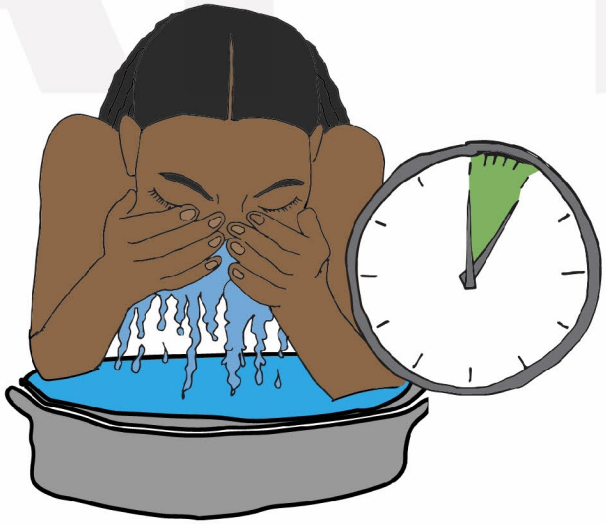
If Contact with Live Bats is Unavoidable



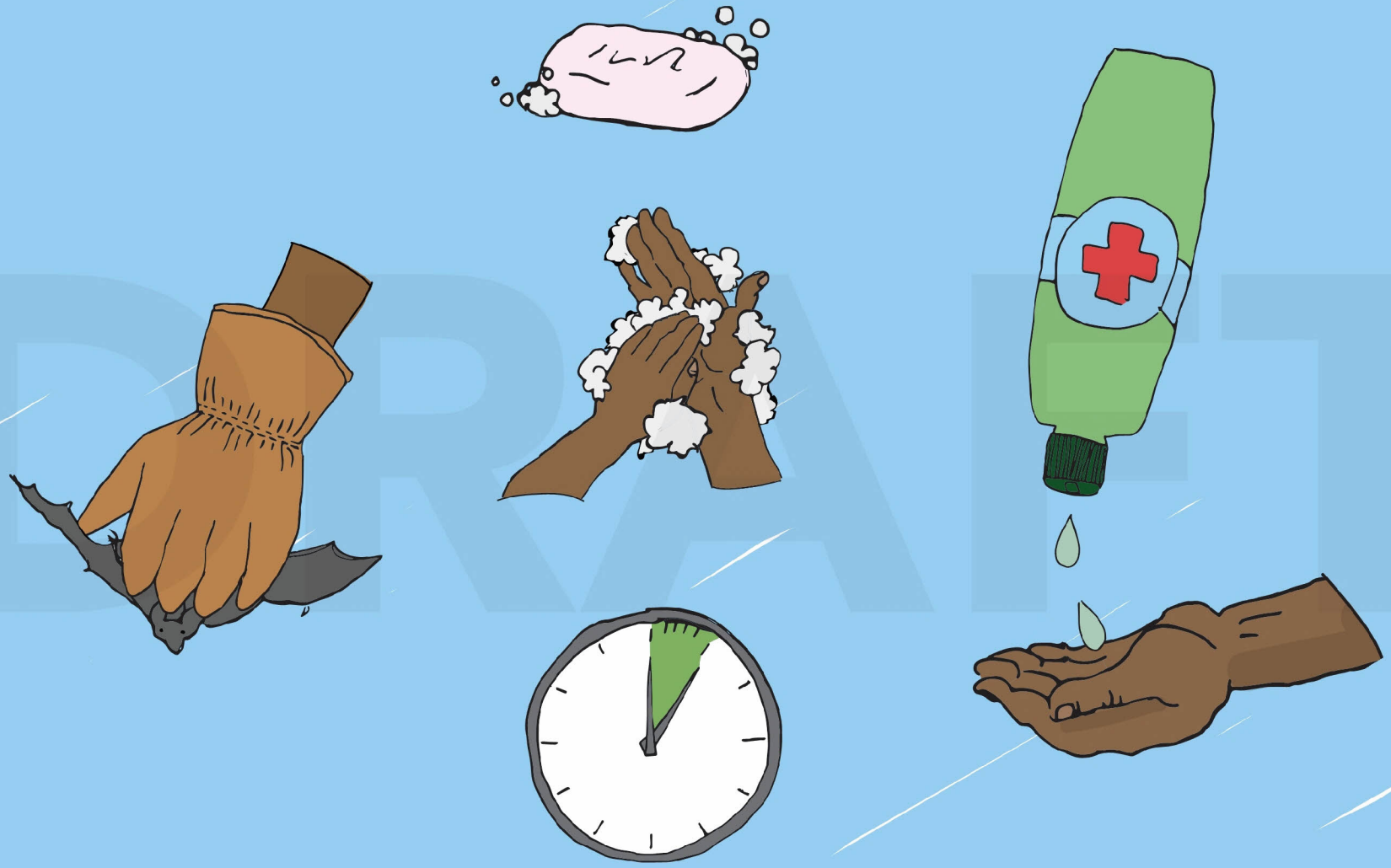
4a. If Contact with Live Bats is Unavoidable

The best way to live safely with bats is to avoid all contact with them. However, if handling live bats is unavoidable, protect yourself from their bodily fluids (such as their spit, blood, urine, or feces). Even if a bat does not look sick, it may still be carrying a disease!

If spit, blood, urine, or feces enters the eyes nose or mouth, the area should be washed very well with water for five minutes. Do your best to wash your skin where you may have had contact, in addition to any other surfaces that may have bat bodily fluids on them.



If Contact with Live Bats is Unavoidable



4b. If Contact with Live Bats is Unavoidable

First Aid if Bitten or Scratched

- Wash hands thoroughly with soap and water for at least five minutes.
- If available, use something that can kill viruses, such as a disinfectant wipe.

If Contact with Live Bats is Unavoidable

4c. If Contact with Live Bats is Unavoidable

First Aid if Bitten or Scratched

Go see a doctor if you have been bitten by a bat to get medical care, including a rabies vaccine.

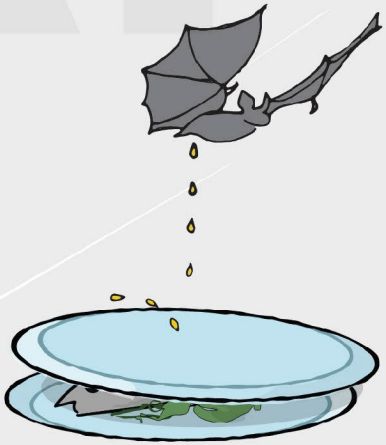
Managing Bats in the Home

5. Managing Bats in the Home

There are many kinds of bats that live around villages or in cities. Most bats that would live inside buildings or houses are smaller, insect-eating bats. Normally, bats are harmless and will not bite or scratch people if left alone. However, on occasion, bats can be infected with viruses such as rabies, which can make them act mean and more likely to bite or scratch, which is how the virus is spread to people.

Because some bats with rabies may also act normally, direct contact with all bats and their bodily fluids should always be avoided. Avoiding contact with bats and their excretions, such as droppings or "guano", is the best way to prevent yourself from getting sick from any disease they may be carrying.

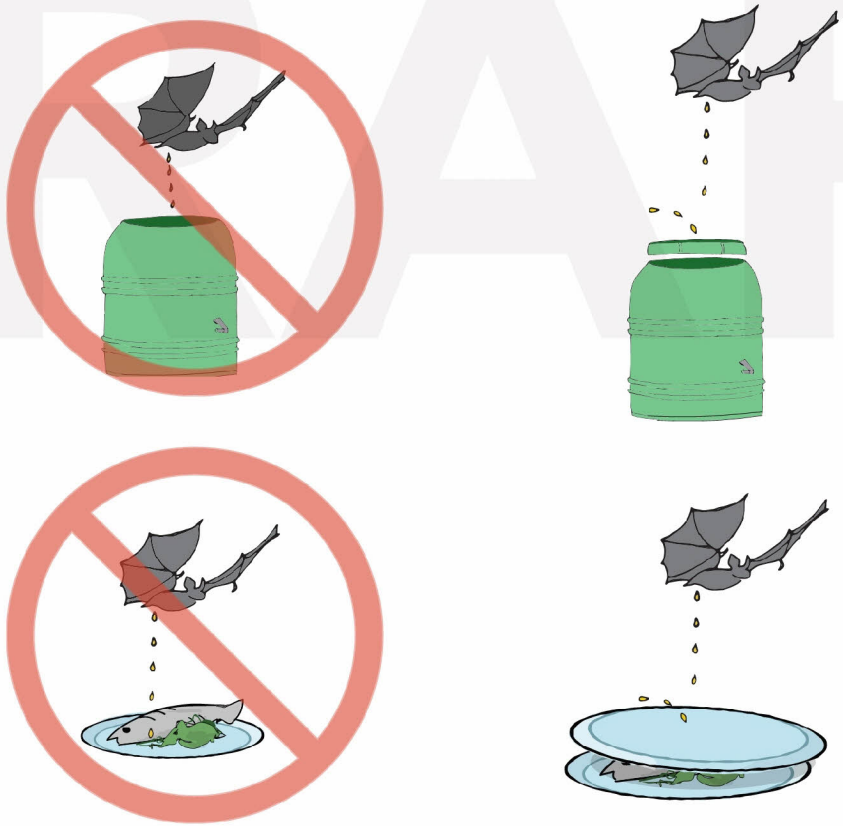
Managing Bats in the Home



5a. Managing Bats in the Home

Removing Things that are Attractive to Bats

Bats may be attracted to uncovered sources of water and other liquids. When bats drink from these sources, they can contaminate it with their spit, urine, or feces. To prevent bats from being drawn to liquids or foods in your home, keep them securely covered.



Managing Bats in the Home

5b. Managing Bats in the Home

Preventing Bats from Entering your Home

The most effective method for preventing bats from entering a building or house is to make sure that there are no holes or extra spaces around your doors, windows, ceiling, and roof where they can enter. Screens or netting can be used to cover windows and doors, and you can use caulk, sealant, stainless steel wool, or wet newspaper to close up any holes.

Managing Bats in the Home

5c. Managing Bats in the Home

Bats typically prefer to hang from the ceiling at the highest point in a room, such as the peak of a pointed ceiling or roof. Many of the bats that roost in buildings are small and dark, so may be hard to see. Smaller bats are able to squeeze into tiny spaces, such as between boards or between thatch and wooden frames. They will often huddle together in groups of either a few (3 or 4) or many (25 to 30) bats.

A sign that there may be bats in your house is the presence of feces on the floor. Bat feces or "guano" appears as small, black rice grain-sized pellets. There may also be visible streaks of urine running down the wall from the ceiling, which is another sign that there may be bats on the ceiling or roof.

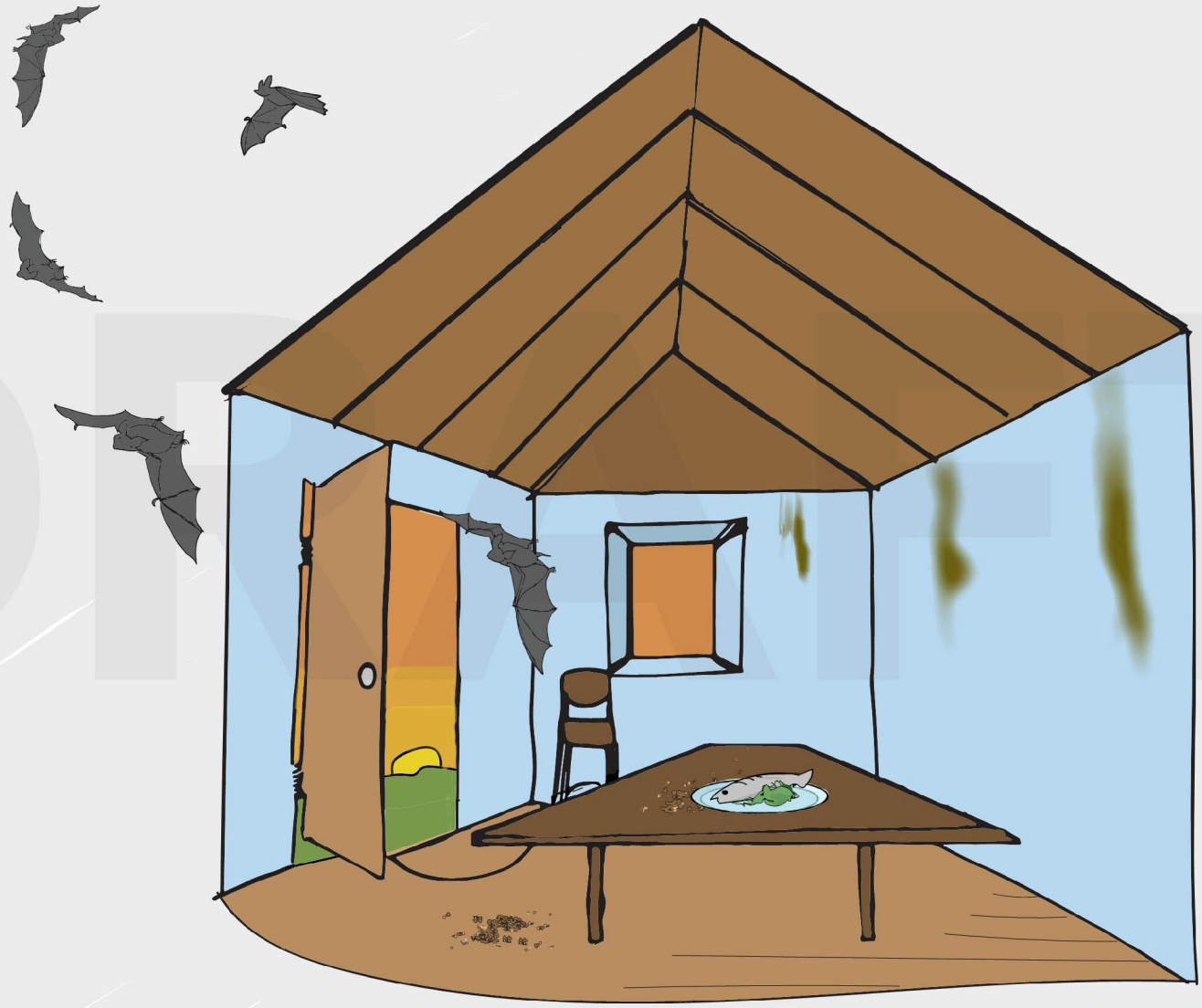
Managing Bats in the Home

5c. Managing Bats in the Home

Removing Bats from a Building or House

If a professional is not available to help you get bats out of your home, there are a few things you can do. First, before cleaning up bat excrement that has fallen from the ceiling and before anything else, use an air tight face mask to reduce the risk of breathing in dust from bat feces that might be harmful for you. If you cannot find a face mask, cover your face with a wet cloth or bandana.

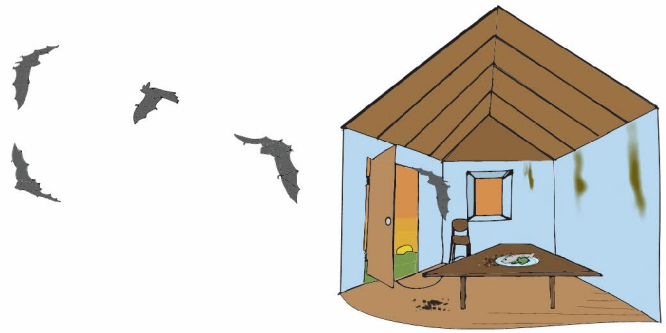
Managing Bats in the Home



5c. Managing Bats in the Home

The best time to remove bats from a building is after dark, when bats have likely left to feed outside. Bats typically become active around dusk, but may leave at any time after sunset. It may be necessary to spend a night or two watching their patterns of exiting the house, as they don't all leave the roost at the same time. It is important to avoid trapping bats inside such that they are unable to leave.

After bats have left to feed outside after dusk, if it is possible, close off all entry points into the house by placing screens or netting across windows and doors, ensuring that there are no holes in the roof that would allow bats to enter. You can use caulk, sealant, stainless steel wool, or wet newspaper to close up holes in or near the ceiling through which bats may enter. Small, insect-eating bats are able to enter buildings through very small openings. Remember that if you are able to fit a small finger into an opening (the finger test), insect-eating bats may be able to enter.



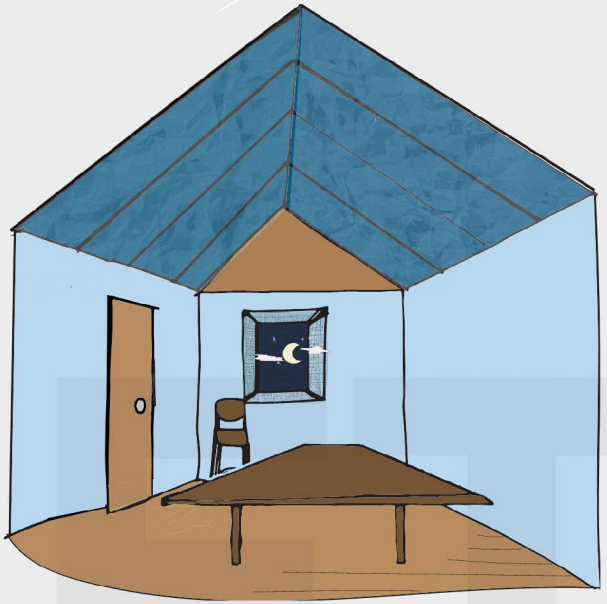
Managing Bats in the Home

5c. Managing Bats in the Home

Removing Bats from a Building or House

Bats have been known to live in buildings especially when they are pregnant. During this time, baby bats (pups) remain at the home while the mother leaves to collect food. You should not try to get rid of bats from a building before the baby bats are able to fly, otherwise they will starve to death when separated from their mothers. Also, when forcefully separated from their babies, bat mothers may look for other ways to get in.

Managing Bats in the Home

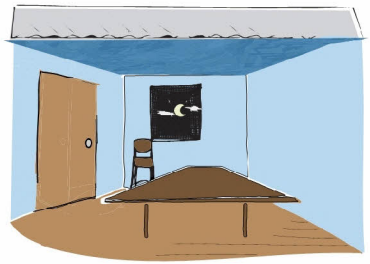


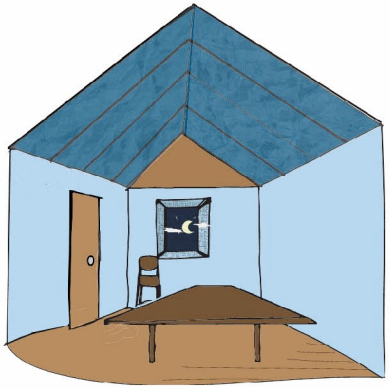
5c. Managing Bats in the Home

Removing Bats from a Building or House

Next, to prevent the bats from making a home in the ceiling, a plastic sheet or tarp can be placed flush against the ceiling to prevent bats from easily finding a stable place to hang. All houses are different, so you may choose different ways of hanging the sheet, depending on the way your house is constructed. For example, if you have a thatched roof and air flow is an issue, you may want to hang the sheet slightly below where bats roost, and above where people live, eat, and play.

It will be important to continue to regularly observe the ceiling and exit points, as well as check the plastic sheet for signs of bat guano, as the bats may be entering holes in the roof and flying between the ceiling and the sheet. If there are still bats, regularly clean the sheet while wearing gloves and covering your face with a mask and continue to close off any entry points as described above until the bats are completely gone.





Managing Bats around the Outside of your Home

6a. Managing Bats around the Outside of your Home

Bats often live and roost in trees around your home. Trees where bats live should be roped or gated off so that farm animals and domestic animals do not directly lie under or eat under these bat roosts. This will help prevent contamination from bat urine or feces and prevent bats from getting into the food that these animals are eating.

Managing Bats around the Outside of your Home

6b. Managing Bats around the Outside of your Home

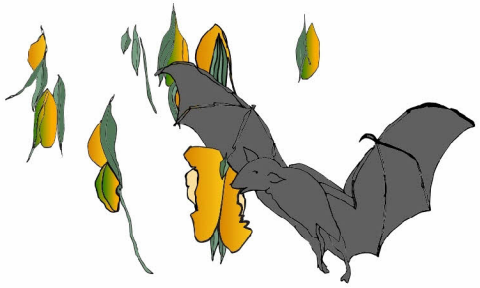
Children should also be discouraged from touching sick or dead bats that may be found on the ground near a tree where they are roosting. Live bats found on the ground are likely sick or hurt, and can be a source of disease. If a dead bat is found on the ground, avoid directly handling it. Instead, follow the advice on pages 15 and 16 on how to handle dead bats.

Managing Bats around the Outside of your Home

6c. Managing Bats around the Outside of your Home

Some bats eat fruit, such as mango or guava. Because fruit dropped by bats may have bat spit on them, eating this fruit may transmit disease to a person or animal. Avoiding direct contact with bats and the food or other materials with which they may have had contact is an important practice to reduce the risks of their viruses being transmitted to people. Bats tend to prefer over ripe fruit, so harvesting fruit on time could reduce the number of fruits that are bitten by bats and reduce the number of bats that feed on your tree.

Remember, to reduce the risk of getting sick, it is best to avoid eating fruit from the ground or feeding it to livestock, especially if the fruit has been partially eaten by another animal. You should never eat or drink something you think bats have come in contact with. Do not try to save food that has been partially eaten by bats by removing the sections with obvious bite marks. The entire item may have been contaminated.



The End.
Any questions?