U.S. China Dialogue and Workshop on the Challenges of Emerging Infections, Laboratory Safety, Global Health Security and Responsible Conduct in the Use of Gene Editing in Viral Infectious Disease Research

Harbin Veterinary Research Institute |Chinese Academy of Agricultural Sciences

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OVERVIEW

To address the interconnected life science challenges associated with combating infectious diseases and further enhance cooperation between China and the United States in the areas of global health, biosafety, and biosecurity, the U.S. National Academy of Sciences (NAS), and the Chinese Academy of Agricultural Sciences (CAAS) in partnership with representatives from other Chinese institutions held the fourth *U.S. China Dialogue on the Challenges of Emerging Infections, Laboratory Safety and Global Health Security* in tandem with a *Workshop on Responsible Conduct in the Use of Gene Editing in Pathogen Research* on January 8-10, 2019 at the CAAS Harbin Veterinary Research Institute (HVRI) in Harbin, China. HVRI is one of three institutes in China that operate a Biological Safety Level-4 (BSL-4) laboratory.

The meeting, ultimately titled *U.S. China Dialogue and Workshop on the Challenges of Emerging Infections, Laboratory Safety, Global Health Security and Responsible Conduct in the Use of Gene Editing in Viral Infectious Disease Research* was the latest in a series designed to share information on research relevant to combating emerging diseases of concern to the U.S. and China, promote common understanding of high biological containment laboratory safety, security and responsible research risk management, and build relationships among members of the U.S. and Chinese research communities who conduct infectious disease research. The special focus on research that uses gene-editing technology gave the opportunity to

participants to discuss ways to create norms and reduce risks when using these tools for research

Several themes emerged during the workshop. The first was the importance of pursuing an integrated, One Health-based approach to understanding the causes and addressing the effects of animal and human diseases. The second was a growing appreciation for the widespread use of gene-editing tools, particularly those based on the CRISPR-Cas9 system, as part of many recent research and development efforts. These tools have been used to facilitate a number of the genetic modifications described during meeting presentations (see Box 1 at the end of this document). The final theme running through several presentations and discussions was the importance of establishing and maintaining institutional cultures that support safe and responsible conduct of science. Appropriate scientific community cultures and norms form part of the foundation necessary for achieving success in high-containment infectious disease research. Many of the sessions, summarized further below, provided the opportunity to highlight this area.

INTRODUCTION

On January 8-10, 2019 approximately 45 scientists from the U.S. and China gathered at the Harbin Veterinary Research Institute for a meeting hosted by the Chinese Academy of Agricultural Sciences, Chinese Academy of Sciences, and U.S. National Academy of Sciences. The meeting was the latest in a series of dialogues aimed at sharing information on relevant research conducted in the partner countries, promoting common understanding of high containment laboratory safety and responsible risk management, and building relationships among members of the U.S. and Chinese research communities who conduct infectious disease research.

The following sections summarize the presentations made during the 2.5 day meeting. The agenda and participant list for the meeting is available at the end of this document.

WELCOME AND OPENING REMARKS

The workshop was opened by **Dr. Zhigao Bu**, director of the Harbin Veterinary research Institute (HVRI) of the Chinese Academy of Agricultural Sciences (CAAS), who chaired the session of remarks from convening organizations. Dr. Bu welcomed participants to the 4th US - China dialogue on emerging infections, biosafety, and biosecurity. He looked forward to the opportunity to exchange ideas and to promote collaboration and cooperation, and expressed his thanks to the members of the organizing committee for their hard work in preparing the program.

Next, Dr**. Kongming Wu**, Vice president of CAAS, welcomed participants to Harbin and expressed his hope that the dialogue and workshop will make important contributions to the prevention and countering of emerging infectious diseases, along with the promotion of bioethics, biosafety, biosecurity, and laboratory safety. China and the U.S. share many common

interests in addressing these challenges. Through this meeting and the ongoing dialogue, China and the US are working together to build a shared community that supports public health security in accordance with principles of One Health, an approach that integrates human, animal, and environmental health concerns. Dr. Wu noted that international cooperation has become ever more important in addressing issues in disease research and promoting innovation. CAAS is ready to support fruitful collaborations with the U.S. and with partners around the world, and he wished the workshop success in its discussions.

Benjamin Rusek from the U.S. NAS welcomed the participants on behalf of the U.S. National Academy of Sciences. He noted that the purpose of the meeting is to improve scientific and technical cooperation to address public health threats of mutual concern and interest to China and the U.S., discuss ways to ensure the safe and secure conduct of life science research and high containment biological laboratories, and to build bridges between Chinese and American scientist and institutions. The meeting was designed to especially focus on ways to reduce risk and create norms for research on pathogens using in gene editing technology. Although the benefits of using gene editing technology are potentially enormous, there is the potential for malevolent application or for the technology to contribute to dangerous bio error. He thanked the CAAS for has assembling an excellent group of scientists and experts to Harbin and noted that the scientists from the U.S. Department of Agriculture would not be able to attend due to the U.S. Government shut down but that other members of the U.S. delegation would give their presentations on their behalf. He encouraged everyone to participate and read and use the discussion questions listed on the agenda during every session.

The final welcome address was provided by **Dr. Diane Griffin** from the Johns Hopkins University Bloomberg School of Public Health and Vice President of the U.S. National Academy of Sciences. She emphasized that, as the 4th dialogue of this series, these meetings have played an important role in bringing together scientists interested in addressing the challenges of emerging infectious diseases. Given the global implications of disease outbreaks, the ability to discuss such issues and to stimulate collaboration among scientists is vital. As the meeting moved into its primary scientific sessions, Dr. Griffin also encouraged participants to ask questions and to engage in active discussions over the course of the multi-day meeting.

KEYNOTE ADDRESSES

Keynote presentations were provided by **Dr. George F. Gao**, Academician and Director-General, Chinese Center for Disease Control and Prevention, **Dr. Linda Saif**, Distinguished University Professor, Ohio State University, and **Dr. Hualan Chen**, Academician of the Harbin Veterinary Research Institute.

Dr. Gao provided an overview of China's preparedness for outbreaks of pandemic diseases, particularly influenza, and a look to the future for disease control. He noted that China plays an important role in global public health, but is a relative newcomer to this area. A key approach to disease preparedness and response, emphasized by Dr. Gao and in multiple talks over the course of the meeting is the concept of One Health, in which the veterinary and agricultural

sciences, as well as human health communities, are collectively engaged to address disease issues.

Influenza remains one of the most serious infectious agent of concern to the China CDC for potential disease pandemics. The 1918 pandemic caused by H1N1, for example, is believed to have originated in China and caused over 40 million deaths worldwide. Pandemics occurred in 1957 (H2N2), 1968 (H3N2) and 2009 (again caused by H1N1, this time emerging in Mexico). Influenza strains can spread rapidly via migrating birds, and as a result, China has focused on developing its disease control capacity, including initiating influenza surveillance systems in the 1950s and joining the World Health Organisation (WHO)'s global surveillance network in the 1980s. Dr. Gao noted that the 2003 outbreak of Severe Acute Respiratory Syndrome (SARS) resulted in further investment and capacity building to support public health, including in laboratory diagnostics and efforts to develop various vaccines, as well as ongoing strong communication with international networks such as WHO and the World Organisation for Animal Health (OIE). In 2010, China collected 200,000-400,000 samples and conducted antigenic analysis on approximately 20,000 viral strains. He pointed to remaining challenges in political commitment and public awareness, including the need for effective risk communication around infectious diseases such as influenza, as well as improved data and sample sharing between animal and human health research communities.

Dr. Saif focused her remarks on coronaviruses, a diverse family of RNA viruses that have four main subtypes (alpha, beta, delta, and gamma) along with a broad host range. The high polymerase error rate and high recombination frequencies in coronaviruses leads to the generation of significant viral diversity and contributes to the emergence of new strains that can have altered tissue tropism, changes in virulence, and ability to infect new hosts. Coronaviruses primarily cause enteric and respiratory infections in mammals and birds, and a number of events have occurred in which viral strains have moved from animal species to humans.

Dr. Saif's presentation emphasized the potential for interspecies transmission of emerging swine coronaviruses. She described aspects of the research conducted by her group on the receptors involved in viral entry into host cells and on genome changes that affect viral properties. For example, the porcine delta coronavirus can infect human cells in culture via the aminopeptidase N (APN) receptor, indicating that there may be possible human susceptibility to this virus. Recently, swine acute diarrheal syndrome was identified in China and may represent a recombination between a bat alpha coronavirus and an unknown beta coronavirus. Her research has also investigated the role of co-infections in disease susceptibility and severity (for example, co-infections with other respiratory pathogens and coronaviruses SARS or MERS). However, many questions remain to be addressed in understanding these and other emerging coronaviruses. There are bird, bat, ruminant, and rodent reservoirs for coronaviruses. Needs include a better understanding of how these viruses circulate among different species and the roles played by the high-density congregation of multiple species and of migratory birds in the process of viral adaptation to new species and the establishment of new reservoirs. Gaining an

increased knowledge of how particular mutations affect virulence and tropism will also be important to underpin the future development of vaccines and improved response strategies.

Dr. Chen's presentation concentrated on the control of influenza outbreaks caused by strains H5N1 (which first emerged in 1996) and H7N9 (which first emerged in 2013). A number of studies were subsequently undertaken to understand the genetic basis for H5N1 viral properties, such as host range, virulence, and transmission, and to use this information as the basis for developing both inactivated virus and recombinant vaccine platforms for use in poultry. Over 230 billion doses of the poultry vaccines have since been used in China as well as in other countries to support poultry disease control and to help prevent spread of H5N1 to humans.

More recently, extensive efforts in H7N9 disease control have been undertaken in China. Following its 2013 emergence, concerns included whether H7N9 can evolve to become highly pathogenic in poultry and whether it could become human-to-human transmissible as suggested by ferret studies. Dr. Chen's research included studies of mutations in viral HA and PB2 proteins that affect virulence in different species. These genetic mutations detected in chickens affected lethality and transmissibility in ferrets and mice, suggesting potential risks for humans. As a result, China conducted large-scale H7N9 surveillance. During 2017, highly pathogenic H7N9 caused a severe outbreak in chickens and a new (5^{th}) wave of infections in humans with 766 cases reported. Dr. Chen and her group drew on the vaccine platforms developed for controlling H5N1 as a basis for developing avian H7N9 and bivalent H5/H7 vaccines, after which a vaccination campaign was undertaken in chickens. Post vaccination surveillance revealed decreased rates of H7N9 isolation and the vaccination appears to have successfully helped prevent a 6^{th} wave of human infection. These examples illustrate the importance of addressing animal health and of using animal vaccination campaigns to help prevent human disease.

Discussion
The discussion addressed questions on conducting environmental surveillance and the challenges of going from genetic sequence to information on structure and function. It was noted that what can be accomplished depends on available resources, although the Global Virome Project (http://www.globalviromeproject.org/) was pointed out as a valuable example of an international effort. Scientific dialogue, as well as the need to improve data and sample sharing was also discussed, and Dr. Gao highlighted China's productive collaboration with Africa CDC

INFLUENZA
The following sessions of the meeting – on influenza and swine fever – were aimed at promoting further understanding of two serious infectious diseases of concern to both China and the U.S.

Dr. Ralph Baric from the University of North Carolina, Chapel Hill delivered the presentation on behalf of **Dr. David Swayne** from the Agricultural Research Service of the U.S Department of

Agriculture, since Dr. Swayne was unable to participate in the meeting. Reservoirs for avian influenza exit in wild aquatic fowl such as ducks, and can spill over to domestic poultry such as chickens through exposure and adaptation. China and the US are the top two global producers of poultry, and have common interests in controlling disease outbreaks. A number of control strategies have been employed to stamp out outbreaks, including diagnostics and surveillance to identify infected poultry, culling to eliminate infected birds, biosecurity measures aimed at preventing movement of strains between farms and infection of naïve farms, and education about responsibilities to prevent and respond to the disease. But a number of questions remain. These include "How do low pathogenicity avian influenza (LPAI) viruses move from wild waterfowl reservoir to domestic agricultural systems? Can we "predict", based on environmental/management conditions at wild bird-agricultural interface and surveillance data, and intervene to prevent such LPAI virus transfers? Can we better understand & intervene to prevent LP → HP viruses in terrestrial poultry? How do we improve AI eradication within agricultural systems?"

In 2017, highly pathogenic avian influenza (HPAI) was detected in the US. Studies showed that insertion of nucleotides from chicken 28S rRNA in the HA cleavage site in the virus genome was a marker of the emergence of HPAI. Further phylogenetic analysis was undertaken to explore common ancestors and virus introduction events across several states. The results showed that low pathogenic avian influenza (LPAI) strains had been circulating in US poultry farms for several months prior to the HPAI emergence.

Dr. Swayne also provided an example of the global spread of highly pathogenic avian influenza H5N8 in 2014. The virus spread and evolved during the wildfowl breeding season in areas such as Siberia, then moved into Europe and the US along the Pacific coast flyway. Viral reassortment with North American avian influenza strains led to outbreaks in 2014-2015. The example shows how strains can spread around the world and evolve in combination with circulating regional strains. Enhanced surveillance along major flyways and in poultry agricultural systems will be critical to better understand the movement of avian influenza viruses and likely outbreaks, which provide a major incentive for countries to collaborate on disease control. Global networks such as the World Organization for Animal Health and Food and Agricultural Organization Animal Influenza Network (OFFLU) are very important in this context.

Dr. Dayan Wang of the National Institute for Viral Disease Control and Prevention of China CDC discussed additional efforts by China to study and control avian influenza outbreaks. China established its national influenza center (CNIC) in 1957 in the wake of the "Asian flu" and has more recently expanded the national influenza surveillance network so that it now includes over 400 network laboratories and multiple sentinel hospitals covering the major cities of the country. Influenza H5N6 is a current concern because of a high positive rate in poultry along with sporadic human cases.

Dr. Wang's presentation highlighted additional examples of research on H7N9 and H1N1. She noted differences in characteristics of human infections between these viruses – with older

people, for example, seeming to be more susceptible to infection with H7N9 and an apparent 2:1 male to female infection ratio whose reasons remain unknown, compared to H5N1 that seemed to infect younger adults. Her research on H7N9 has explored genetic variants, receptor binding capacity, tissue tropism, transmissibility in various animal species, and phylogenetic analysis of outbreak strains. Similarly, her laboratory has conducted research on the pathogenicity, tropism, and other viral properties associated with two genotypes of a European (EA) variant of H1N1, in which 12 cases of human infection have been identified since 2010. These two genotypes have similar tropism but show differences in virulence, although there is no clear evidence yet for human-to-human transmission. These ongoing studies will help to clarify the genetic basis for properties of avian influenza viruses and inform additional future research. Dr. Wang also noted that her institute has launched a new journal of Biosafety and Health sponsored by the Chinese Medical Association, and that this may be a publication of interest to the workshop.

Dr. Chengjun Li, a professor at HVRI, delivered the final presentation in this session. He highlighted the virus life cycle and key proteins encoded by the influenza virus genome, as well as host factors that interact with the virus at various stages of the infection process.

For example, the influenza ribonucleoprotein (RNP) complex is a critical functional unit required for viral transcription and replication. The host enzyme phospholipid scamblase 1 (PLSCR1) interacts with both the viral nucleoprotein (NP) and the host protein importin alpha. PLSCR1 interactions impede the interaction of importin alpha with importin beta, which are involved in the import pathway of the virus into the host cell nucleus, inhibiting this process. A second host factor investigated by Dr. Li, the enzyme PIAS1, also interacts with proteins in the import complex. PIAS1 interferes with assembly of the viral RNP complex and inhibits viral polymerase activity. Generation of heterozygous PIAS1 knock out mice results in enhanced pathogenicity as measured by titer and post-infection mortality. Finally, Dr. Li reported on mutations in the H7N9 polymerase that are key determinants of the adaptation of avian influenza virus to mammals. He reported that a mutation in the influenza PB2 protein at position 627 from glutamic acid (E) to lysine (K) was often acquired by avian H7N9 during replication in mammals. Different avian influenza viruses appear to differ in their capacity to acquire this mutation. Research is continuing to investigate mechanisms for this mutation along with other polymerase mutations and interactions with host factors as part of efforts to better understand the biology of the disease.

Discussion

Differences in the ability to control outbreaks of H5N6 versus H7N9 were discussed, including the role of host immune factors and the question of whether the viruses can escape from the inhibition provided by the host immune response. Strategies to control influenza outbreaks were also discussed, including the use of vaccination in chickens compared to culling. Culling has been commonly employed as a strategy in the US. Participants from China indicated that active animal surveillance programs identified influenza virus continuing to circulate even with culling, leading to the decision to undertake vaccination. It was noted that mass vaccination

campaigns can place new selection constraints on a virus, and it will be valuable to continue disease surveillance and research to understand the effects over time.

SWINE FEVER

The first three presentations in the session focused on African swine fever. The final presentation discussed advances in generating transgenic pig hosts for resistance to classical swine fever.

Dr. Xiaoxu Fan delivered the first presentation on control of African swine fever in China on behalf of **Dr. Xiaodong Wu**, professor at the China Animal Health and Epidemiology Center, as Dr. Wu was unable to attend the meeting. Although Africa Swine Fever (AFS) does not produce disease in humans, it produces virtually 100% mortality in infected animals with no available treatment or vaccine. Endemic in some African countries, AFS has spread globally; it is a notifiable disease by the World Organisation for Animal Health (OIE). China has a large pork industry and the country has undertaken efforts to prevent and control the spread of the virus as well as to develop diagnostics and vaccines. However, the clinical signs of AFS can be similar to classical swine fever and to several other swine diseases, which complicates surveillance and early response. By early 2019, cases of AFS had been reported in 19 provinces.

A number of possible transmission routes to domestic pigs are possible, including from wild boars and imported poultry. The Chinese government released technical guidelines for AFS in 2015 and a contingency plan in 2017. After an outbreak in August 2018, over 30 new disease-related policies were enacted. Efforts to prevent and control outbreaks include the deployment of field working groups, culling of over 600,000 pigs, and an awareness campaign for farmers, veterinarians, and the public. The government has also increased incentives for reporting, such as by increasing compensation. Two factors were identified as key in outbreak transmission – swill feeding and movement of live pigs and pig products. Banning swill feeding, reducing long distance/cross-province movement of animals, and cracking down on animal smuggling through customs have been important parts of control strategies. Other policies require slaughterhouses to run ASF tests on pig products before sale, cracking down on practices such as illegal slaughtering, and strengthening efforts such as confirmation testing to combat cases in domestic pigs and wild boar. Strong multisector collaboration will be needed to address ASF, including international collaboration on prevention and control strategies with partners such as the UN Food and Agriculture Organization (FAO), OIE, and other countries.

Dr. Zhigao Bu of HVRI provided additional information on the genetic analysis and properties of African Swine Fever. AFS is a large double-stranded DNA virus whose host is domestic pigs and wild boars. AFS is the number one killer of pigs, with approximately 58,000 cases in 22 countries in 2018. Because China has over 50% of the pigs on hand and pigs to market worldwide, this disease is of significant concern. Dr. Bu discussed an outbreak at several sites in Heilongjiang province in 2018, and reported phylogenetic comparison of the strains with those from other AFS outbreaks. In addition, researchers at HVRI successfully isolated the virus and conducted infection tests in pigs in high containment laboratory facilities to study properties such as

incubation time, viremia/titers, virus shedding, and time to death. As expected, the AFS strain was highly lethal.

Dr. Bu noted reports of two deletion mutations - delta CD2V and delta DP148R – that might attenuate the virus and could be candidates in the development of vaccines. However, results suggest that the mechanisms of pathogenicity are complicated and the mutant strains did not necessarily result in attenuated virulence plus protection against ASF challenge in pigs.

Dr. Pei-Yong Shi of the University of Texas Medical Branch, Galveston delivered the final presentation on African Swine Fever. He presented on behalf of **Dr. Douglas Gladue** of the U.S. Department of Agriculture's Plum Island Animal Disease Center, who was unable to attend. As noted in the prior presentation, the immune mechanisms associated with protection from African Swine Fever have not yet been identified, no commercial vaccine is available, and the mechanisms of protection associated with experimental vaccines are not well understood.

Dr. Gladue's research includes strategies to more efficiently develop and purify recombinant ASF virus as part of the development of live attenuated viral vaccines. Current processes can take up to two years. Dr. Gladue's group incorporated a fluorescent reporter gene to improve identification of the recombinant plasmid and used CRISPR/Cas9 editing to delete the locus 8DR. The protein encoded by this locus is involved in viral rosette formation and the deletion enabled them to quantify the extent to which use of CRISPR/Cas9 increased the rate of obtaining desired genetic changes. Dr. Gladue reported that combining the florescent marker and 8DR deletion enabled them to achieve significant increases in the efficiency and speed of generating, identifying and purifying recombinant virus, which could thus be produced in 3-4 months instead of years. These results demonstrates the possibilities enabled by using gene editing to engineer AFS. Finally, Dr. Gladue provided information on the use of live attenuated viral vaccines to combat the current AFS outbreak strain. Multiple deletion mutations have been created and studied, including delta 9GL, delta MFG, the double mutant delta 9GL/MFG, delta UK and the double mutant delta 9GL/delta UK, and delta CD2 for both viral attenuation and the ability to induce protection when administered as a live vaccine. However, alternatives will need to continue to be explored along with further collaborations to combine the advantages of various platforms.

The last presentation in the session was provided by **Dr. Hongsheng Ouyang**, a professor at Jilin University, who focused on the generation of genome-edited pigs as part of efforts to understand and control classical swine fever virus. Dr. Ouyang's research makes use of the tools enable by CRISPR/Cas 9 to edit genes. There are currently two main methods for generating genome edited pigs – direct microinjection of Cas9 mRNA and single guide (sg)RNA into zygotes and somatic cell nuclear transfer. Edited embryos are then implanted into surrogate pigs, resulting in the birth of edited piglets.

Dr. Ouyang focused on efforts to design and screen a short hairpin RNA (shRNA) that, when expressed in the pig genome, would act to target and silence the infecting virus. A plasmid containing the sequence that would produce this shRNA along with the CRISPR/Cas9 machinery

was introduced into the nucleus of a pig cell via electroporation, followed by somatic cell nuclear transfer and the generation of edited piglets whose cells produce the designed interfering RNA molecule. The aim was to generated edited pigs that would be resistant to classical swine fever. Challenge experiments were conducted in which both wild type and edited pigs were exposed to a swine fever-infected pig. During swine fever infection, the transgenic pigs exhibited greater antiviral responses than the wild type, unedited pigs. Dr. Ouyang also investigated whether mutations or deletions in the LamR receptor, which is the cellular receptor for classical swine fever, could result in pigs resistant to swine fever infection. The generation of transgenic pigs and initial tests in cell culture showed some resistance but more remains to be investigated.

Discussion

Questions were directed to the issue of what needs to be done to prepare for and control African Swine Fever. To date, there have been limited studies on T cell responses and neutralizing antibody titers are not closely correlated with protection, indicating that significant work remains to understand the biology and immunology of ASF infection. The need to implement traditional control strategies was noted, while research toward vaccine development continues. For example, swill feeding and long distance pig transportation have been factors in prior outbreaks, leading to changes to policies and practices.

UNDERSTANDING AND ENGINEERING VIRAL PATHOGENS WITH PANDEMIC POTENTIAL

The remainder of Day 1 focused on science and responsibility in studying pathogens with pandemic potential. The sessions included a discussion of challenges and opportunities in altering pathogen properties as part of research into understanding and combating disease, as well as the responsible planning and conduct of such research so that it can be conducted safety and securely.

Dr. Ralph Baric, a professor at the University of North Carolina Chapel Hill and an expert in coronaviruses, provided an overview of the selection and design of pathogen properties, along with information gaps and barriers. He noted the rapid pace of advance and decreasing cost of nucleic acid synthesis; the first coronavirus to be synthesized cost roughly $42,000, a price that would now be $6,000. The largest genome currently synthesized is a 520kb mycobacterium, indicating that it is now possible to synthesize the genomes of most RNA and DNA viruses. In addition, high fidelity sequences are available for many viruses, rendering it possible to synthesize viral genomes and recover viable virus for many strains.

Studies to alter pathogen properties of viruses can use several approaches, including selection pressure to drive evolution toward a phenotype as well as deliberate design. Potential opportunities might include building chimeric viruses with altered structures for the receptor for viral entry, or those that incorporate changes to other virulence determinants or that modulate host-pathogen interactions. Dr. Baric noted the example of adding the spike protein

from a mouse coronavirus to a bat coronavirus, which can produce a strain that is more virulent in mice. But he cautioned that a combination of techniques, including selection pressure from passaging in mice, is generally needed to generate virulent strains in a host and reminded the audience of the law of unintended consequences and the possibility of surprise.

Dr. Baric also noted that predictive modeling of protein interfaces and the use of such models for structure-guided virus design has recently improved, providing advancing capabilities for affecting host-virus interactions and altering antigenic properties. Similarly, he noted that the "shopping list" of virulence determinants continues to grow as more is learned about host-virus interfaces and how they evolve as viruses move through different species. As examples of both potential opportunities and ongoing barriers to effective engineering of viral properties, Dr. Baric referenced studies to develop a self-replicating alphavirus containing HIV sequences for use as an HIV vaccine, an approach that was abandoned when the virus was too pathogenic in mice. He also noted the barrier provided by a glycosylation site that makes it relatively easy to create mouse-adapted SARS strains but not mouse-adapted MERS. And the use of newer tools such as CRISPR/Cas-based gene editing to create a mouse model by introducing the human virus binding receptor domain into the mouse. On the other hand, creating a virus that is super-adapted to a particular host can actually result in an attenuation of virulence, if the virus interacts overly strongly with a cellular receptor. This shows the complexity of deliberate design as well as a potential sweet spot for pathogenicity. Other challenges to deliberate design include the fact that a large number of virus-host protein interactions occur, and changing one can have unexpected effects across the interaction networks as virus-host interactions are usually a "highly coordinated co-evolved process." Viral packaging constraints, the effects of host genetic variation on disease severity, and other factors all add to the complexity and confound the utility of predictive design models.

The second presentation in the session was delivered by **Dr. Weiwen Zhang**, professor at Tianjin University, who spoke about cutting-edge biotechnology, the potential for rapid changes in science and technology capabilities to outpace ethical and regulatory measures, and the need for appropriate governance. He provided the example of recent synthesis and preparation of horsepox virus from starting genetic sequence as illustrative of what could now be achieved at relatively low cost with fairly standard technical approaches. Past oversight has been based primarily on physical control of materials such as viral strains, but access to information such as gene sequences is of increasing importance. Many countries have made significant investments in synthetic biology R&D – in China, for example, a national key program for 2018-2011 will support 50-60 projects of $3-4M each. But achieving the right balance of research innovation and governance to prevent ethical, security, or other misuses of biotechnology is challenging and it can be difficult to separate positive uses from potential misuses (a "dual use dilemma"). A number of reports and continuing global dialogues address ethics and safety associated with new technologies such as synthetic biology and genome editing, and consider related aspects such as laboratory precautions and development of strategies that could mitigate harmful effects (for example, to reverse genetic changes). Following debate, the 2016 Human Genome Project-Write (HGP-W) has become Genome Write (not just human) and the project established an international bioethics and biosafety working group. The international genetically

engineered machines (iGEM) competition for students has also focused increasingly on ensuring biosafety, biosecurity, and risk management of projects undertaken by iGEM teams.

China has undertaken a number of efforts to promote and support responsible research and governance. For example, the synthetic biology society of China formed an ethics committee in 2018. The Ministry of Science and Technology has also established an ethical and regulatory framework for synthetic biology with goals of (1) establishing norms for research and market access and making government regulatory policy recommendations; (2) proposing effective safety management norms and implementation methods; (3) establishing public channels of communication and public participation; and (4) developing intellectual property mechanisms relevant to synthetic biology. Tianjin University established programs on ethics associated with new biotechnology in 2018. China and Pakistan have also put forward a proposal to the Biological Weapons Convention on a code of conduct for biological scientists. Dr. Zhang noted that identification and management of potential dual use issues should occur early in a relevant research project as well as various stages throughout the research life cycle stages, with responsibilities by multiple stakeholders; existing regulatory policies and laws are also frequently under discussion. These conversations within the scientific and government communities are ongoing.

A separate discussion session was not held following these talks; see the panel discussion "Science and Ethics in Research with Pathogens with Pandemic Potential," below

BIOETHICS AND RESPONSIBLE PLANNING FOR PATHOGEN RESEARCH

Dr. Zhiming Yuan, professor at the Wuhan Institute of Virology, discussed the value of learning from outbreaks such as SARS and Ebola and the role of the network of global high containment laboratories in efforts to improve global health security and strengthen public health systems. The BSL-4 laboratory at Wuhan, which announced its operation in early 2018, is part of this overall system. Prior to operation, significant time was spent on design and validation of the laboratory, which successfully received accreditation by China National Accreditation Service (CNAS). Projects being undertaken at the laboratory include on Crimean-Congo hemorrhagic fever virus and Nipah virus, along with synthetic biology studies manipulating proteins from Ebola and Nipah viruses. The facility also provides services in virus preservation, diagnostics, animal model development, education, and training.

Dr. Yuan echoed comments made by prior presenters emphasizing the role of governance for dual use research of concern (for example, the use of use of biotechnology to alter host responses to pathogens). He noted the existence of Chinese laws such as Article 331 Criminal Law of the People's Republic of China, relevant regulations and guidelines, and foundational ethical principles such as non-malfeasance, and stated that "ethics is a necessary supplement to the law" in preventing misuse. As part of the responsible planning and conduct of pathogen research, both national and institutional ethics review committees are commonly established. Dr. Yuan noted, however, that many bioethical committees still have a limited focus, such as on the protection of human subjects in biomedical research. Biorisk extends beyond this narrow

focus. It is a multi-stakeholder issue and there are a number of questions to consider when assessing benefits versus risks of a research effort. Biorisk management thus exists at an intersection that draws on biosafety, biosecurity, policy, ethics, society, economics, and science, animal and human health. Key players who need to be involved in these discussions include scientists, publishers, funders, accreditation bodies, and others while biorisk management occurs at multiple stages of a project from conception, preparations, application, and implementation to publication. He supported the need to establish a code of conduct for scientists and the importance of establishing a global network of biosafety laboratories to enable sharing of best practices, collaboration, and information exchange.

Dr. David Relman, a professor at the Stanford School of Medicine, continued the discussion of benefits and risks in pathogen research. He noted the imperative to understand and anticipate emerging infectious diseases in order to prepare for, prevent, and respond to them, along with the growing technical capabilities in the biosciences.

Dr. Relman highlighted the importance of the critical combination of pathogenicity and transmissibility, which affect the harm something can do and the means by which it can be disseminated. He also noted the need to understand things that have arisen in nature compared to things that have been created by human design. Dr. Relman suggested that the scientific community can learn from the global virome project, which is expanding viral discovery and generating large amounts of sequence information. The project will provide a more complete understanding of biodiversity and may inform diagnostics and therapeutics development. For example, analysis of the data collected may identify new pathogens or help to predict potential virulence factors. But in an era in which most viruses can be synthesized from a genetic sequence, the discovery new viruses and elucidation of their properties may present both biosafety and biosecurity concerns. As scientific and technical capabilities continue to expand, Dr. Relman emphasized the power and responsibility of the individual, along with recognition that the decisions investigators make about the research they undertake can have consequences. It is very difficult to quantify risks and benefits or to gain agreement on what should be done, but he suggested that a focus of assessment and decision-making should be the purpose of the work and end state product, not the means or process by which it is achieved. Dr. Relman noted that scientists routinely make decisions on experiments to undertake, or not to conduct, for all sorts of scientific and economic reasons. This type of calculus should be going on with regard to security as well. He supports transparency, the use of experimental design choices aimed at minimizing risks, and establishing research norms. There are roles in these efforts not only for individual researchers but also professional organizations, academia, and industry; national leadership; and international organizations. Finally, he noted that no matter how much we prepare some failures will occur. We need to be prepared to respond to such situations, emphasizing complementary strategies for risk reduction.

Finally, **Dr. Yang Xue**, Associate Professor at Tianjin University, highlighted the announcement in late 2018 of CRISPR-edited human babies and the resulting scientific and social criticism. Both international ethical frameworks and national regulations are relevant to the

considerations of biosecurity management and the encouragement of a culture of responsibility in life sciences. In the first case, documents such as the Declaration of Helsinki from the World Medical Association (2013) and International ethical guidelines for biomedical research involving human subjects (CIOMS 1993) support respect for individual welfare. There are also a number of relevant policies, regulations, and guidelines in China that could apply to the human genome editing situation. These include Ethical Guiding Principles for the Research on Human Embryonic Stem Cells (2003), Interim Measures for the Administration of Human Genetic Resources (2012), the Ethical Review of Biomedical Research Involving People (2016), and others. Institutions involved in biomedical research should set up ethics committees and file information with the practicing registration authority. In addition, they should follow Administrative Measures for the Safety of Biotechnology Research and Development Cell such as abiding "laws and administrative regulations, respect social ethics, and not harm…" (2017). Dr. Xue also presented the draft model code of conduct proposed by China and Pakistan to the Biological Weapons Convention. Finally, Dr. Xue shared some thoughts on pressures and factors that may have affected the project to create CRISPR edited babies, including publicity achieved by Dr. Junjiu Huang of Sun Yat-Sen University in 2015 for his use of genome editing for thalassemia in non-viable, non-implanted embryos. Rapid development and regulatory systems that have not necessarily kept pace with capabilities may also play roles.

A separate discussion session was not held following these talks; see the panel discussion "Science and Ethics in Research with Pathogens with Pandemic Potential," below

SCIENCE AND ETHICS IN RESEARCH WITH PATHOGENS WITH PANDEMINC POTENIAL (PANEL DISCUSSION)

The panel and meeting participants discussed the importance of institutional and scientific community cultures, along with research oversight systems, in promoting safe and ethical research.

Speakers and participants indicated that the research community has a number of responsibilities in this regard, including an awareness of potential risks and careful experimental planning. Research with pathogens can have unintended consequences because living systems are complex and unanticipated results can arise from experimental changes. There have been examples in which pathogenicity has been accidentally enhanced though experiments, thus it is important for researchers to recognize that they may not fully understand the systems they are changing, to reflect on potential risks during the experimental design stage, and to manage for the possibility of risks though use of appropriate containment levels.

Panelists and participants also discussed the role of researcher motivations and community norms for responsible conduct of research. It was suggested that researchers are often motivated by scientific curiosity, a quest for knowledge, and a desire to help others through their research, as well as a desire for respect and recognition from their scientific contributions. But it was also noted that researchers can be under pressure to produce results and

publications, and may also be motivated by financial interests and a desire for fame and influence. One participant suggested that the scientific community has not had enough discussion about how the varying motivations, incentives, and disincentives for researchers influence the ethical, safe, secure, and responsible practice of science. Other participants agreed and suggested that researchers need to keep in mind not only scientific aims but also the broader objectives of the research and the ethical and regulatory context for their experiments.

It was suggested that research laboratories and the scientific community need to continue to foster cultures that recognize that unintended results can occur from experiments, that promote the need to think through how to respond when unintended findings arise, and that provide community and peer pressure to behave within expected norms of ethics, biosafety, and biosecurity. A number of panelists and participants suggested an important role for education and training for scientists in these issues. One participant also noted that relevant education and training will need to reach beyond students from traditional life sciences disciplines to include those with engineering, computational sciences, or other backgrounds who are increasingly involved in biotechnology projects.

In addition, speakers and participants discussed the importance of institutional oversight systems for research. It was suggested that one important question for the scientific community and research institutions remains how to go about deciding what experiments to conduct and not to conduct. Through review boards, research institutions have important oversight roles as part of this process. It was suggested that institutions need to have review meetings and debates so that the institution is able to consider relevant experiments, such as those involving pathogens. In addition, review boards need to have the expertise to ask detailed questions on proposed protocols. And it was noted that there need to be strict consequences for researchers who breach the institutional policies. Finally, several participants noted the need for balanced oversight systems and regulations that will enable the conduct of appropriate experiments and research projects while preventing or minimizing misuse. It was suggested that responsible research infrastructures and cultures are already well-established in certain settings, but that there is an important role for continuing to further develop and foster these systems in other settings.

Finally, the panelists and participants briefly considered the DIY bio community and the question of what experiments could be conducted with basic scientific knowledge outside of traditional laboratory settings. For example the potential to synthesize fentanyl was raised. On the other hand, it was noted that research with pathogens still remains largely within more advanced laboratories. In general, however, it was suggested that as biological technologies become more and more accessible, there will be a continued need for outreach on review procedures around which experiments are conducted and under what conditions, as well as the broad promotion of norms of biosafety and biosecurity.

VECTOR AND HOST ENGINEERING: SAFETY AND SECURITY

The second day of the conference opened with a session on research in vector and host engineering. **Dr. Qian Han**, professor at Hainan University, provided the first presentation. He discussed the mosquito innate immune system, referred to as melanization, a process that is also involved in wound healing and cuticle formation in addition to response to infection. Dr. Han presented the conversion pathway of the key substrate tyrosine to melanin, which includes enzymes prophenoloxidase (PPO) and dopachrome conversion enzymes (DCE). Mosquitos have "mosquito specific" PPO genes that catalyze tyrosine reactions, along with other, typical insect PPOs. DCE converts dopachrome to 5,6 dihydroxyindole (DHI), a molecule with high antibacterial activity. The enzyme arylalkylamine N-acetyltransferase (aaNAT) is also involved in melatonin synthesis and perhaps other functions; similar to PPO mosquitos have a greater number of aaNAT and aaNAT-like enzymes than other insects. The substrate specificity for mosquito-type aaNAT remains unknown and the laboratory is continuing to study this area.

Dr. Han's lab has used CRISPR/Cas9 genome editing tools to generate Aedes aegypti mosquitos with nucleotide deletions leading to knock out of DCE activity. They have also generated knock out aaNAT1 mosquitos. The laboratory is interested in studying whether the editing affects the mosquito's immune response to viral infection and are looking for collaborators in this area, as they do not have the facilities for viral infection studies.

Dr. Stephen Higgs, professor in the College of Veterinary Medicine at Kansas State University provided an overview of best practices in safely and security when conducting laboratory experiments with insect vectors. He provided an extensive list of reference papers on the topics of his talk (that was provided to HVRI after the meeting).

A number of excellent references are available on the design of laboratory insectories (including a recent paper from 2007) and the arthropod containment guidelines (2003). Insectories at BSL-3, for example, need to include practical safety and security features such as airflow specifications, secure access, signage, self-closing doors, prevention of two doors being open at same time, and screens to prevent insect escapes. Other references address experimental best practices and protocols for studies using insect vectors of disease (such as a 2006 guide). For example, an artificial blood meal chamber on top of insect cages can now be used to better simulate warm blood feedings. A number of resources are also available on practices in dissecting mosquitos, quantifying virus titer, collecting saliva, and conducting analysis, among other topics.

As projects involving transformed insect vectors potentially move out of the laboratory environment and into controlled field release trials, additional considerations become important. Dr. Higgs noted 2002, 2008, and 2014 guidance discussing planning for controlled trials, including trial design and cage placement, the types of data that should be collected, and the importance of community engagement. Since the emergence of CRISPR/Cas9 genome editing, progress in engineering various insect species has been rapid; by 2018 a gene edited mosquito for reduced malaria competence had been created. Editing to create gene drive modified vectors presents special circumstances (see Box 1) and a 2017 publication discusses

operating procedures for gene drive research. Finally, he noted that a revised version of the key 2003 arthropod containment guidelines will be published in 2019 to include the latest context of technical advances. A special issues of the journal *Vector Borne and Zoonotic Diseases* will also be released in early 2019 and may be of interest to the workshop.

Dr. Tongyan Zhao from the Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences discussed vector competence for mosquito borne viruses including Dengue, West Nile, Western Equine Encephalitis, and Zika. Dengue has become a particular issue in the tropical forest border area of the Yunnan province, and the laboratory was interested in investigating whether the mosquito species Culex pipiens quinquefasciatus could serve as a vector (along with known vectors Aedes albopictus and Aedes aegypti). Blood feeding experiments revealed a virus binding protein in the midgut of Aedes species that was not identified in Culex. They also found that Dengue virus could survive in dipausing Aedes albopictus eggs, a strategy to survive overwintering. As a result, they have not found evidence to suggest that Culex is a vector for Dengue.

Similarly, the laboratory has investigated whether Culex species are potential vectors for West Nile Virus, WEE virus, and Zika virus through blood feeding and transmission studies. Culex tritaeniorhynchus, found widely in China, showed the highest vector competence for Dengue among the Culex species studies. Aedes species are also vectors for this disease. All mosquito species tested were susceptible to infection with WEE virus, including Culex tritaeniorhynchus, which is also an efficient transmission vector for Japanese Equine Encephalitis. For Zika, Aedes aegypti showed higher infection rates in salivary glands, midgut and ovary than Ae. albopictus and Cx. pipiens quinquefasciatus, though species such as Cx. pipiens quinquefasciatus can also transmit Zika to baby mice. This information on which mosquito species can be infected and transmit particular viral diseases will be valuable in informing the development of control strategies.

Finally, **Dr. Gong Cheng**, a professor at Tsinghua University, discussed arbovirus infection in Aedes and Culex mosquitos. During a typical arbovirus lifecycle, virus in host blood enters the mosquito gut environment, which has an abundant community of commensal bacteria. Dr. Cheng's laboratory thus investigated whether and how mosquito gut microorganisms affect viral replication. Use of antibiotics to eliminate gut bacteria in Ae. Aegypti led to reduced Dengue virus replication, while oral reintroduction of the isolated cultivatable bacterial species identified the bacteria Serratia marcescens as facilitating infection. The laboratory identified a secreted protein peptidase, named M60 Viral Enhancin, as key to the mechanism of action. Introducing this protein into the gut of antibiotic-treated mosquitos enhances infection with Dengue virus as well as other arboviruses such as Zika. Engineering the bacteria to knock out the enhancin protein abolished this infection-enhancing effect.

Dr. Cheng reported that the enhancin protein was first identified in baculoviruses, where it digests mucin polysaccharides and enables virus to more easily reach gut epithelial cells. It appears that the S. marcescens enhancin functions in a similar way. Both elimination of gut mucins and oral introduction of S. marcescens in field-derived mosquitos enhances viral

infection. S. marcescens has been identified in the gut of field caught mosquitos, and gaining further knowledge of this and other mechanisms that promote arbovirus infection in mosquitos is valuable for future public health and disease control research.

Discussion

During discussion a question was asked on the generation of transgenic insects in which the melanin enzymes were knocked out. It was noted that the survival rates were low, and there was a suggestion that pathways involving melanin enzymes are also involved in eggshell formation. A question was also asked on whether other gastrointestinal commensal bacteria that Serratia affect viral infection. It was noted that a number of bacteria in addition to Serratia encode an enhancing protein, or components in host blood such as microRNA might affect replication; a number of different avenues are being studied. The issue of managing ticks under biocontainment was raised and it was noted that ticks are more difficult to work with than insects such as mosquitoes because of their longer lifecycles and existence of few artificial feeding systems to aid in replacing the need to feed on animals. The emphasis in biocontainment is on preventing ticks, particularly larval ticks, from escaping. Finally, the release of gene drive modified organisms was discussed. The regulatory process for releasing gene drive modified organisms can be quite complicated, as approvals may need to be sought not only from the country of release but also from adjacent countries. There have been efforts to develop guidelines, but with the likely near-term releases of gene drive modified mosquitoes it is very important to continue to develop agreement around internationally recognized guidelines, norms and standards.

UPDATE ON ACCUTE FLACCID MYELITIS

The next session provided an update on a topic that has been receiving significant recent attention in US because of an ongoing outbreak – the disease acute flaccid myelitis. **Dr. Diane Griffin**, a professor at the Johns Hopkins University Bloomberg School of Public Health, reviewed what is known about the etiology of this disease, which is primarily identified in children and can cause rapid onset of paralysis with incomplete recovery. Gray matter spinal cord lesions are observed on MRI, particularly in the cervical area and upper extremity, though effects in the lower regions are seen as well. A prodromal febrile illness is also a common feature prior to onset of the weakness. Developing a definition that distinguishes acute flaccid myelitis from alternative diagnoses such as transverse myelitis and spinal cord ischemia has been important and a revised "restricted" definition was published in 2018. Disease outbreaks appear to have biennial periodicity with case peaks in the summer and fall of 2012, 2014, 2016, and 2018. Cases have occurred across the US as well as in ~20 countries.

Scientists and clinicians are still exploring the cause of this disease and extensive culturing and sequencing of samples from patients have been undertaken. No consistent cause has yet emerged. Although the illness presents similarly to Polio, it is not cause by poliovirus. The leading hypothesis so far centers on enterovirus EV-D68, which was first detected in California in 1962 as a cause of respiratory illness. Declared a "reemerging pathogen of disease concern,"

there are some indications that EV-D68 has acquired mutations that make it more neurovirulent than the virus observed in early isolates. Should the two year pattern continue, the medical community might expect another outbreak in 2020. It will be important to continue to study and understand this disease, and to consider whether a vaccine should be developed.

Discussion

The question was raised on the existence of animal models for investigating acute flaccid myelitis. It was noted that a mouse model exists, involving inoculation of baby mice with the virus. However, more work needs to be done in developing mouse models to better understand viral pathogenesis and the roles of the acquired mutations in affecting disease characteristics.

BEST PRACTICES IN LABORATORY SAFETY AND SECURITY FOR PATHOGEN RESEARCH

Dr. Joseph Kanabrocki, a professor of microbiology and associate vice president for research safety at the University of Chicago, opened the session. He emphasized that having a scientific understanding of the research being conducted is important for appropriate oversight and encouraged institutions to employ scientists in roles such as his. In his view, this expertise is important in helping establish a positive institutional culture and the right balance of oversight.

Dr. Kanabrocki provided a brief review of the US policy landscape in biosafety and biosecurity. The BMBL and NIH guidelines for recombinant and synthetic nucleic acid long provided two core documents on laboratory safety. Concerns surrounding access to certain pathogens led to the creation of Select Agent policies in the 1990s. Since 2012, the US has also enacted policies for federal and institutional oversight of "dual use research of concern" (DURC). Debate over "gain of function" experiments with influenza H5N1 resulted in a funding pause on a subset of research projects. Subsequent scientific and government discussions eventually led to new policy guidance on "Potential Pandemic Pathogen Care and Oversight (P3CO)." Implementation of this guidance by the Department of Health and Human Services, the primary U.S. agency that would be involved in relevant studies, resulted in a framework that includes agency review, reporting, and risk management processes for projects that make use of the 15 agents and toxins currently designated "potentially pandemic pathogens."

Dr. Kanabrocki turned to how the University of Chicago has enacted these policies as a major research-conducting institution, providing an example of how an institution can undertake responsible safety and security oversight of its activities. In response to the US policies, the University created a DURC task force with members from its existing two institutional biosafety committees (IBCs). Task force members include investigators and biosafety and biosecurity experts, as well as those in university administration, providing valuable cross representation. Significantly, the university expanded its screening to include projects beyond the list of 15 mandated P3CO agents and toxins. Investigators complete online questions on the nature of their research and may have further conversations with the DURC task force based on their answers. As part of its institutional management of DURC research, the task force also provides

risk management recommendations in areas such as the design and conduct of projects, often in consultation with the US government funding agency. Reviews for DURC are conducted at the grant proposal and manuscript preparation stages and on an annual basis for those projects determined to have DURC components. These reviews focus on the potential for the research to yield information that could be misused to cause threats to public health and safety or to national security, as well as the benefits of the research. How to communicate the results of studies identified as DURC is one area in which it is important to be careful and considerate. For example, it is important in public communication to convey the beneficial reasons why the research was undertaken as well as the biosafety and biosecurity measures that were followed; certain information may also need to be redacted in light of security concerns.

In addition, Dr. Kanabrocki noted that the University requires all investigators working with the pathogens and toxins designated as Select Agents (a longer list than the 15 P3CO pathogens) to adhere to an ethical Code of Conduct that is signed annually and discussed during annual interviews. The code expects investigators to report mishaps including "near misses." The university is also considering requiring reporting of unanticipated laboratory results. The University has developed an iPhone/Android app for easily reporting incidents and quickly responding to them. These, along with other factors, have been important in establishing an institutional culture in which investigators can seek out DURC and biosafety advice in a collaborative fashion and that contribute to early identification and mitigation of any potential concerns.

Dr. David Franz, the retired former commander of the US Army Medical Research Institute of Infectious Diseases (USAMRIID) provided his perspective on the role of leaders in promoting laboratory safety. He emphasized the importance of building safety into the system and of the leader communicating that safety is a priority. The scientific community has both a tradition and an important responsibility of self-governance. In the US, however, various incidents associated with laboratory accidents or carelessness, as well as concerns over potential misuse, have been followed by new guidelines or regulations that attempt to prevent such incidents from occurring. Thus, "a malevolent or thoughtless act by an individual or small group can slow the entire enterprise."

For the leader of an institution, safety is one of many responsibilities. As a result, Dr. Franz emphasized that, in his experience, leaders need to rely on the skills of a competent biosafety professional to help scientists problem-solve when needed and to empower them to do their work well. This culture of trust and support among the leader, the biosafety professional, and the researchers is critical. An organization's culture should make people feel comfortable asking questions and seeking help when needed, while not being overly punitive. A research system needs to strike the right balance among safety and security, scientific progress, and regulatory control. Dr. Franz suggested that creating such cultures might play a role in strengthening regulatory authorities' view that the scientific community is behaving responsibly and help to reduce potential bureaucratic overreach.

Dr. Franz concluded by sharing how leaders can build this culture of trust at their institution by leading with science. Leaders also need to be technically competent to be able to understand what people are doing.

Finally, **Dr. Peijun Zhai**, a professor from the China National Accreditation Service for Conformity Assessment (CNAS), provided an overview of biosafety laboratory management and accreditation in China. He began by briefly reviewing biosafety regulations. These include "Biosafety regulation on pathogenic microorganism laboratories," which were first published in 2004 and have been subsequently revised. Under Article #20, these regulations include that BSL 3 and BSL 4 laboratories will be accredited by an authorized body. Article 37 of "Regulation on certification and accreditation" provides that the accreditation body will be designated by the accreditation regulatory department of State Council.[1] In China, CNAS plays this laboratory accreditation role. Dr. Zhai noted that this system is different that the US, in which there are many accreditation bodies.

There are a number of additional Chinese regulations relevant to biosafety laboratories, such as "Law on the Prevention and Control of Infectious Disease," "Law on Evaluation of Environmental Effects," "Law on Sanitation & Quarantine on Country Board," and others. Dr. Zhai noted that there are categories of laboratory accreditation, including for testing laboratories, calibration laboratories, medical laboratories, proficiency testing laboratories, and producers of reference materials, as well as for biosafety level laboratories. For some of these types of laboratories, ISO, IEC or other international standards exist. The accreditation standard CNAS follows for BSL laboratories is based on China national standards GB19489-2008 and GB50346-2011, and follows the CNAS guidance documents CNAS-RL05 (rules for accreditation of laboratory biosafety) and CNAS-CL05 (further criteria and guidance documents). The general process of BSL accreditation is in accordance with the standard ISO/IEC17011n and includes an application from the laboratory, audit and on site assessment process, approval, and the granting of a certificate. Periodic surveillance and reassessment is also required. For BSL 4 (P4) laboratories, surveillance is conducted every 12 months, with reassessment for accreditation taking place every 5 years. Dr. Zhai concluded by emphasizing, "accreditation is playing an important role in normalizing laboratory management and evaluating laboratory competence."

Discussion

The discussion addressed the challenges of balancing potentially competing interests in achieving scientific results as well as ensuring safety and security. The important roles of laboratory management, biosafety professionals, scientists, and students and trainees were all noted. The importance of creating an interactive and integrated culture was highlighted, in which safety should not be seen as impeding the best science – these reinforce each other as part of responsible science.

ONE HEALTH IN CHINA

[1] https://wwwnc.cdc.gov/eid/article/25/5/18-0220_article

The utility of a One Health concept in addressing disease was a theme underlying a number of presentations and discussions during the meeting. **Dr. Harish Menghwar** provided an overview of the role of One Health in the Guangdong province of China on behalf of **Dr. Jiahai Liu**, a professor at Sun Yat-sen University, who was unable to attend.

The Guangdong province has a subtropical climate, diverse wildlife and livestock populations, is on migratory bird flyways, hosts a number of disease vector species, and has a food culture that consumes an array of animal products. Dr. Menghwar provided examples of recent outbreaks. For example, the source for SARS in 2002 was contact among infected bats and uninfected civets in markets while outbreaks of H7N9 are closely linked to markets with live poultry. Brucellosis, which causes disease in cattle, goats and sheep is considered endemic in the province. Guangdong is also the province most affected by Dengue virus, with all four subtypes identified in samples. The geographic range of Dengue has increased in recent years, and Guangdong has served as a hot spot enabling disease spread to other areas. Control strategies, such as closure of poultry markets in the case of H7N9, have had only temporary effects with cases reemerging when markets reopen. As a result, there is a general need to identify additional interventions and means of control for disease outbreaks. Dr. Menghwar emphasized the need for a multidisciplinary approach to the challenge of emerging infectious diseases. This approach will need to incorporate medical, ecological and ecosystem health, and animal health experts, to be cross-sectoral, and to occur across regional interfaces. Active surveillance of human – animal – environment interfaces and exposures will be important. Such an integrated approach can help to better identify causes and achieve reductions in transmission of diseases.

CHINESE BIOSAFETY, BIOSECURITY, AND BIOETHICS

The final two presentations on day 2 highlighted additional efforts by Chinese researchers and institutions to address laboratory safety issues.

Dr. Jiancheng Qi, a professor at the National Biological Protection Engineering Center, provided the first presentation. China has released a number of biosafety standards and has developed laboratory and research equipment to support the conduct of experiments in accordance with such safety guidelines. This includes personal protective equipment (PPE), related equipment such as masks, isolators, and biosafety cabinets, and decontamination and waste treatment equipment. They have also developed mobile laboratories, including a vehicle-mounted BLS 3 laboratory. Dr. Qi noted that the design and successful commissioning of BSL 4 laboratories such as the one at the Harbin Veterinary Research Institute reflects China's independent ability to provide for the highest biosafety level research. China is also assuming a global role in facilitating the safe conduct of research in partner countries. For example, equipment such as class II biosafety cabinets have been provided to laboratories in Sierra Leone and to a Sino-Kazakhstan laboratory, while the equipment for high temperature alkaline hydrolysis has been provided to Cuba for use in its vaccine laboratories. A mobile laboratory also was deployed in Africa during the Ebola outbreak.

Dr. Yunzhang Hu, a professor at the Institute of Medical Biology in Kunming, further discussed the development of high containment laboratory capacity in China. He noted that China's 2006-2020 national plan on prevention and control for important infectious diseases included pillars on the development of new drugs and vaccines and on an emergency response system. High containment laboratory infrastructure is critical to these research and development efforts. China has established three BSL 4 (P4) laboratories - in Wuhan, Harbin, and Kunming. The laboratories at the Institute of Virology in Wuhan and at the Harbin Veterinary Research Institute are operational, while the "Kunming National Primate Research Center of High Level Biosafety" at the Institute of Medical Biology is proceeding through the process. It just received its certificate from the China National Accreditation Service (CNAS), the designated laboratory accreditation body. The facility will include laboratories at BSL (P) 2, 3, and 4. The P4 laboratory will include 7 principle investigators and 4 functional departments, including departments of biosafety management and of training.

Dr. Hu shared information on plans for the P4 laboratory once it is operational. It is intended that the laboratory will support national emergency response capacity for emerging infectious diseases. It will provide a platform for vaccine research and development, which is a traditional focus of the Institute; the new P4 capacity will enable the Institute to combine high containment research with the vaccine platform components. The desire is for the facilities to provide an efficient pipeline that includes virus isolation, animal model testing, studies to understand pathogenesis, and development of rapid detection methods. These will feed into downstream vaccine processes such as evaluations of safety and efficacy, clinical trials, new drug certification, and GMP production and marketing. The facility will also serve as pathogen seed bank and information center for important infectious diseases. Finally, the facility can be a regional resource and support opportunities through China's One Belt One Road efforts. As the high containment laboratory completes its final processes and becomes operational, Dr. Hu emphasized that ensuring a culture of biosafety will be very important to their success.

Discussion

During discussion, a question was asked on the process for developing the new vaccines at the Kunming Institute. It was indicated that basic research directed toward vaccine development is done within the Chinese Academy of Medical Sciences or in collaboration with others, depending on the particular diseases, and that the researchers are working only with rhesus monkeys, not in other types of primates. A question was also asked about emergency management. It was noted that China CDC has primary responsible for emergency response and management, and technicians from the Institute can accompany China CDC into the field as needed.

CHINESE PERSPECTIVES ON EMERGING INFECTIOUS DISEASES

The last day of the meeting provided an opportunity to learn more about innovative research on infectious diseases being conducted by laboratories in China.

Dr. Changjiang Weng, professor at HVRI, discussed the inflammatory response in host cells resulting from entry of RNA viruses. A protein complex in host cells (the inflammasome) binds to viral genomic RNA and activates downstream enzymes leading to the production of the cytokine Il-1 beta. Dr. Weng's laboratory explored in more detail the interaction of RNA from porcine reproductive and respiratory syndrome virus (PRRSV) with the protein NLRP3 as part of the inflammasome complex. His group identified a helicase protein called DDX19A that binds to both components. In addition, the laboratory studied whether DDX19 could regulate production of the immune system cytokine interferon (IFN). In this case, experiments showed that DDX19 disrupts phosphorylation of the cellular protein IRF3 and blocks its interaction with another protein as part of the IFN signaling pathway. Thus, DDX19 leads to suppression of IFN while DDX19 knockdown experiments lead to enhanced IFN production. In future directions, the laboratory will study additional cellular inflammasome complexes affecting production of inflammatory cytokines in response to RNA virus infection.

Dr. Zhengli Shi, a professor at the Wuhan Institute of Virology, provided information on Middle East respiratory syndrome (MERS)-related coronavirus and cases in China. MERS emerged in recent years in the Arabian Peninsula, though it may have been circulating in its natural reservoirs for some time. It shares a number of genomic similarities with SARS virus, including the importance of the viral spike protein that binds to cellular receptors as part of viral entry. For MERS, the human cellular receptor to which the spike binds is DPP4.

There are multiple MERS-related coronaviruses, including strains in clades A and B that cause human infection, camel MERS viruses, and bat MERS-related coronaviruses including the related bat coronavirus HKU4. Dr. Shi's laboratory explored diversity in the MERS spike binding domain for its role in interspecies infection ability. A recombinant MERS containing the spike binding domain from HKU4 could replicate in human cells. Many strains of MERS-related coronaviruses are prevalent in multiple bat host species in China. The potential for interspecies transmission highlights the need for long-term surveillance of this group of viruses.

Dr. Wen Dang of the Lanzhou Veterinary Research Institute provided a presentation on foot and mouth disease (FMD) vaccine development. He delivered the presentation on behalf of **Dr. Haixue Zheng**, a professor at the Lanzhou Institute, who was unable to participate. FMD is a highly infectious virus that affects cloven-hoofed animals such as pigs and cattle. Current FMD vaccines have low efficacy and outbreaks can have significant economic cost; in addition to vaccination, current control strategies include quarantine and slaughter of animals. An improved FMD vaccine that can provide rapid and long lasting protection has been needed.

Dr. Dang discussed the Lanzhou laboratory's efforts to design attenuated seed viruses for use in FMD vaccine production. They explored the effects of a number of deletions in regions of the virus including 3A and PK, and in particular the effects of 43-nucleotide and 86-nucleotide deletions to create strains rO-DPKs43 and rO-DPKs86. These showed decreased pathogenicity when tested in cattle, but not in pigs. Studies of a mutation in region VPI found decreased pathogenicity in swine, so the laboratory tested a strain that included both PKS and VP1 mutations. They also examined the effects of a mutation in VP3 that may increase the host

immune response to FMD, resulting in earlier response and higher antibody titers in pigs. These efforts led to the design of various master seed stocks and cell lines for FMD vaccine production. The Institute has produced bivalent (O and A serotypes) and trivalent vaccines (O, A, Asia 1 types) against FMD. Dr. Dang indicated that FMD vaccine safety and efficacy have been improving and outbreaks of FMD in China have been declining, with China currently free of type Asia 1 and aiming to be free of type A by 2020.

Dr. Longding Liu from the Institute of Medical Biology of the Chinese Academy of Medical Sciences discussed enterovirus 71 (EV71), which causes outbreaks of hand, foot and mouth disease (HFMD) worldwide. The disease primarily causes lesions on skin and oral mucosa although serious side effects such as encephalitis can occur in some cases. As part of efforts to understand the biology of this virus and to develop a vaccine, a neonatal rhesus monkey model has been developed.

Dr. Liu's laboratory created a fluorescently labeled EV71 virus. During early stages of infection, the virus appeared in tracheal and bronchial epithelial cells, was subsequently identified in pulmonary lymph nodes, and by later stages could be identified around blood vessels of the olivary nucleus of the brainstem. Studies indicate that EV71 may have the ability to degrade cell junction proteins to increase penetration into the brain. There is also a suggestion that the subset of dendritic cells positive for CD141 are involved in transferring the virus from areas such as lymph nodes to the endothelial cells of the blood brain barrier. EV71 appears to proliferate in astrocytes rather than in neurons. The Institute subsequently created a viral vaccine seed stock that underwent human clinical trials and demonstrated protection in children. Market approval was obtained and Dr. Liu reported decreased mortality in China from HFMD.

Dr. Chengfeng Qin from the Institute of Microbiology and Epidemiology at the Academy of Military Medical Sciences provided the final presentation in this session, which focused on Zika virus. Zika is a flavivirus related to Dengue, West Nile, and yellow fever. It was first isolated from the Zika forest in Uganda around 1950. Since 2007, there have been infections in over 80 countries. Recent outbreaks have highlighted concern over cases of microcephaly, and direct injection of Zika virus into mouse brain can lead to reduced cerebral size, enlarged ventricles, and thinner cortex in infected mice. Zika virus replicates in the ventricular zone and infects neural progenitor cells but not differentiated glial cells and neurons. Analysis of Zika strains, particularly mutation S139N that first appeared in 2013 and subsequently spread in the Americas, shows enhanced neurovirulence compared to older strains.

Using this knowledge, Dr. Qin's laboratory has explored whether Zika virus could have application in the treatment of the cancer glioblastoma, which is an aggressive and hard to treat tumor that can show chemo- and radiotherapy resistance and recurrence. The laboratory created a live attenuated Zika virus vaccine strain, ZIKA-LAV. Testing in mice has shown promise including extended glioblastoma survival time. Studies tracking the virus in mice show it localized to mouse brain. These results suggest that it may be possible to develop this approach

further and to combine it with chemo and radiotherapy as part of efforts to provide glioblastoma treatment options.

Discussion

During discussion, a question was asked about disease surveillance in camels for MERS. It was indicated that MERS CoV was not detected in camels in China, but that Chinese camels are a different species from dromedary camels. With regard to the EV71 vaccine, the question was asked about testing for possible interference with other vaccines on the immunization schedule. It was answered that yes, testing to understand any possible effects in relation to the current schedule of childhood vaccines in required as part of the licensing process. A question was asked about the number of strains against which the FMD vaccine cross protects. It was noted that China's primary focus is on addressing the type A strain, and cross protection has not yet been investigated against other global strain types. Finally, a question was asked on the structural biology of S139. It was noted that a mutation can affect the interaction of S139 with a receptor on neuronal progenitor cells and affect viral replication, but research is still ongoing to explore this question.

SPECIAL SESSION (SYNTHETIC BIOLOGY)

The last session of the workshop covered two areas of progress in synthetic biology. **Dr. Chunbo Lou**, a professor at the Institute of Microbiology of the Chinese Academy of Sciences, first provided the first presentation. Methods enabling the manipulation of large nucleic acid fragments (over 30kb) are of assistance in the construction of gene circuits and organisms using synthetic biology. This scale of manipulation, for example, overlaps with a number of natural product gene clusters (10-150kb) and viruses (5-200kb). Dr. Lou's laboratory has focused on a method called Cas 9-assisted targeting of chromosome segments (CATCH). The positive rate of obtaining recombinant plasmid obtained through their process of RNA-guided Cas9 cleavage depends on the insert size; for a 50kb fragment, for example, the positive rate is roughly 60%. Dr. Lou also reported simultaneous editing to insert promoters or make other changes to five target locations in a gene cluster, with a 10% success rate.

Based on these tools, the laboratory is aiming to manipulate components of gene clusters in bacteria to affect transcription, translation, and/or cell-cell communication by turning on or off gene expression. They have designed components including a promotor, a chimeric transcription factor, and DNA binding site with the aim of eventually using these as part of a biosensor. Quorum sensing is a type of cell-cell communication in which changes in gene expression are affected by signaling molecules released and detected in response to cell density. The laboratory undertook de novo design of signaling molecules to turn on and off the gene for, made alterations to HSL molecules, and undertook directed evolution to generate significantly greater sensitivity than the natural system. They hope to combine multiple signaling molecules in three- and four- signal cellular communication systems in future efforts to design and control bacterial sensors. Such sensors can be engineered to make the bacteria

produce or recognize something, with the details dependent on the specific applications selected.

The second presentation also addressed fundamental research in synthetic biology and was provided by **Dr. Xiaoli Xue**, a professor in the Laboratory of Synthetic Biology at the Shanghai Institute of Plant Biology. Her research focused on eukaryotic chromosome evolution and the question of whether the number of chromosomes in eukaryotic cells can be changed, using yeast as an example organism. By fusing the 16 chromosomes found in budding yeast, her laboratory sought to generate a viable, single chromosome organism. She reported using CRISPR-Cas9 editing and homologous recombination to delete the extra yeast chromosome centromeres and telomeres and to ligate the chromosomes in pairwise fashion. There was also concern not to affect the expression of genes nearby these altered regions. Ultimately, a single chromosome yeast strain SY14 was created.

Fusing the wild type yeast chromosomes into a single, large chromosome resulted in drastic changes to the 3-D structure of the genome, from distinct chromosomes to a twisted globular configuration. Although wild type inter-chromosomal interactions were lost, local chromatin interactions appeared to be similar in the SY14 strain. The laboratory identified only 28 differentially expressed genes. The yeast was able to grow and showed similar overall morphology to wild type, although competition experiments showed that SY14's growth was slower. The creation of SY14 was recently published; Dr. Xue noted that the laboratory of Dr. Jef Boeke at New York University published a paper in the same journal issue reporting creation of a two-chromosome yeast, although the two groups used different approaches in their efforts.

Finally, Dr. Xue reported on further experiments to fuse the ends of the large single chromosome to create a yeast circular chromosome. This effort resulted in the creation of strain SY15. SY15 cells grew slower than SY14, showed a higher number of abnormal cell shapes, and were unable to produce viable spores. However, they did not exhibit the telomerase-dependent cellular senescence that wild type and SY14 strains did. She wondered whether efforts to understand circular eukaryotic chromosomes such as the SY15 might ultimately help to understand rare human ring chromosome syndromes.

Discussion

The question was asked about potential applications associated with the single chromosome yeast strain and the ongoing synthetic quorum sensing research. That the single chromosome yeast strain has been provided so far to over 30 research groups who have requested it, and it was noted further information on applications may arise. With regard to the quorum sensing system, the researchers hope to further develop their synthetic system in the future by exploring the ability to build a system similar to a multicellular organelle.

THEMES, SUMMARY AND NEXT STEPS

Several themes emerged during the workshop. The first was a focus on the importance of pursuing an integrated, One Health-based approach to understanding the causes and addressing the effects of animal and human diseases. Some of the issues raised included the short-term intended and unintended effects of recent disease control strategies, as well as a need for collaboration amongst experts and across regions on medical, ecological and ecosystems science to ensure human and animal health.

The second was a growing appreciation for the widespread use of gene-editing tools, particularly those based on the CRISPR-Cas9 system, that are being integrated into many new research efforts. New gene editing techniques to cut, edit, activate and repress genes with greater accuracy and at much lower costs go well beyond longstanding methods of selective breeding of hosts and pathogens and allow researchers to quickly explore changes not readily achieved in the past. Although the benefits to society are potentially enormous, there is the potential for malevolent application or for the technology to contribute to dangerous "bioerror," that is, for researchers to inadvertently create a modified organism that has an unintended disastrous impact. Many of the technical talks including those on vaccine development, vector engineering, host engineering, and synthetic biology included details on the researchers' use of gene editing tools and techniques. During the presentations the importance of gene editing to facilitate genetic modifications were highlighted but participants also discussed how they assessed risks and untended consequence of using the tools and best practices to ensure the appropriate and safe use of this technology in the future. See box on Gene Editing below.

A third theme was the importance of establishing and maintaining strong institutional cultures and norms to support the safe and responsible conduct of science. The sessions included several discussions on possible collaborations between Chinese and American scientists and institutions on the responsible conduct of science and other ways to ensure that gene editing techniques are used safely and ethically across the life science enterprise in the future.[2]

Throughout the workshop and during tours of the HVRI security and training centers, experts also shared information to ensure the safe and secure operation of high containment biological laboratories. Topics discussed included combating emerging infectious diseases in livestock, safely doing research on insect vectors in high biological containment, inactivation/decontamination procedures for African swine fever virus, improving the public health response to disease outbreaks, training and vetting high containment laboratory workers, and the safe and sustainable operation of high containment laboratories.

[2] Although the meeting agenda was not designed to focus on human gene editing, the research project led by He Jiankui, formally an associate professor in the Department of Biology of the Southern University of Science and Technology in Shenzhen, China that lead to the birth of two children with edited DNA was universally condemned by the meeting participants.

NAS has helped to shape the international dialogue on the risks and the multitude of challenges associated with planning for, building, sustaining, and safely and securely running high containment laboratories. Before the meeting the directors of the Galveston National Laboratory (of the University of Texas Medical Branch) and the Wuhan Institute of Virology (of the Chinese Academy of Sciences) jointly published an editorial in Science that calls for the creation of a global network to bring together the leaders of institutes that operate laboratories at the highest biological safety level, BSL-4, to ensure the safe, secure and sustainable operation of these facilities. The final session of the meeting included a discussion on the concept led by the authors of the editorial. During the discussion the director of China's Centers for Disease Control and the leadership of the Chinese institutes that operate China's three BSL-4 labs indicated that they support the idea and will work with NAS to develop and create a network. Although there's a tremendous network of BL-3 labs in the U.S., in China and around the world, the group agreed to limit the network to the BL-4 community. Many BSL-4 labs are being constructed around the world, and not all of them are in the most robust economies, if there is a problem or accident in any one lab other labs will suffer. Some of the people that are building these laboratories have government support to build the building but don't have a good idea of what they're going to work on after construction ends and they don't necessarily have the understanding of the incredible cost of keeping the doors open. Operating BSL-4 facilities can be used to show potential operators how to work under BSL-4 conditions. NAS and CAS will work together to planning an international meeting of laboratory leaders to take place later in 2019.

The group also discussed future bilateral meetings. Going forward the groups should continue to think in terms of real partnership, with 50-50 joint funding, joint personal involvement by partners and joint sharing of credit. While safety and security are core issues that we need to continue to discuss, it's really important now that we focus on scientific collaboration and what we can we do together that is going to be of joint interest and beneficial to both China and the U.S. and to our society in general. The meetings should continue to reflect the interests of China and not be a paternalistic relationship, but a true partnership. The next bilateral meeting will likely take place in Kunming, China at the Kunming National Primate Research Center, another Chinese institute that operates a BSL-4 laboratory, later in 2019, it is too difficult to meet in the U.S. due to visa constraints.

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BOX 1
GENE EDITING

Gene editing involves altering the DNA of a cell or organism. Modifications can include deletions, insertions, or other changes to the genetic code, generally carried out to change characteristics of a gene's expression. A particular edit might aim to "knock out" the gene and render it non-functional. Alternatively, precise changes to the nucleic acid sequence may be

made to affect properties of the protein that results from the gene's transcription and translation. Edits are carried out for a number of other purposes, as well.

Researchers have been making alterations to nucleic acid sequences for many years, but tools developed since approximately 2012 – particularly the CRISPR-Cas9 editing system – have significantly increased the flexibility, efficiency, accuracy, speed, and ease (including lower cost) of making these changes. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9 (CRISPR-associated protein 9) were discovered in bacteria, where they form part of an immune mechanism for responding to infection. The basic system has since been adapted and repurposed as a key research tool and the use of gene editing is now widespread in research laboratories (Doudna and Charpentier 2014). A designed guide RNA sequence aligns with and binds to a target DNA sequence in the genome. The Cas9 protein then makes a double strand DNA cut. This cut can be repaired in various ways, including end joining or filling in with a donor DNA template to lead to sequence alterations.

Approximately a third of the presentations during the workshop referred to CRISPR and Cas9-based gene editing tools in some fashion. The key areas discussed during the workshop included (see presentation summaries for more detail):

- **Vaccine development efforts**. Use of gene editing to generate an attenuated version of a double-stranded DNA virus (Gladue)
- **Vector engineering**. Use in generating a mosquito that lacks an immune system associated enzyme (Han)
- **Host engineering**. Introduction of a human receptor domain into the mouse to create a model for viral infection (Baric); Creation of a pig designed to be resistant to a viral disease of interest (Ouyang)
- **Synthetic biology**. Design of gene circuits in bacteria to produce or respond to signaling molecules (Lou); Changes to eukaryotic genome organization to create a single chromosome yeast (X. Xue).
- **Ethics**. Considerations for responsible use and governance of gene manipulations in synthetic biology (W. Zhang) and in human genome editing applications (Y. Xue).

Professor Steven Higgs also briefly discussed a special use of CRISPR-Cas9 in the creation of a gene drive. Gene drives "are systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced" (NASEM 2016). The design of these systems includes a mechanism in which an edited gene can be copied to its homologous chromosome and will continue to spread as the organism reproduces (Esvelt et al. 2014). The advent of CRISPR-Cas9 genome editing enabled scientists to create a gene drive and the field has continued to progress rapidly, with gene drive modified mosquitos reported by late 2015 (Gantz et al., 2015). Scientists are exploring genetically modified and/or even gene drive modified insect vectors as one strategy for control of devastating insect-borne diseases. But if used in this way, modified insects ultimately would be released from the laboratory into an ecosystem. Special considerations arise from use of modified species in open field trials and potential deployment. Researchers will need to

understand not only how a modification affects the target population, but also how it affects the broader ecological community. A number of reports have discussed scientific, ethical and social considerations, particularly around gene drives (for example, NASEM 2016), while funders and scientists are developing norms to guide responsible use (Emerson et al. 2017). Dr. Higgs highlighted these special considerations in his presentation on the use of insect vectors for infectious disease research.

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Sessions and Presentations

Keynote addresses

- Oversight for Research on Viral Pathogens with Pandemic Potential – the Perspective from China
- Emerging Swine Coronaviruses and their Interspecies Transmission
- Vaccination in Poultry Eliminated Human Infections with H7N9 Virus in China

Influenza

- Collaborating on Influenza Research from the U.S. perspective
- Human Infection with Zoonotic Influenza
- Interactions between Influenza Viral RNP Complex Proteins and Host Cellular Factors

African swine fever

- Current Situation and Control Strategy of African Swine Fever in China
- Characterization of African Swine Fever Virus Isolated from China
- ASFV Vaccine Status: Rapid Production of Recombinant ASFV for Live Attenuated Vaccines
- Genome-edited Pigs Protected From Classical Swine Fever Virus

Understanding and engineering viral pathogens with pandemic potential

- Advances in Understanding and Altering Pathogen Properties
- Cutting Edging New Biotechnology: Strategies and Tools for Engineering

Bioethics and responsible planning for pathogen research

- Biosafety and Bioethics in Medical Research
- Bioethical Considerations for the Conception and Design of pathogen Research and Approaches for Assessing Potential Risks and Benefits of Research
- Biosafety and Bioethics Considerations in Dual-Use Risks Management - China's Efforts and Future Works

Vector and host engineering: safety and security

- Biochemical Pathway of a Mosquito Innate Immunity, Melanization
- Best Practices in Safety and Security for Working with Arthropods that Serve as Pathogen Vectors, Including Responsible Research with Gene Drive Modified Vectors
- Vector Competence of Emerging Mosquito Borne Virus in China
- A Gut Commensal Bacterium Promotes Arbovirus Infection in Mosquitoes

Acute Flaccid Myelitis

- Review of what is currently known about the cause of Acute Flaccid Myelitis

Best practices in laboratory safety and security for pathogen research

- Institutional Review and Oversight for Research on Viral Pathogens with Pandemic Potential - a U.S. Perspective
- The Role of Leaders in Laboratory Safety: Culture and governance to steer toward behavior changes
- Overview of Laboratory Biosafety Management System in China

One Health in China

- Understanding how a One Health Approach is Shaping Research to Understand and Address Viral Pathogens in China

Chinese biosafety, biosecurity, and bioethics

- Development and Application of Laboratory Biosafety Equipment in China
- Kunming National Primate Research Center of High-Level Biosafety

Chinese perspectives on emerging infectious diseases

- DDX19, A novel Viral RNA Sensor, Regulates IFN Signaling upon Viral Infection
- Risks of MERS-cluster Coronaviruses in China
- Rational Design of Master Seed Virus to Improve the Safety and Effectiveness of Foot and Mouth Disease Vaccine
- Development of Inactivated EV71 Vaccine (Human Diploid Cell) and Pathogenic Study of EV71 Infection in Rhesus Macaques
- Zika Virus Neurotropism: the Bad and the Good

Special session (gene editing and synthetic biology)

- Large Gene Cluster and Regulatory Manipulation for Activating the Cryptic Natural Product Clusters in Streotomvces
- Creating a Functional Single-chromosome Yeast

China-U.S. Collaboration: Opportunities to Prevent Future Pandemics

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