

Wildlife Markets: Ten years following the SARS-CoV outbreaks, there is little information available on the *current* diversity of bats and other mammals available in the wet markets in southern China. One study found that 91 species of vertebrates, including 40 mammal species, were being traded in Guangxi, China (75). Further, little data is available on the origin of wild animals brought into the market system. In some cases, animals may be locally collected, while in other cases animals may be imported from Southeast Asia, including adjacent Vietnam (74-76) – factors which will affect the diversity of CoVs. Captive and free-ranging rodents are found in markets and may be an additional host for CoVs (77). We have worked with Yunnan Institute of Endemic Diseases Control and Prevention since June 2012 (see **Letters of Support**). We have conducted initial surveillance in Nujiang, Baoshan Denong and Xishuangbanna prefectures and Ruili, which is a major wildlife trade gateway between Myanmar and China (**Fig. 3**). We have collected 187 small mammals from markets in Yunnan and tested them for coronaviruses using a 1-step PCR assay (78), finding 2/21 shrews (*Crocidura attentuata*) are CoV-positive.



Figure 3: Map of wildlife trade routes from Southeast Asia into China. Modified from (79).

Other animal samples available for this project: To date, our group has collected more than 90,000 high quality specimens from 15,000 animals representing key wildlife reservoirs for zoonoses such as bats, rodents and primates under our USAID-EPT PREDICT project. Clinical samples include blood, throat swabs, feces and urogenital swabs and represent animals from 10 different countries including Bangladesh, India, Malaysia, Thailand, Indonesia, China, Brazil, Bolivia, Colombia, Peru, and Mexico. 50,000 of these samples originate from Asia, and are currently being screened for novel coronaviruses (See Section C2a, Fig. 6). **We have also collected more than 500 bat specimens representing seven species from the Kingdom of Saudi Arabia in collaboration with Saudi Arabia's Ministry of Health and Columbia University.** Nearly 20,000 of our samples come from bats, and will be used to analyze CoV diversity along with novel CoVs we identify.

Identifying novel CoVs in wild bats in China: We have already conducted significant CoV surveillance in China for bats, other wildlife and humans. For this, we use pan-coronavirus PCR protocols based on conserved RNA-dependent RNA polymerase (RdRp) motifs A and C to screen samples at Wuhan Institute of Virology (80). Besides a large number of SL-CoVs, we have detected several novel bat-CoVs including strains closely related to CoV HKU4/5, CoV 1A & 1B, CoV HKU 2, 6, & 8. For the first time, we have also isolated and characterized a bat-CoV from China that uses ACE2 receptors (see Section C3a preliminary data) (28). **In all, we have identified sequences from 268 novel bat-CoVs (140 from China alone) from bat species collected in Bangladesh, Thailand, Mexico, Brazil and China (See Section C2a, Fig. 6).** We have an additional 5,000+ clinical samples from free-ranging bats and rodents from Guangdong province, from an ongoing study which are being screened for viral pathogens, including CoVs at Guangdong Entomological Institute.

Survey of people highly exposed to wildlife in Guangdong, China: We have worked with Guangdong CDC since 2008, under a currently active IRB protocol, to interview and sample people working in live animal markets, hunters and restaurant workers with a high level of exposure to animals. We have interviewed volunteer participants about the nature and frequency of animal interactions; collected biological samples (blood, feces, sputum), and trained participants to collect animal blood samples (dried blood spots on filter paper) from animals they butchered or hunted. We enrolled 1300 participants across 12 sites within Guangdong Province (**Fig. 4**).



Figure 4: Sites of current human sample collection by Guangdong CDC for zoonotic pathogen surveillance in Guangdong Province, Southern China. Each star represents a large wildlife market where we have enrolled market and restaurant workers (total = 1,300) for our zoonotic pathogen spillover study. Seventeen people had IgG antibodies to SARS-CoV and a follow-up study is underway.

Samples have been tested for antibodies to animal pathogens, including SARS-CoV. **Of the 1300 serum samples screened using a SARS-CoV ELISA, 17 were positive for IgG antibodies to SARS-CoV.** These patients were not acutely ill at the time of sample collection, and this finding suggests one of three possibilities: 1) that SARS-CoV is still circulating in Guangdong markets; 2) that these people may have been exposed during the time of the 2002-3 outbreak; or 3) that the ELISA used is cross-reacting to another CoV. Review of their history of wildlife exposure is currently underway. In Shanghai, the Shanghai Municipal Center for Disease Control and Prevention (see **Letters of Support**) currently conducts surveillance on people with influenza-like illness in rural communities surrounding Shanghai. We will develop a similar study of people in