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Surveillance for Ebola Virus in Wildlife, Thailand

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DOI: http://dx.doi.org/10.3201/eid2112.150860

To the Editor: Active surveillance for zoonotic pathogens in wildlife is particularly critical when the pathogen has the potential to cause a large-scale outbreak. The recent outbreak of Ebola virus (EBOV) disease in West Africa in 2014 was initiated by a single spillover event, followed by human-to-human transmission (*1*). Projection of filovirus ecologic niches suggests possible areas of distribution in Southeast Asia (*2*). Reston virus was discovered in macaques exported from the Philippines to the United States in 1989 and in sick domestic pigs in the Philippines in 2008 (with asymptomatic infection in humans) (*3*). Dead insectivorous bats in Europe were found to be infected by a filovirus, similar to other members of the genus *Ebolavirus* (*4*).

Although EBOV has historically been viewed as a virus from Africa, recent studies found that bat populations in Bangladesh and China contain antibodies against EBOV and Reston virus recombinant proteins, which suggests that EBOVs are widely distributed throughout Asia (*5*,*6*). Thus, an outbreak in Asian countries free of EBOV diseases may not only be caused by importation of infected humans and/or wildlife from Africa but may arise from in-country filovirus–infected wildlife. Serologic and molecular evidence for filoviruses suggests that members of the order Chiroptera (bats) may be their natural reservoir (*7*).

As part of a proactive biosurveillance program, we conducted a cross-sectional study for EBOV infection in bats and macaques in Thailand. We screened 500 *Pteropus lylei* bats collected from 10 roosting sites during March–June 2014 (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/12/15-0860-Techapp1.pdf) for antibodies against EBOV antigen by using an ELISA validated by the Centers for Disease Control and Prevention (Atlanta, GA, USA) (*8*).

Bats and macaques were captured with permission from the Department of National Parks, Wildlife and Plant Conservation. The Institutional Animal Care and Use Committee at the University of California, Davis (protocol #16048) approved the capture and sample collection protocols.

To further screen a wide range of wildlife species in Thailand for active EBOV infection, we sampled and tested 699 healthy bats, representing 26 species, and 50 long-tailed macaques (*Macaca fascicularis*). Additional bat species were randomly captured (≥50/site) in 6 provinces in Thailand during 2011–2013 and identified by morphologic traits. Macaques were captured and sampled in March 2013 from 1 site at Khao Chakan, Sa Kaeo Province, and released at the same site. Blood, saliva, urine, and feces were collected from anesthetized macaques or nonanesthetized bats. All animals were released after sample collection. Details on species screened, sample sizes, and trapping localities are provided in the Table.

All nonblood specimens were collected in nucleic acid extraction buffer (lysis buffer) and transported on ice to the World Health Organization Collaborating Centre for Research and Training on Viral Zoonoses laboratory (Bangkok, Thailand) for storage and testing. Three types of specimen (saliva, urine, and serum) were collected from individual animals and pooled.

Nucleic acid was then extracted with NucliSENS easyMAG (bioMérieux, Boxtel, the Netherlands) and analyzed by reverse transcription PCR (RT-PCR). A consensus RT-PCR was used to screen for all known species of Ebola virus and Marburg virus, including EBOV (*9*). In total, 5 RT-PCRs were performed on each specimen, a regimen that included 4 sets of primers specific to known filoviruses and 1 degenerate primer set to detect novel viruses in this family. The sensitivity of RT-PCR on synthetic standard was 50–500 copies/reaction (*9*). We ran 3,745 PCRs, covering a range of assays, to increase detection sensitivity. All specimens examined were negative for filoviruses by EBOV ELISA and PCR (Table). For *P. lylei* ELISA screening, optical density values for all 500 bats ranged from 0.000 to 0.095, well below the potential positive cutoff value of 0.2.

Assuming a population size of ≈5,000 bats/roost and a sample size of 50 bats/site, we have 95% confidence that

Table. Overview of bats and macaques tested by Ebola virus IgG ELISA or PCR for filoviruses, Thailand, 2011–2014

Species	Host family	No. tested (no. positive)	Test method*	Specimen type†	Location‡
Chiroptera					
Pteropus lylei	Pteropodidae	500 (0)	ELISA	Serum	a
Cynopterus brachyotis	Pteropodidae	10 (0)	PCR	Pooled	b
C. sphinx	Pteropodidae	4 (0)	PCR	Pooled	b
Eonycteris spelaea	Pteropodidae	12 (0)	PCR	Pooled	b
Macroglossus sobrinus	Pteropodidae	2 (0)	PCR	Pooled	b
Megaerops niphanae	Pteropodidae	1 (0)	PCR	Pooled	b
Rousettus amplexicaudatus	Pteropodidae	3 (0)	PCR	Pooled	b
Hipposideros armiger	Hipposideridae	113 (0)	PCR	Pooled	b
H. cineraceus	Hipposideridae	4 (0)	PCR	Pooled	b
H. larvatus	Hipposideridae	33 (0)	PCR	Pooled	b, c
H. lekaguli	Hipposideridae	158 (0)	PCR	Pooled	b
Megaderma lyra	Megadermatidae	1 (0)	PCR	Pooled	b
Miniopterus magnate	Vespertilionidae	132 (0)	PCR	Pooled	b, c
M. pusillus	Vespertilionidae	1 (0)	PCR	Pooled	b
M. schreibersii	Vespertilionidae	22 (0)	PCR	Pooled	b
Myotis horsfieldi	Vespertilionidae	6 (0)	PCR	Pooled	b
M. muricola	Vespertilionidae	1 (0)	PCR	Pooled	b
Rhinolophus shameli	Rhinolophidae	44 (0)	PCR	Pooled	b
R. coelophyllus	Rhinolophidae	7 (0)	PCR	Pooled	c
R. luctus	Rhinolophidae	1 (0)	PCR	Pooled	b
R. malayanus	Rhinolophidae	4 (0)	PCR	Pooled	c
R. microglobosus	Rhinolophidae	1 (0)	PCR	Pooled	b
R. pusillus	Rhinolophidae	1 (0)	PCR	Pooled	b
Scotophilus kuhlii	Vespertilionidae	1 (0)	PCR	Pooled	b
Taphozous longimanus	Emballonuridae	27 (0)	PCR	Pooled	b
T. melanopogon	Emballonuridae	110 (0)	PCR	Pooled	b
Total		699 (0)			
Primate					
Macaca fascicularis	Cercopithecidae	50 (0)	PCR	Pooled	d

*ELISA for IgG against Ebola virus.
†Nucleic acid extraction from Pooled saliva, serum, and urine.
‡a, Central Thailand; b, Eastern Thailand; c, Chaing Mai Province; d, Kao Chakan, Sa Kaeo Province.

if >6% of the population had antibodies against EBOV antigen, we would have detected it. If we assume that all 500 animals are part of 1 large panmictic population, and we have 95% confidence that if EBOV were circulating in >0.5% of the population, we would have detected it. Therefore, although we cannot rule out infection of this species with 100% confidence, *P. lylei* bats, the most abundant species of large pteropid bats in Thailand, are highly unlikely to be reservoirs for EBOV.

Our sample sizes for PCR screening of other bat species in this study were much smaller, and we had no supported serologic data, but these negative results could add to the knowledge of filovirus infection in nontissue specimens from healthy bats. Previous studies have detected Ebola virus–like filovirus RNA in lung tissue of healthy *Rousettus leschenaultia* bats in China (*10*) and from organs and throat and rectal swab specimens from a die-off of *Miniopterus schreibersii* bats in Spain (4). In our study, which included 22 *M. schreibersii* and 132 *M. magnate* bats, none of the bats tested positive for filoviruses.One limitation of the cross-sectional sampling strategy used here, however, is that PCR-negative findings do not necessarily mean that the bats were not infected in the past. Although we found no evidence of filovirus infection in wildlife species tested in Thailand, we believe that continuing targeted surveillance in wildlife should enable early detection and preparedness to preempt emerging zoonoses.

This study was supported by a research grant from Department of National Parks, Wildlife and Plant Conservation, the Thailand Research Fund (RDG5420089), the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530148-HR), Health and Biomedical Science Research Program by National Research Council of Thailand and Health System Research Institute, the Research Chair Grant, the National Science and Technology Development Agency, Thailand, and the Naval Health Research Center (BAA-10-93) under the Cooperative Agreement no. W911NF-11-2-0041, and the United States Agency for International Development Emerging Pandemic Threats PREDICT project.

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Probability of Spirochete *Borrelia miyamotoi* Transmission from Ticks to Humans

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DOI: http://dx.doi.org/10.3201/eid2112.151097

To the Editor: Borreliosis caused by *Borrelia miyamotoi* is an emerging disease transmitted by *Ixodes* ticks (*1*). Each year in the Netherlands during 2007–2009, ≈70,000 bites by hard ticks occurred per 1 million inhabitants (*2*). In the Republic of Udmurtia, Russia, ≈10,000 hard tick bites per 1 million inhabitants are reported annually among persons seeking medical help. Recent studies indicate that almost 3% of *I. ricinus* ticks in the Netherlands and 2%–6% of *I. persulcatus* ticks in Russia are infected with *B. miyamotoi* (*1*,*3*,*4*). Human exposure is substantial, and comparable exposure to *B. miyamotoi* is expected in many Eurasian countries and in North America (*4*,*5*). The probability of *B. miyamotoi* transmission from ticks to humans requires examination to estimate the true risk for human disease.

In Izhevsk (population 650,000), a city in European Russia (Republic of Udmurtia), we identified 95 human cases of *B. miyamotoi* infection during 2010–2014 (*6*). In this city, primarily because of concern about tickborne encephalitis (TBE), all patients with suspected tickborne infection are hospitalized in the Republican Hospital of Infectious Diseases (RHID). A service also enables tick-bitten persons to bring the removed tick for PCR for TBE virus (TBEV) and *B. burgdorferi* sensu lato. We supplemented that with PCR testing for *B. miyamotoi* (*3*).

In June 2014, twenty-four persons (≈5% of those bitten by ticks subjected to PCR-based investigation for TBEV, *B. burgdorferi* sensu lato, and *B. miyamotoi*) were bitten by adult *I. persulcatus* ticks infected with *B. miyamotoi* only. We informed these persons of their results and about the clinical features of *B. miyamotoi* infection and recommended self-observation during 2 months (twice the maximum incubation period for *B. miyamotoi* infection [*3*,*6*]). These persons were advised to contact their medical supervisor at RHID (D.S. Sarksyan) if fever, fatigue, erythema migrans, or any other possible symptom of a tickborne infection developed. In 2 patients, such symptoms developed: one 12 days (patient 1), the other 16 days (patient 2), after the tick bite. *B. miyamotoi* DNA was detected by PCR in their blood on admission to RHID. Neither IgM nor IgG were found by a nonspecific ELISA (Omnix, St. Petersburg, Russia [*7*]) that reacts with serum from *B. burgdorferi* sensu lato–infected and *B. miyamotoi*–infected persons. However, *Borrelia* IgM and IgG were detected in serum obtained 12 and 45 days after illness onset from patient 1 and 6 and 39 days later from patient 2. Clinical characteristics were typical of *B. miyamotoi* infection: chills, sweating, headache, dizziness, fatigue, thirst, nausea, vomiting, fever (axillar temperature 38.8°C in patient 1 and 39.0°C in patient 2). Erythema migrans was absent. Both patients had modest thrombocytopenia (134 [patient 1] and 118 [patient 2] × 10^9 platelets/mL [reference range 150–400 × 10^9 platelets/mL]) and increased band neutrophils (10% [patient 1] and 8% [patient 2] of leukocytes [reference range 1%–5%]). Patient 2 had clinical and laboratory signs of kidney dysfunction (oliguria, leukocytes, erythrocytes, and epithelial cells in a urine sample), a complication observed in 18% of 95 patients with *B. miyamotoi* disease (*4*). Both patients were