In this study, we detected Zika virus RNA in 2 pools of engorged *Ae. aegypti* mosquitoes that were collected during a mosquitoborne virus surveillance program in Rio de Janeiro. Information regarding Zika virus infection rates is lacking for female and male mosquitoes trapped in the field. However, experiments performed in the laboratory demonstrated transovarial transmission of Zika virus among *Ae. aegypti* mosquitoes and revealed a minimal filial infection rate of 1:290 (*10*). Mosquitoborne virus surveillance provides an early warning for arbovirus circulation, points out high-risk areas for virus transmission, and provides data for directing control measures. Furthermore, future surveillance-based studies should further illuminate Zika virus ecology and patterns of spatial dynamics.

In conclusion, we showed the presence of Zika virus in engorged *Ae. aegypti* mosquitoes trapped in Rio de Janeiro before the first case of autochthonous Zika virus disease was diagnosed in the city (*3*). This finding emphasizes the importance and benefit of routine entomologic surveillance programs to public health in terms of ensuring timely implementation of disease prevention and control measures. Furthermore, considering that the analyzed Zika virus from Rio de Janeiro clustered in different lineages, our phylogenic analysis suggests multiple introductions of Zika virus from other regions of Brazil, rather than from outside the country, and an early presence (2013) of Zika virus in Rio de Janeiro State.

Acknowledgments

We acknowledge the Sequencing Platform Network, Technological Development Program in Materials for Health–PDTIS (Fiocruz, Brazil), for viral genomic sequencing. We thank Alexandra Bialonski for excellent technical assistance in next-generation sequencing.

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro-FAPERJ (grant nos. 401542/2015-7, E-26/102.241/2013, and E-26/010.001610/2016) and Fundação Oswaldo Cruz.

Dr. Ayllón is a postdoctoral reseacher in the Instituto Nacional de Infectologia Evandro Chagas–Fiocruz. Her research interests include entomology, virology, and immunology, with special focus on mosquitoborne viruses.

References

1. Zammarchi L, Tappe D, Fortuna C, Remoli ME, Günther S, Venturi G, et al. Zika virus infection in a traveller returning to Europe from Brazil, March 2015. Euro Surveill. 2015;20:21153. http://dx.doi.org/10.2807/1560-7917.ES2015.20.23.21153
2. Zanluca C, Melo VC, Mosimann AL, Santos GI, Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. 2015;110:569–72. http://dx.doi.org/10.1590/0074-02760150192
3. Calvet GA, Filippis AM, Mendonça MC, Sequeira PC, Siqueira AM, Veloso VG, et al. First detection of autochthonous Zika virus transmission in a HIV-infected patient in Rio de Janeiro, Brazil. J Clin Virol. 2016;74:1–3. http://dx.doi.org/10.1016/j.jcv.2015.11.014
4. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, et al. Differential susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika virus. PLoS Negl Trop Dis. 2016;10:e0004543. http://dx.doi.org/10.1371/journal.pntd.0004543
5. Jöst H, Bialonski A, Maus D, Sambri V, Eiden M, Groschup MH, et al. Isolation of usutu virus in Germany. Am J Trop Med Hyg. 2011;85:551–3. http://dx.doi.org/10.4269/ajtmh.2011.11-0248
6. dos Reis IC, Honório NA, Codeço CT, Magalhães MA, Lourenço-de-Oliveira R, Barcellos C. Relevance of differentiating between residential and non-residential premises for surveillance and control of *Aedes aegypti* in Rio de Janeiro, Brazil. Acta Trop. 2010;114:37–43. http://dx.doi.org/10.1016/j.actatropica.2010.01.001
7. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008;14:1232–9. http://dx.doi.org/10.3201/eid1408.080287
8. Wæhre T, Maagard A, Tappe D, Cadar D, Schmidt-Chanasit J. Zika virus infection after travel to Tahiti, December 2013. Emerg Infect Dis. 2014;20:1412–4. http://dx.doi.org/10.3201/eid2008.140302
9. Naccache SN, Thézé J, Sardi SI, Somasekar S, Greninger AL, Bandeira AC, et al. Distinct Zika virus lineage in Salvador, Bahia, Brazil. Emerg Infect Dis. 2016;22:1788–92. http://dx.doi.org/10.3201/eid2210.160663
10. Thangamani S, Huang J, Hart CE, Guzman H, Tesh RB. Vertical transmission of Zika virus in *Aedes aegypti* mosquitoes. Am J Trop Med Hyg. 2016;95:1169–73. http://dx.doi.org/10.4269/ajtmh.16-0448

Address for correspondence: Jonas Schmidt-Chanasit, Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany; email: jonassi@gmx.de

Scrub Typhus Outbreak in a Remote Primary School, Bhutan, 2014

Tshokey Tshokey, Stephen Graves, Dorji Tshering, Kelzang Phuntsho, Karchung Tshering, John Stenos

Author affiliations: Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan (T. Tshokey); University of Newcastle, Australia (T. Tshokey, S. Graves); Australian Rickettsial Reference Laboratory (T. Tshokey, S. Graves, J. Stenos); Bajo Hospital, Wangduephodrang, Bhutan (D. Tshering); Royal Centre for Disease Control, Ministry of Health, Thimphu (K. Phuntsho, K. Tshering)

DOI: https://doi.org/10.3201/eid2308.162021

Scrub typhus in Bhutan was first reported in 2009. We investigated an outbreak of scrub typhus in a remote primary school during August–October 2014. Delay in recognition and treatment resulted in 2 deaths from meningoencephalitis. Scrub typhus warrants urgent public health interventions in Bhutan.

Scrub typhus, caused by the intracellular parasite *Orientia tsutsugamushi*, is a miteborne infection that largely occurs in the "tsutsugamushi triangle" (online Technical Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/23/8/16-2021-Techapp1.pdf), where Watt et al. estimated ≈1 million cases occurred annually in 2003 (*1*). Infected persons commonly have fever, headache, conjunctival congestion, myalgia, lymphadenitis, rashes, and eschars with and without complications (*2*). Among untreated persons, the case-fatality rate is 6%–35% (*3*). In scrub typhus–endemic areas, central nervous system involvement occurs in ≈25% of patients (*4*). Consequently, scrub typhus should be considered in the differential diagnosis of aseptic meningitis.

During January–October 2016, Nepal reported scrub typhus in 37 districts, resulting in 8 deaths (*5*). Himachal Pradesh, India, reported 700 case-patients, 20 of whom died (*6*). In Bhutan, scrub typhus gained clinical attention after an outbreak in 2009 (*7*); earlier cases might have been missed because of low awareness.

During August–October 2014, a scrub typhus outbreak occurred in Singye Namgyal Primary School (SNPS), a remote community boarding school in the Wangduephodrang district of Bhutan (online Technical Appendix Figure 2). On August 17, three girls from SNPS reported 5–6 days of fever, headache, cough, and body aches and were treated symptomatically by the visiting health assistant from Kamichu Basic Health Unit (KBHU). Two of the girls recovered; the third was admitted to the KBHU on August 20 and transferred to Bajo Hospital (BH) the next day. By August 26, she experienced neck stiffness, irritability, and disorientation. Viral encephalitis was suspected, and she was referred to the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Thimphu on August 27. On admission, a serum sample tested positive for *O. tsutsugamushi* by rapid diagnostic test (RDT); she died the next day.

Another girl and her brother from SNPS were admitted to the Punakha district hospital on September 1 with similar symptoms. The boy was sent home with medications and recovered; his sister had meningeal symptoms and severe thrombocytopenia and was transferred to the JDWNRH on September 2, where she died on September 28. Specimens from both patients were *O. tsutsugamushi*–positive by RDT.

On September 22, a 10-year-old girl from SNPS was referred to JDWNRH with similar symptoms. Her serum specimen was *O. tsutsugamushi* positive, but she recovered with treatment.

After linking the 2 deaths and other cases, an investigation team visited the school during October 2–4. Case-patients were defined as any person from SNPS with fever, headache, and body ache with or without hemorrhagic manifestations currently or in the previous 2 weeks. Forty-one cases related to the outbreak were listed (online Technical Appendix Figure 3); blood samples were drawn from 21 students, 12 of whom were acutely ill, and 10 local residents. Results for all 31 were negative for malaria and dengue; the Widal test of serum samples for enteric fever from 1 student and 2 local residents showed high antibody titers against *Salmonella enterica* serotype Typhi. Serum samples from the 12 acutely ill students were also tested for *O. tsutsugamushi* by RDT (SD Bioline Tsutsugamushi Test; Standard Diagnostics, Yongin, South Korea) in the Bhutan Public Health Laboratory; all were positive. The 12 samples were taken to the JDWNRH for routine blood tests; results showed anemia in 5 patients, thrombocytopenia in 4, neutropenia in 3, lymphocytosis in 2, and neutrophilia in 2 (online Technical Appendix Table). The samples were also sent to the Australian Rickettsial Reference Laboratory (http://www.rickettsialab.org.au/), where they were tested for antibodies against *Orientia* by an indirect microimmunofluorescence assay (mIFA) (*8*). Of the 12 samples, 9 were positive (≥1:512 for IgM or ≥1:256 for IgG) (Table), including samples from all 6 students who had eschars. All samples from the 12 students were negative for *Orientia* DNA by using quantitative PCR.

Table. Antibody titers by indirect microimmunofluorescence assay of 9 students with diagnosis of scrub typhus, Bhutan, 2014*

		Orientia tsutsugamushi							
		Gilliam		Karp		Kato		*O. chuto*	
Patient ID	Age, y/sex	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
2	6/M	256	512	256	512	256	128	<128	128
3	9/F	8,192	8,192	8,192	8,192	8,192	8,192	4,096	4,096
4	6/M	512	128	512	256	512	128	128	128
5	10/F	1,024	128	1,024	128	1,024	128	512	128
6	13/M	1,024	256	512	128	256	128	256	128
7	15/M	1,024	128	512	128	512	128	<128	<128
9	7/F	2,048	4,096	2,048	4,096	2,048	2,048	<128	<128
11	10/F	1,024	512	1,024	512	1,024	512	256	256
12	14/F	128	1,024	256	512	128	512	256	256

*ID, identification.

Of the acutely ill patients who had positive mIFA results, 67% had pathognomonic eschars, confirming the clinical diagnostic value in this sign of systemic infection. Thrombocytopenia as a sign of scrub typhus could be useful but is a less specific diagnostic indicator (*9*). There was only a 75% agreement between the rapid test kit and the precise mIFA, but RDTs were shown to be more useful in early detection (*10*).

The deaths of 2 children in this outbreak could have been prevented if the public had greater awareness of the signs and symptoms of scrub typhus. Lapses of 7–10 days from symptom onset to initial medical consultation and >1 month until the outbreak was investigated demonstrate the importance of training school health coordinators to identify and report incidences of abnormal medical findings to public health agencies, especially in remote, hard-to-reach areas. Parents delayed seeking medical advice, and in some cases, school staff had to persuade them to take their children for medical evaluation. Rapid medical care during illnesses should be encouraged through better community education.

Despite inadequate identification and reporting, there is increasing evidence of endemic scrub typhus in Bhutan. Outbreaks may be common but unrecognized, and past outbreaks may have been missed. Scrub typhus warrants a dedicated public health program or incorporation into the existing vectorborne disease control program in this country.

Acknowledgments

We thank the Wangduephodrang district health administration, Singye Namgyal Primary School authorities, students and their families, and the local community. We thank Chelsea Nguyen, Mythili Tadepalli, Gemma Vincent, and Hazizul Hussain-Yusef for laboratory support.

Dr. Tshokey is a clinical microbiologist in the Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan. He is currently pursuing a PhD at the University of Newcastle, Australia, and undertakes academic laboratory work in the Australian Rickettsial Reference Laboratory.

References

1. Watt G, Parola P. Scrub typhus and tropical rickettsioses. Curr Opin Infect Dis. 2003;16:429–36. http://dx.doi.org/10.1097/00001432-200310000-00009
2. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*). PLoS Negl Trop Dis. 2015;9:e0003971. http://dx.doi.org/10.1371/journal.pntd.0003971
3. Mahajan SK. Scrub typhus. J Assoc Physicians India. 2005;53:954–8.
4. Abhilash KP, Gunasekaran K, Mitra S, Patole S, Sathyendra S, Jasmine S, et al. Scrub typhus meningitis: An under-recognized cause of aseptic meningitis in India. Neurol India. 2015;63:209–14. http://dx.doi.org/10.4103/0028-3886.156282
5. 59 new cases of scrub typhus in Chitwan, Nepal. myRepublica, Nepal. 2016 [cited 2016 Dec 10]. http://www.myrepublica.com/news/7173
6. Dutt A. Scrub typhus outbreak in Himachal affects 700, kills 20. Hindustan Times. 2016 Sep 27, 2016 [cited 2016 Dec 10]. http://www.hindustantimes.com/india-news/scrub-typhus-outbreak-in-himachal-affects-700-kills-20/story-6k5RvlnFLCIdePB-g2cxKAK.html
7. Tshokey T, Choden T, Sharma R. Scrub typhus in Bhutan: a synthesis of data from 2009 to 2014. WHO South East Asia J Public Health. 2016;5:117–122.
8. Graves SR, Dwyer BW, McColl D, McDade JE. Flinders Island spotted fever: a newly recognised endemic focus of tick typhus in Bass Strait. Part 2. Serological investigations. Med J Aust. 1991; 154:99–104.
9. Tsay RW, Chang FY. Serious complications in scrub typhus. J Microbiol Immunol Infect. 1998;31:240–4.
10. Zhang L, Yu Q, He S, Wang S, Yu H, Li X, Zhang D, Pan L. Comparison of a rapid diagnostic test and microimmunofluorescence assay for detecting antibody to Orientia tsutsugamushi in scrub typhus patients in China. Asian Pac J Trop Med. 2011;4:666-8.

Address for correspondence: Tshokey Tshokey, Clinical Microbiologist, Department of Laboratory Services, Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan; email: doc_tshokey@yahoo.com

Scrub Typhus as a Cause of Acute Encephalitis Syndrome, Gorakhpur, Uttar Pradesh, India

Mahima Mittal, Jeromie Wesley Vivian Thangaraj, Winsley Rose, Valsan Philip Verghese, C.P. Girish Kumar, Mahim Mittal, R. Sabarinathan, Vijay Bondre, Nivedita Gupta, Manoj V. Murhekar

Author affiliations: BRD Medical College, Gorakhpur, India (Mahima Mittal, Mahim Mittal); National Institute of Epidemiology, Chennai, India (J.W.V. Thangaraj, C.P. Girish Kumar, R. Sabarinathan, M.V. Murhekar); Christian Medical College, Vellore, India (W. Rose, V.P. Verghese); National Institute of Virology, Gorakhpur (V. Bondre); Indian Council of Medical Research, Delhi, India (N. Gupta)

DOI: https://doi.org/10.3201/eid2308.170025

Outbreaks of acute encephalitis syndrome (AES) have been occurring in Gorakhpur Division, Uttar Pradesh, India, for several years. In 2016, we conducted a case–control study. Our findings revealed a high proportion of AES cases with *Orientia tsutsugamushi* IgM and IgG, indicating that scrub typhus is a cause of AES.

Article DOI: https://doi.org/10.3201/eid2308.162021

Scrub Typhus Outbreak in a Remote Primary School, Bhutan, 2014

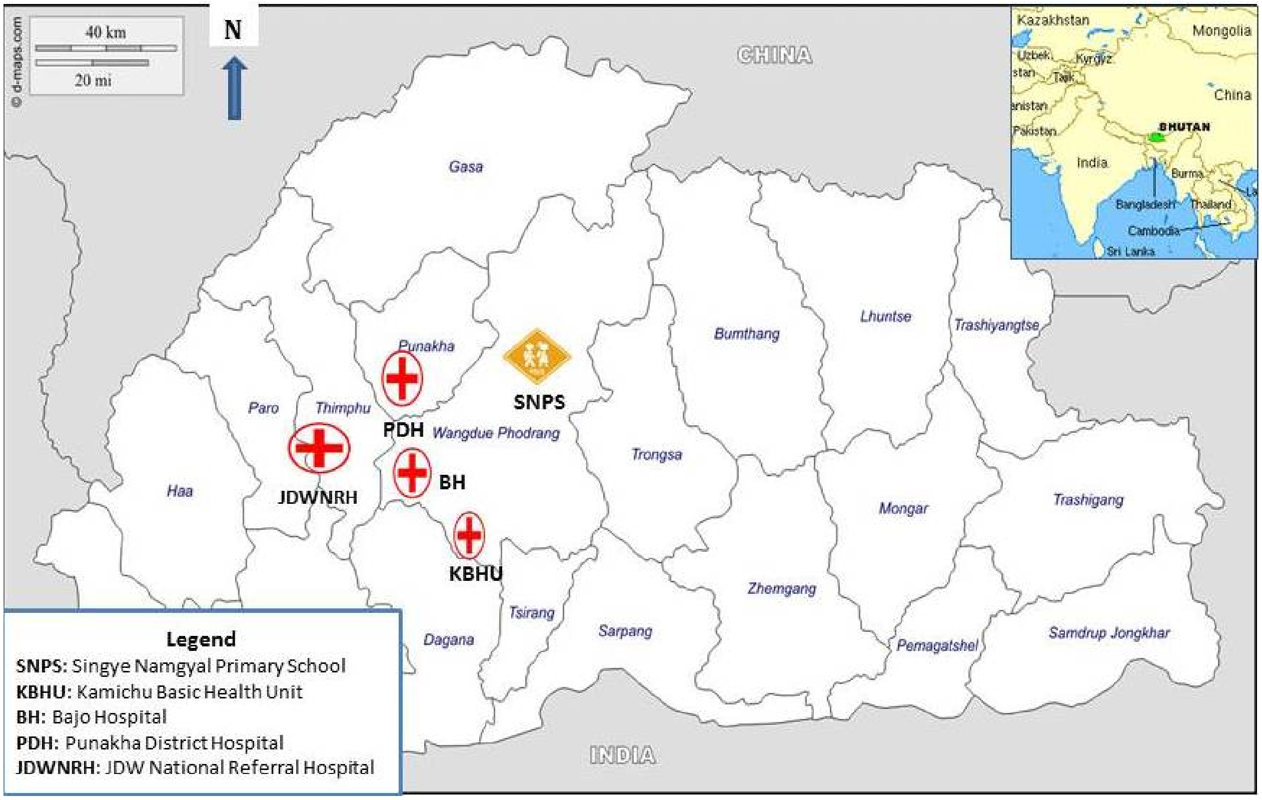
Technical Appendix

Technical Appendix Table. Complete blood count of 12 acutely ill children infected with scrub typhus, Bhutan, 2014*

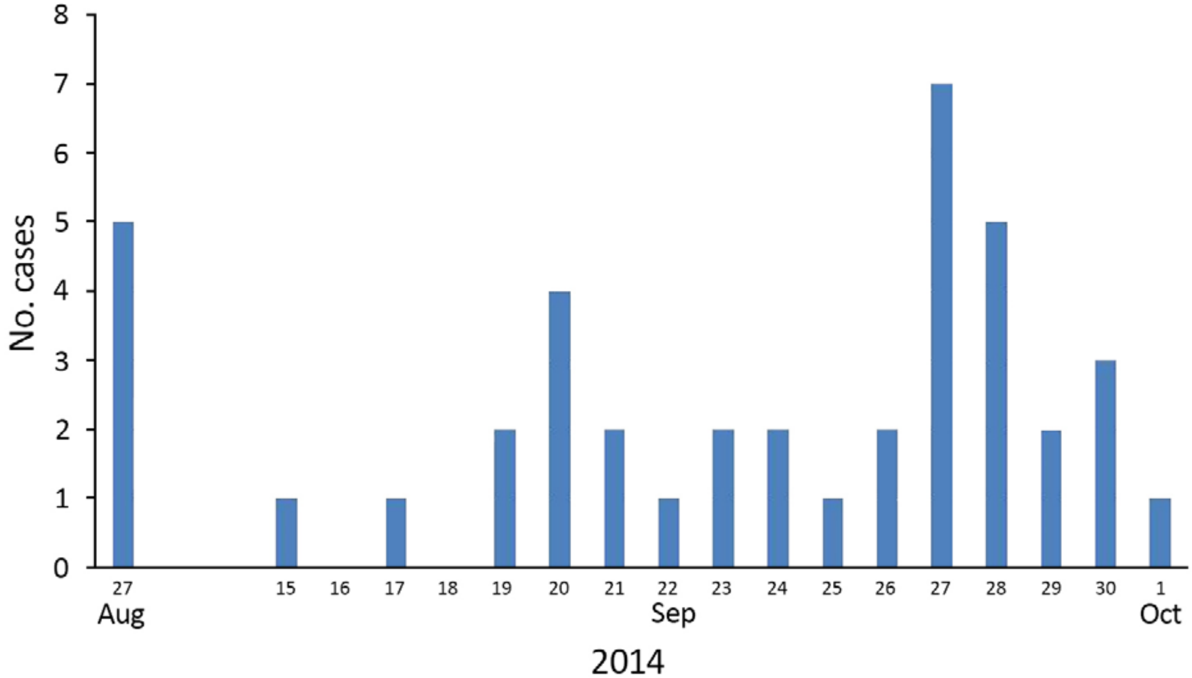
			Full blood count					
Pt. ID	Age (y)	Sex	**Total leucocyte count (10^3/µL) (NR=4–12)**	**Lymphocyte (%) (NR=20–50)**	**Neutrophil (%) (NR=40–65)**	**Platelet (10^3/µL) (NR=150–450)**	**Haemoglobin (g/dL) (NR=11–16)**	**Haematocrit (%) (NR=33–45)**
1	7	F	9.5	46	40	298	10.2	25
2†	6	M	6.5	47	39	272	10.0	30
3†	9	F	9.4	30	56	329	11.2	35
4†	6	M	8.1	34	45	169	12.0	36
5†	10	F	12.8	28	56	251	11.3	35
6†	13	M	4.5	21	70	121	13.2	41
7†	15	M	6.0	22	68	137	12.7	39
8	14	M	7.1	43	43	187	12.5	41
9†	7	F	7.9	31	58	92	12.2	35
10	8	M	4.6	57	31	193	9.3	29
11†	10	F	5.0	58	28	76	8.4	27
12†	14	F	11.4	32	59	209	9.9	30

*NR, normal range, Pt. ID, patient identification.
†Positive for scrub typhus by microimmunoflorescence.

Technical Appendix Figure 1. The tsutsugamushi triangle (in red). More than 1,000,000 cases of scrub typhus, which is a mite-borne infection caused by the bacterium, *Orientia tsutsugamushi*, were reported in this area during 2003.



Technical Appendix Figure 2. Map of Bhutan showing the school and health centers relevant to outbreak of scrub typhus.



Technical Appendix Figure 3. Clinical cases of scrub typhus identified among students of Singye Namgyal Primary School in the Wangduephodrang district of Bhutan during August 27–October 1, 2014. Symptom onset among the first 3 case-patients began 5–6 days before being reported on August 17; the first incidence of scrub typhus in this cohort was diagnosed on August 27.