

Epidemiologic Survey of Japanese Encephalitis Virus Infection, Tibet, China, 2015

Hui Zhang, Mujeeb Ur Rehman, Kun Li, Houqiang Luo, Yanfang Lan, Fazul Nabi, Lihong Zhang, Muhammad Kashif Iqbal, Suolangsi Zhu, Muhammad Tariq Javed, Yangzom Chamba, Jia Kui Li

Author affiliations: Huazhong Agricultural University College of Veterinary Medicine, Wuhan, China (H. Zhang, M.U. Rehman, K. Li, H. Luo, Y. Lan, F. Nabi, L. Zhang, M.K. Iqbal, J.K. Li); Tibet Agriculture and Animal Husbandry College, Linzhi, Tibet, China (S. Zhu, Y. Chamba, J.K. Li); University of Agriculture, Faisalabad, Pakistan (M.T. Javed)

DOI: <https://dx.doi.org/10.3201/eid2306.152115>

We investigated Japanese encephalitis virus (JEV) prevalence in high-altitude regions of Tibet, China, by using standard assays to test mosquitoes, pigs, and humans. Results confirmed that JEV has spread to these areas. Disease prevention and control strategies should be used along with surveillance to limit spread of JEV in high-altitude regions of Tibet.

Japanese encephalitis virus (JEV) is the causative agent of a viral encephalitis that is a major public health threat in most parts of East and Southeast Asia (1,2). Tibet, China, has been considered a nonendemic area for JEV because its mean elevation is >3,100 m, and the relatively low temperatures at the elevation do not support JEV transmission between mosquitoes, reservoirs, and amplifying hosts (2). However, this situation in Tibet has probably changed due to increased pig farming in the region and increased travel between Tibet and areas where JEV is endemic (3–5). To test our hypothesis, we tested for JEV in mosquitoes and measured JEV-specific IgM in swine and humans living in a high-altitude region of Tibet.

We conducted the study in Nyingchi District (elevation 3,100 m) in southeastern Tibet. The mean temperature during the study period ranged from 11.3°C to 22.4°C. Swine and humans included in the study had no history of travel to JEV-endemic areas.

To determine whether mosquitoes were infected with JEV, we collected 8,330 mosquitoes (belonging to 9 genera and 4 species) near pig sties and human residences in Nyingchi, Mainling, and Gongbo'gyamda Counties. From those 8,330 mosquitoes, we chose 2,655 JEV vector mosquitoes: 330 (3.96%) *Culex tritaeniorhynchus*, 45 (0.54%) *C. bitaeniorhynchus*, and 2,280 (27.37%) *C. pipiens*

mosquitoes. To detect JEV, we used the TIANamp Virus DNA/RNA Kit (TianGen, Beijing, China) with pools of whole-body *Culex* mosquito extracts; we analyzed the samples by reverse transcription PCR (RT-PCR) amplification using the Quant One-Step RT-PCR Kit (TianGen) and primers specific for the JEV nonstructural 1 gene (6). Of 11 *C. tritaeniorhynchus* and 69 *C. pipiens* mosquito pools, 7 (63.6%) and 2 (2.9%), respectively, were positive for JEV by RT-PCR; the 1 *C. bitaeniorhynchus* mosquito pool was not positive for JEV (Table).

To determine the origin of JEV in pigs, we collected a total of 454 serum samples from 1- to 6-month-old pigs from local slaughterhouses. We analyzed the samples for JEV IgM by using a commercial ELISA kit as previously described (7). We used the χ^2 test and SPSS software (SPSS Inc., Chicago, IL, USA) to analyze all data; $p < 0.05$ was considered significant.

The overall seroprevalence of JEV IgM in the pigs was 5.07%. The percentage of positive samples from Nyingchi County (3.25%) was significantly lower than that from Mainling County (7.81%); no serum samples from Gongbo'gyamda County were JEV-positive (Table). The difference in seroprevalence of JEV in male (4.62%) and female (5.67%) pigs was not statistically significant.

To determine the prevalence of JEV infection among persons, we collected blood samples from 364 healthy human volunteers residing in the 3 counties and analyzed the samples for JEV IgM by using a commercial ELISA kit as previously described (8). JEV seroprevalence was 11.71% for the 1- to 23-year-old age group, 13.43% for the 24- to 45-year-old age group, and 4.20% for the >45-year-old age group (Table). Seroprevalence was significantly higher among persons 1–23 or 24–45 years of age, compared with persons >45 years of age, suggesting that 1) younger persons may have greater exposure risks than persons >45 years of age, 2) JEV is a relative new introduction in the area as a result of the changing (i.e., warmer) climate, and 3) younger persons may travel more frequently than older persons to lower elevations where JEV is endemic. An IgG-based survey might identify more JEV disease, even in persons who showed no symptoms of the disease.

JEV seroprevalence was significantly higher in rural populations (6.87%) compared with urban population (3.02%). The spread of JEV may be increased by amplifying hosts (pigs); thus, the lower prevalence of JEV in urban residents may be associated with a lower number of pig farms in urban areas compared with rural villages in Nyingchi District.

In conclusion, we found that JEV infection is prevalent in a high-altitude region of Tibet that was previously considered to be free from JEV. Factors such as increased tourism (9), increased mean summer temperatures, increased

Table. Japanese encephalitis virus IgM-positive pigs and humans and Japanese encephalitis virus-positive *Culex* mosquitoes in Nyingchi District, Tibet, China, 2015*

Category, variable	No. IgM positive/no. tested (%)		Total no. positive/total no. tested (%)
	Male pig or person	Female pig or person	
Pigs			
County location			
Nyingchi	3/146 (2.05)	5/100 (5.00)	8/246 (3.25)
Mainling	9/105 (8.57)	6/87 (6.90)	15/192 (7.81)
Gongbo'gyamda	0/9	0/7	0/16
Total	12/260 (4.62)	11/194 (5.67)	23/454 (5.07)
Humans			
County location			
Nyingchi	6/66 (9.09)	11/93 (11.83)	17/159 (10.69)
Mainling	8/58 (13.79)	9/65 (13.85)	17/123 (13.82)
Gongbo'gyamda	1/43 (2.33)	1/39 (2.56)	2/82 (2.44)
Total	15/167 (8.98)	21/197 (10.66)	36/364 (9.89)
Age group, y			
1–23	6/57 (10.53)	7/54 (12.96)	13/111 (11.71)
24–45	7/54 (12.96)	11/80 (13.75)	18/134 (13.43)
>45	2/56 (3.57)	3/63 (4.76)	5/119 (4.20)
Total	15/167 (8.98)	21/197 (10.66)	36/364 (9.89)
<i>Culex</i> mosquitoes†			
<i>C. tritaeniorhynchus</i>	ND	ND	7/11 (0.636)‡
<i>C. bitaeniorhynchus</i>	ND	ND	0/1‡
<i>C. pipiens</i>	ND	ND	2/69 (0.029)‡

*Except as indicated, data are no. (%). ND, Not done.

†Denominator indicates no. in pool.

‡Maximum likelihood estimates (no. pools positive for JEV by reverse transcription PCR).

movement between the study area and nearby JEV-endemic regions, inadequate public health systems, increased pig farming, increased migration of water birds, and the absence of a compulsory immunization policy may contribute to emergence of this disease (3,4,9,10). Disease prevention and control strategies should be used along with surveillance to limit the spread of JEV in high-altitude regions of Tibet.

This study was supported by the Key Science Fund of the Science and Technology Agency of Tibet Autonomous Region, the Twelfth Five-Year National Science and Technology Support Project (grant no. 2012BAD3B03), and the Sustentation Fund (the serology of *Toxoplasma gondii* in Tibetan pigs) of the Huazhong Agricultural University (grant no. 52209-814121).

Mr. Zhang is a PhD scholar in the Department of Clinical Veterinary Medicine, Huazhong Agricultural University, Wuhan, China. His research has focused on the surveillance of emerging infectious diseases by molecular epidemiology analysis and the analysis of pathogenicity mechanisms.

References

- Misra UK, Kalita J. Overview: Japanese encephalitis. *Prog Neurobiol*. 2010;91:108–20. <http://dx.doi.org/10.1016/j.pneurobio.2010.01.008>
- Centers for Disease Control and Prevention (CDC). Japanese encephalitis surveillance and immunization—Asia and the Western Pacific, 2012. *MMWR Morb Mortal Wkly Rep*. 2013;62:658–62.
- Li YX, Li MH, Fu SH, Chen WX, Liu QY, Zhang HL, et al. Japanese encephalitis, Tibet, China. *Emerg Infect Dis*. 2011;17:934–6. <http://dx.doi.org/10.3201/eid1705.101417>
- National Bureau of Statistics of the People's Republic of China. National data. Number of hogs at year-end in Tibet from 1995 to 2014 [cited 2015 Dec 29]. <http://data.stats.gov.cn/english/easyquery.htm>
- Liu Q, Cao L, Zhu XQ. Major emerging and re-emerging zoonoses in China: a matter of global health and socioeconomic development for 1.3 billion. *Int J Infect Dis*. 2014;25:65–72. <http://dx.doi.org/10.1016/j.ijid.2014.04.003>
- Hua RH, Liu LK, Chen ZS, Li YN, Bu ZG. Comprehensive mapping antigenic epitopes of NS1 protein of Japanese encephalitis virus with monoclonal antibodies. *PLoS One*. 2013;8:e67553. <http://dx.doi.org/10.1371/journal.pone.0067553>
- Pant GR, Lunt RA, Rootes CL, Daniels PW. Serological evidence for Japanese encephalitis and West Nile viruses in domestic animals of Nepal. *Comp Immunol Microbiol Infect Dis*. 2006;29:166–75. <http://dx.doi.org/10.1016/j.cimid.2006.03.003>
- Moore CE, Blacksell SD, Taojaikong T, Jarman RG, Gibbons RV, Lee SJ, et al. A prospective assessment of the accuracy of commercial IgM ELISAs in diagnosis of Japanese encephalitis virus infections in patients with suspected central nervous system infections in Laos. *Am J Trop Med Hyg*. 2012;87:171–8. <http://dx.doi.org/10.4269/ajtmh.2012.11-0729>
- Cao L, Fu S, Gao X, Li M, Cui S, Li X, et al. Low protective efficacy of the current Japanese encephalitis vaccine against the emerging genotype 5 Japanese encephalitis virus. *PLoS Negl Trop Dis*. 2016;10:e0004686. <http://dx.doi.org/10.1371/journal.pntd.0004686>
- Prosser DJ, Cui P, Takekawa JY, Tang M, Hou Y, Collins BM, et al. Wild bird migration across the Qinghai–Tibetan plateau: a transmission route for highly pathogenic H5N1. *PLoS One*. 2011;6:e17622. <http://dx.doi.org/10.1371/journal.pone.0017622>

Address for correspondence: Jia Kui Li, Veterinary Clinical Medicine, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei 430070, China; email: lij210@sina.com