

## Original Article



# Serological Survey of Zika Virus in Humans and Animals in Dejiang Prefecture, Guizhou Province, China\*

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## Abstract

**Objective** The current outbreak of Zika virus (ZIKV) poses a severe threat to human health. Two ZIKV strains were isolated from mosquitoes collected from the Dejiang prefecture in China in 2016, which was the first isolation of ZIKV in nature in China.

**Methods** In this study, serum samples were collected from 366 healthy individuals and 104 animals from Dejiang prefecture in 2017, and the plaque reduction neutralization test (PRNT) was used to evaluate the seroprevalence of ZIKV.

**Results** None of the 366 residents from whom the samples were collected were seropositive for ZIKV. None of the 11 pigs from whom the samples were collected were seropositive for ZIKV, while 1 of 63 (1.59%) chickens and 2 of 30 (6.67%) sheep were seropositive for ZIKV.

**Conclusions** The extremely low seropositivity rate of ZIKV antibodies in animals in the Dejiang prefecture, Guizhou province in this study indicates that ZIKV can infect animals; however, there is a low risk of ZIKV circulating in the local population.

**Key words:** Zika Virus; Serological survey; Humans; Animals; China; Plaque reduction neutralization test

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## INTRODUCTION

Zika virus (ZIKV) is a mosquito-borne virus that was first identified in a monkey in Uganda in 1947<sup>[1,2]</sup>. Subsequently, ZIKV spread eastward across the Pacific Ocean to the Americas, resulting in the 2015–2016 epidemic<sup>[3]</sup>. ZIKV has been detected in many species of *Aedes* mosquito, along with *Anopheles coustani*, *Mansonia uniformis*, and *Culex perfuscus*; however, this alone does not incriminate these mosquitoes as vectors of ZIKV<sup>[4]</sup>. The infection often causes no symptoms or symptoms similar to a very mild form of dengue fever<sup>[1]</sup>. ZIKV is mainly transmitted through infected mosquito bites (*Aedes* spp.), but it can also spread through sexual contact and from a pregnant woman to her fetus<sup>[4,5]</sup>. ZIKV infections in adults can trigger Guillain-Barré syndrome<sup>[6]</sup> and cause microcephaly, and other severe brain anomalies in infants<sup>[7]</sup>. ZIKV belongs to the genus *Flavivirus* of family *Flaviviridae*, and is related to dengue, Japanese encephalitis (JE), yellow fever, and West Nile viruses. Like other flaviviruses, ZIKV is enveloped and icosahedral with a non-segmented, single-stranded, 11-kilobase, positive-sense RNA genome<sup>[8]</sup>.

China has reported several cases of imported ZIKV infection in 2016, and the risk of autochthonous circulation following the imported ZIKV spreading will be inevitable due to the distribution of *Aedes* mosquitoes in southern China<sup>[9,10]</sup>. There were no previous reports of ZIKV being isolated from a vector in China until the isolation of two strains of ZIKV from *Culex pipiens quinquefasciatus* and *Armigeres subalbatus* in 2016 from Dejiang prefecture (107°36'–108°28' E, 28°00'–28°38' N) in northeast Guizhou Province in China<sup>[11]</sup>. Guizhou is located on the Yunnan Guizhou Plateau in southwest China and has a sub-tropical climate with abundant rainfall that is suitable for breeding for a variety of hematophagous insects. Guizhou Province has had a high prevalence of JE<sup>[12,13]</sup>, and many JE viruses (JEVs) and Getah viruses have been isolated from mosquito samples collected from Guizhou<sup>[14]</sup>. It is not clear whether ZIKV circulates in local population and animals. In this study, we examined serum from healthy humans and domestic animals for ZIKV infection to determine the local ZIKV transmission risk.

## MATERIALS AND METHODS

### Ethics Statement

This study was approved by the institutional

review board of the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention. All study participants provided informed consent before blood collection. No animals were killed to conduct this study.

### Cells and Viruses

Baby Syrian hamster kidney (BHK-21) cells were preserved in our laboratory and maintained in Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 20,000 U/mL penicillin-streptomycin (PS) at 37 °C with 5% CO<sub>2</sub>. The cells were cultivated in PS-MEM with 2% FBS when infected with the virus. The ZIKV strain GZDJ1685 (GenBank MF099651) used in this study was isolated in our laboratory from *C. p. quinquefasciatus* specimens collected in 2016 from Dejiang prefecture in Guizhou Province<sup>[11]</sup>. The JEV P3 strain (GenBank JEU47032) was preserved in our laboratory<sup>[15]</sup>.

### Collection of Serum Samples

Blood from humans and livestock was collected in October 2017 at Shidatou Village in Dejiang prefecture. Blood samples were collected from residents of all ages. Blood was collected from the livestock that were mainly raised locally. The blood samples were centrifuged to obtain serum and refrigerated for future analysis after aliquoting.

### Detection of Neutralizing Antibody of ZIKV

We used the 90% plaque reduction neutralization test (PRNT90) to detect neutralizing antibodies against ZIKV. The serum was inactivated at 50 °C for 30 min, and then diluted to 1:10 for prescreening ZIKV-neutralizing antibodies. Then, it was mixed with an equal volume of 200 pfu ZIKV suspension and incubated at 37 °C for 1 h. The mixture was added to BHK-21 cells plated in 6-well culture plates and incubated at 37 °C for 1 h, and then overlaid with 1.1% methylcellulose-MEM culture medium with 2% FBS for 4–5 days. Crystal Violet staining was performed to calculate the number of plaques. Samples that showed protective capacity against ZIKV infection in prescreening were diluted from 1:10 to 1:1,280 in two parallel wells to determine the antibody titers. A PRNT90 titer is the dilution of a sample at which 90% reduction of plaques is observed as compared to a virus backdropping control. The neutralizing antibody titers of JEV were detected in parallel to any ZIKV protective

antibody-positive specimens. The specimens were judged seropositive when PRNT90-neutralizing antibody titer was  $\geq 1:10$ . When the PRNT90 anti-ZIKV antibody titer was at least 4-fold greater than that of the corresponding anti-JEV antibody titer in the same specimen, it was identified as ZIKV infection rather than JEV infection. If the PRNT90 anti-JEV antibody titer was at least 4-fold greater than that of the corresponding anti-ZIKV antibody titer in the same specimen, it was identified as JEV infection<sup>[16]</sup>. If the antibody titers of the two viruses differed by less than 4-fold in the same specimen, it was identified as coinfection with ZIKV and JEV.

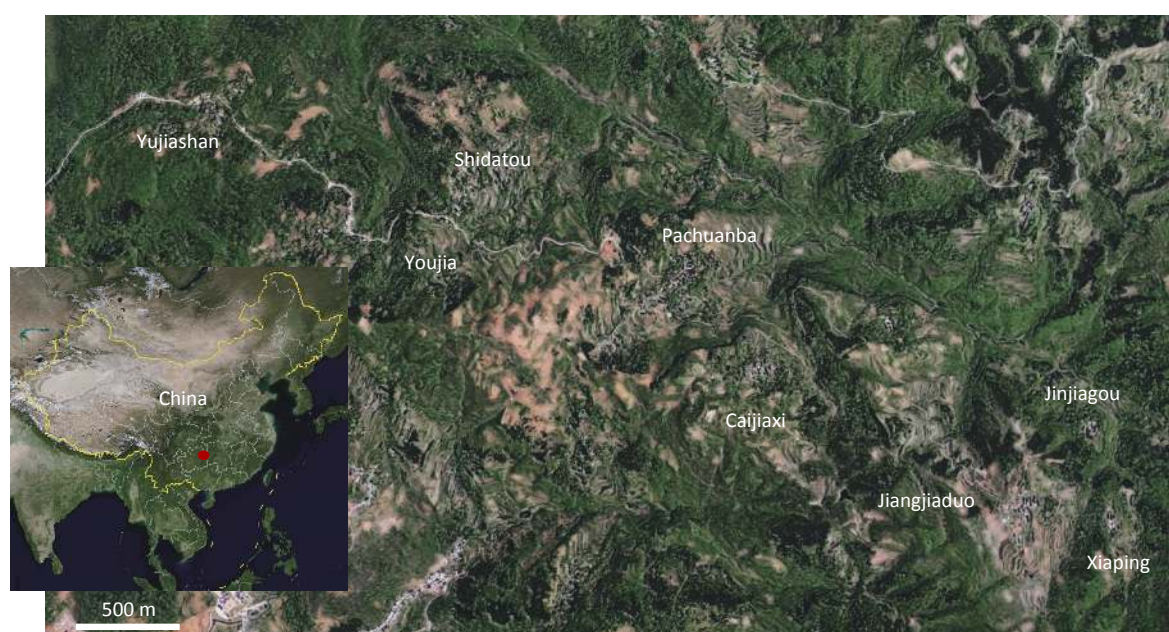
## RESULTS

### Serum Collection

Blood was collected from October 27 to November 2, 2017 at Shidatou village in Dejiang prefecture in Guizhou Province. This study analyzed the samples from 366 healthy individuals collected from eight groups in Shidatou (the Yujiashan, Youjia, Shidatou, Pachuanba, Caijiayi, Jinjiagou, Jiangjiatuo, and Xiaping groups) and 104 domestic animals (63 chickens, 11 pigs, and 30 sheep) from the Yujiashan, Shidatou, and Jiangjiatuo groups (Figure 1, Table 1). Of the human subjects, 29.9% were 0–19 years old, 28.1% were 20–49 years old, and 42.0% were  $\geq 50$  years old.

**Table 1.** Summary of the collected serum of human health and domestic animal

Variable	Number (n)	Constituent ratio (n%)
Human		
Age group (Year)		
0–9	69	18.8
10–19	40	10.9
20–29	26	7.1
30–39	31	8.5
40–49	46	12.5
50–59	54	14.7
60–69	63	17.2
> 70	37	10.1
Sex		
Man	171	46.7
Woman	195	53.2
Nationality		
Tu	364	99.4
Han	2	0.6
Total	366	
Animal		
Chicken	63	60.5
pig	11	10.5
sheep	30	28.8
Total	104	



**Figure 1.** Locations in China where sampling was performed are indicated by red solid circles.

### Serological Evidence of ZIKV Infection

None of the human serum specimens tested positive for ZIKV PRNT90, while three animals (chicken D32, sheep D56, and sheep D61) tested positive, with neutralizing antibody titers of 1:10, 1:20, and 1:40, respectively (Table 2). The three positive animal specimens were further tested for JEV neutralizing antibody titers: D32 was negative, while D56 and D61 were positive for JEV PRNT90 neutralizing antibody, with titers of 1:40 and 1:80, respectively (Table 2). As the titers of the two viruses did not reach a 4-fold difference in the same specimen, D56 and D61 were identified as being coinfecting with ZIKV and JEV.

In summary, all 366 residents and 11 pigs whose samples were collected, tested negative for ZIKV neutralizing antibody; 1 of 63 chickens (1.59%) was positive for ZIKV neutralizing antibody but negative for JEV, and 2 of 30 sheep (6.67%) were positive for both ZIKV and JEV neutralizing antibodies (Table 3).

### DISCUSSION

This study follows up on the first isolation of a ZIKV strain in 2016 from Guizhou Province in China. It assessed the local risk of ZIKV infection in Dejiang prefecture in Guizhou Province. Shidatou Village, the region from where the ZIKV GZDJ1685 was isolated from a mosquito and this serological survey was conducted, covers an area of 8.3 square kilometers

dominated by karst landforms<sup>[11]</sup>. Because of poor local infrastructure, the residents live in dispersed groups (the Yujiashan, Youjia, Shidatou, Pachuanba, Caijiayi, Jinjiagou, Jiangjiatuo, and Xiaping groups). Most derive their incomes from agriculture, farm animals (pigs, sheep, and chickens) and aquaculture. The registered population of the village is 1,278, but young and middle-aged laborers have moved to urban areas and the local population is decreasing. The residents are mainly the elderly and children under 15 years of age. In this study, no ZIKV infection was seen in humans in the Shidatou region, while three of the animals sampled had suspected ZIKV infection.

ZIKV belongs to the flaviviruses, and its antigenic structure is very similar to that of the other flaviviruses<sup>[17]</sup>, whose main antibody-binding sites are prM protein, E protein, and secretory non-structural protein 1 (NS1); this also applies to ZIKV<sup>[18]</sup>. Antibodies to ZIKV persist in the serum of infected individuals after ZIKV infection. There may be cross-reactivity between different flaviviruses, so an accurate serological diagnosis of ZIKV has always been problematic. The criterion classifies recent infection as samples with a 4-fold difference between two flaviviruses in the same specimen<sup>[19]</sup>.

We conducted PRNT90 test only for ZIKV and JEV in this study as Guizhou Province is a traditionally epidemic region for JE<sup>[12,13]</sup>, and no dengue virus or other flaviviruses were detected in local arbovirus

**Table 2.** Neutralizing antibody titer to ZIKA and JEV infection among domestic animals in Shidatou village

Sample ID	Collection site	Host species	PRNT90 titer*	
			ZIKV	JEV
D32	Yujiashan	chicken	1:10	–
D56	Shidatou	sheep	1:20	1:40
D61	Shidatou	sheep	1:40	1:80

**Note.** \* PRNT 90 titer, 90% plaque reduction neutralization test titer.

**Table 3.** The seroprevalence of ZIKV in human and domestic animals in Shidatou village

Host species	No. case	No. PRNT90 positive		Seroprevalence (%)
		ZIKV	JEV	
Human	366	–	–	–
Chicken	63	1	–	1.59
Pig	11	–	–	–
Sheep	30	2	2	6.67



survey in 2016 except JEV and ZIKV<sup>[11]</sup>. The results showed that only one animal (D32) was suspected of having ZIKV infection and showed no protection against JEV. Another two animals (D56 and D61) might have had ZIKV and JEV coinfection.

While virus detection from field vector samples is important for identifying putative vectors of the virus, the natural blood feeding pattern of the candidate vector species is also critical for understanding the role of a putative vector species in pathogen transmission. The two ZIKV strains isolated in Shidatou were from *C. p. quinquefasciatus* and *A. subalbatus*. In this area, the three dominant mosquito species are *Anopheles sinensis*, *C. p. quinquefasciatus*, and *A. subalbatus* while no *Aedes* mosquitoes have been found locally. The strain used for the PRNT90 test in this study was GZDJ1685 strain isolated from *C. p. quinquefasciatus* in 2016<sup>[11]</sup>. Our results showed an extremely low seropositive rate in local animals. The local Center for Disease Control and Prevention is also investigating Guillain-Barré syndrome and microcephaly in Dejiang prefecture, but no cases have been discovered (data not shown). Therefore, we consider that ZIKV has not developed an effective transmission cycle between mosquitos and local animals or humans. In nature, *C. p. quinquefasciatus* and *A. subalbatus* may be incompetent vectors for ZIKV transmission<sup>[20]</sup>.

The primary mosquito vectors for ZIKV belong to the genus *Aedes*, most notably *Ae. aegypti* and *Ae. albopictus*<sup>[21-24]</sup>. ZIKV is believed to be mainly transmitted by the human-biting mosquito *Ae. aegypti*<sup>[25,26]</sup>. Experimental infections with epidemic ZIKV strains have demonstrated that populations of *Ae. aegypti* and *Ae. albopictus* are associated with the risk of local transmission<sup>[27]</sup>. ZIKV has been isolated from many other mosquito species and genera, but those mosquito species were incompetent for ZIKV transmission<sup>[20,28-32]</sup>. The biological characteristics of different mosquito species differ markedly. The anthropophilic mosquitoes of *Aedes* feed during the day, while *Culex* and *Armigeres* feed at dawn and dusk. *Aedes* likes fresh water while *Culex* likes dirty water and *Armigeres* likes manure pits. *Aedes* specializes in feeding on humans while *Armigeres* prefers cows and humans and *Culex* prefers birds. A highly competent vector that does not feed on a competent host species will not contribute to virus transmission in nature<sup>[33]</sup>. With regard to ZIKV, for which humans are competent hosts, since both *C. p. quinquefasciatus* and *A. subalbatus* obtain a relatively low percentage of blood meals from

humans, they are unlikely to serve as the principal transmission vectors of the virus. As there is no competent vector to support the replication of ZIKV and the risk of ZIKV transmission in the target region, we conclude that there is a low risk of ZIKV transmission in the Dejiang prefecture and even in Guizhou. The results of this ZIKV serological survey support this view.

## CONCLUSION

In summary, this study conducted a serological survey of ZIKV infection in animals and humans in Shidatou village, where ZIKV-positive *C. p. quinquefasciatus* and *A. subalbatus* mosquitoes were reported previously. None of the humans tested positive for ZIKV PRNT90 while three livestock specimens tested positive for ZIKV PRNT90. The results revealed a low risk of local ZIKV transmission in Dejiang prefecture in Guizhou province in China. However, further mosquito surveillance should be performed locally on an annual basis to better understand the transmission dynamics and public health threat of ZIKV in this community.

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## AUTHOR CONTRIBUTION

LI Fan, WANG Huan Yu, and ZHANG Yan Ping designed the study; WANG Ding Ming, LI Fan, ZHOU Jing Zhu, TIAN Zhen Zao, SHAO Nan, and TANG Guang Peng collected the serum samples; LI Fan and FU Shi Hong performed the PRNT experiments; FU Shi Hong, LEI Wen Wen, and HE Ying performed virus culture; LI Fan, WANG Qi, and LI Dan processed the data and created the figure; LI Fan, ZHOU Jing Zhu, ZHOU Lei, and LIANG Guo Dong contributed to drafting and editing the paper; all authors reviewed the manuscript.

## CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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