Tahyna Virus Infection, a Neglected Arboviral Disease in the Qinghai-Tibet Plateau of China

Wenjuan Li^{1,2,5,*} Yuxi Cao^{1,*} Shihong Fu¹ Jinglin Wang¹ Minghua Li¹ Suangying Jiang³
Xuewen Wang³ Shiyong Xing⁴ Linghui Feng⁴ Zhiqun Wang³ Yan Shi³
Shencang Zhao⁴ Huanyu Wang¹ Zhiyu Wang² and Guodong Liang¹

Abstract

Tahyna virus (TAHV) was first isolated from mosquitoes collected in the suburbs of Geermu city in the Qinghai-Tibet Plateau of China in 2007. Since then, TAHV antibodies have been detected in local livestock in Geermu, Qinghai. To determine whether the disease caused by TAHV was present in local residents, an investigation was conducted in the summer of 2009. During this investigation, ward inspections were conducted in rural clinics, and clinical information and specimens were collected from patients who complained mainly of acute fever. The collected samples were tested by serological and molecular methods. The results showed that four samples were positive for TAHV immunoglobulin M and had four-fold or higher levels of TAHV-neutralizing antibody titers between convalescent-phase and acute-phase, and that TAHV nucleotide sequences were detected in two acute sera. Clinical features of TAHV infection commonly included fever, accounting for 100%. Among all other symptoms, the one with the highest frequency was pharyngitis (80%), followed by malaise, inappetence, arthralgia, headache, and drowsiness. Follow-up surveys revealed that all cases recovered in 2–5 days after onset, and no serious or deadly cases were observed. This is the first time that the disease caused by TAHV infection has been reported in China. TAHV infection is another known mosquito-borne arboviral disease in China.

Key Words: Tahyna virus—Infectious disease—Qinghai-Tibet Plateau.

Introduction

TAHYNA VIRUS (TAHV; BUNYAVIRIDAE, *ORTHOBUNYAVIRUS*) belongs to the California complex of bunyaviruses (Bennett et al. 2011). TAHV was originally isolated from mosquitoes in Slovakia in 1958 (Bardos et al. 1959), and the disease caused by TAHV was confirmed in the 1960s (Hubálek 2008). Both the virus and the disease it causes have been documented in many countries in Eastern and Western Europe (Sluka 1969, Korobeinikova et al. 2003, Hubálek et al. 2004, Hannoun et al. 1966, Hubálek et al. 2008). The disease in humans mainly presents as fever, gastrointestinal disorders, sometimes as atypical pneumonia, and rarely as meningoencephalitis (Mayerova et al. 1966, Simková et al. 1973, Bárdos et al. 1975, Lundstrom 1999). The disease has occurred annually in Eastern European countries, such as the Czech Republic

(Hubálek et al. 2008). Previous studies have revealed high TAHV antibody prevalence (60–80%) among inhabitants of endemic foci, suggesting that TAHV has become widespread in Europe (Bárdos et al. 1975, Gratz 2004, Hubálek et al. 2008).

In 2007, an investigation of mosquitoes and mosquito-borne arboviruses was conducted in the Qinghai-Tibet Plateau, China. TAHV was isolated from mosquitoes collected in the suburbs of Geermu city, and TAHV antibodies were found among local cows, sheep, and swine (Li et al. 2010). This was the first isolation of TAHV and the first evidence of TAHV infection in local livestock in the Qinghai-Tibet Plateau. However, little is known about the pathogenicity of TAHV or the clinical symptoms and prognosis of the related disease in local residents.

In the summer of 2009, an investigation was performed in the suburbs of Geermu city in the Qinghai-Tibet Plateau to

¹State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Institute for Viral Disease Control and Prevention, China CDC, Changping District, Beijing, P.R. China.

²School of Public Health, Shandong University, Jinan, China.

³Qinghai Center for Disease Control and Prevention, Xining, China.

⁴Geermu Center for Disease Control and Prevention, Geermu, China.

⁵School of Forensic and Laboratory Medicine, Jining Medical University, Jining, China.

*These authors contributed equally to this article.

determine whether TAHV human infection cases occurred there and to describe their clinical features. Clinical cases caused by TAHV were identified among acute fever patients based on observed symptoms, household investigations, and laboratory tests. Here, we describe cases of TAHV-related disease in China for the first time.

Methods

Investigation setting

Geermu city is located on the north side of the Kunlun Mountains; local residents live along Ge-Mang Road. We selected the village where TAHV was isolated in 2007 as the survey center, and the survey area was extended from east to west along Ge-Mang Road. A total of six villages were included in this study, covering an area of approximately 240 square kilometers (40 km × 6 km) and a population of more than 12,000 persons (Fig. 1).

Case identification and household surveys

Wards in clinics in all six villages were inspected every day during the study period. Any patient who complained mainly of acute fever ($\geq 37^{\circ}\text{C}$) was screened, because humans with TAHV infection typically initially present with fever (Mayerova et al. 1966, Simková et al. 1973, Hubálek et al. 2008). Clinical symptoms and epidemiological data were collected by local doctors. Cases diagnosed with bacterial infections or other common acute respiratory or intestinal infections were ex-

cluded. After receiving informed consent, blood samples (2 mL) were collected from patients with fever during the acute phase (within 10 days after the onset of fever). These patients were followed-up regularly to collect information about clinical signs and the duration and severity of symptoms. Patients were interviewed at home every 3 days and were asked for more information about symptoms, course, and recovery. For patients in whom we detected TAHV immunoglobulin M (IgM) antibodies, paired serum samples were taken in the convalescent phase, usually 14–30 days after symptom onset.

Laboratory analysis

Indirect immunofluorescence assay. TAHV strain QH07060, which was isolated from *Aedes detritus* in Qinghai in 2009 (Li et al. 2010), was used to infect BHK-21 cells, and slides were prepared for use in an indirect immunofluorescence assay (IFA). Suspensions of infected cells were applied to Teflon-coated 10-well slides (Feizhou, Inc., Hangzhou, China) with TAHV antigen, and uninfected cells were used as controls. Serum samples at a dilution of 1:50 were tested for antibodies against TAHV, as reported previously (Lv et al. 2009, Li et al. 2010).

Plaque-reduction neutralization test. Serum samples were tested for neutralizing antibodies to TAHV QH07060 using the 90% plaque-reduction neutralization test (PRNT₉₀) (Lv et al. 2009, Li et al. 2010). Sera were tested with serial two-fold dilutions starting at 1:5. Diluted sera were mixed with

FIG. 1. The black square on the map shows the survey area in Geermu, Qinghai, China.

equal volumes of minimum essential medium (Hyclone, Inc., South Logan, UT) containing TAHV (100 plaque-forming units [pfu]) and were incubated at 37°C for 1 h. Six-well plates of confluent BHK-21 cells were inoculated with the serum-virus mixtures and incubated at 37°C in a 5% CO₂ incubator for 1 h. The plates were overlaid with 3 mL of medium containing 0.8% agarose, then with 2.5 mL of a second overlay medium containing Neutral Red vital stain (Sigma-Aldrich, Co., St. Louis, MO), as described previously (Lv et al. 2009, Li et al. 2010). The neutralizing antibody titer was identified as the highest serum dilution that reduced the number of viral plaques in the test by 90% or more.

RNA extraction, cDNA synthesis, reverse transcription polymerase chain reaction, and sequence analysis. Extraction of viral RNA from samples was performed using TRIzol reagent, and first-strand cDNA was synthesized using Ready-To-Go You-Prime First-Strand Beads (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) according to the manufacturer's instructions. Serum samples were examined by nested polymerase chain reaction (PCR) with primers designed for the TAHV S segment (outer primer, THV162F, 5'-TAAACATGGGAGGCAATC-3', THV871R, 5'-GGGGAACCTTTTGGA TAG-3'; inner primer, THV178F, 5'-ATCAATCTCCATTCCG TTAGG-3', THV735R, 5'-GAGACCACCTCTTCCCCAC-3'). Amplified DNA fragments were detected by electrophoresis in 1% agarose gels. Positive DNA fragments were extracted using a TaKaRa DNA Fragment Purification Kit (Takara Bio, Inc., Otsu, Japan) and were sequenced by a service provider (Beijing Genome Institute, Beijing, China). Initial sequence analysis was conducted using SeqMan (www.dnastar.com; DNASTAR, Inc., Madison, WI). Identity and alignment analyses were carried out using Clustal X (v. 1.8; www.clustal.org) and MegAlign (DNASTAR) software. MEGA 4 (www.megasoftware.net; Center for Evolutionary Medicine and Informatics, The Biodesign Institute, Tempe, AZ) software was used for phylogenetic analysis and tree construction based on the neighbor-joining method with a bootstrap value of 1000.

Laboratory-confirmed case definition

We considered a patient to have confirmed TAHV infection (Duffy et al. 2009, Kay et al. 2009) if: (1) The patient had IgM antibodies against TAHV on the basis of IFA and a ratio of TAHV PRNT₉₀ titer between convalescent-phase and acute-

phase samples of ≥ 4 , or (2) TAHV RNA was detected in the serum.

Results

Laboratory-confirmed case identification

From July 9 through September 10, 2009, a total of 229 patients with acute fever were enrolled. In total, 229 sera samples from the acute phase and four samples from the convalescent phase were collected. All of the acute-phase samples were tested for TAHV IgM antibodies and viral nucleotide sequences. TAHV IgM antibodies were detected in five patients (Table 1). Paired sera collected from four IgM antibody-positive patients were simultaneously tested for neutralizing antibodies. They showed four-fold or greater increases in TAHV neutralizing antibody titer (Table 1). TAHV nucleotides with partial S segments were detected in acute-phase serum samples from two of 229 patients using nested reverse transcription PCR (Table 1). The sequences from cases GEM09112 and GEM09121 were both 549 bp in length, and the identity of these two nucleotide sequences was 99.5%. In addition, these two sequences were 91.1% and 91.3% identical with the TAHV strain Bardos 92, respectively. Phylogenetic analysis showed that the nucleotide sequences from these two patients were in the same evolutionary branches of TAHV (Fig. 2), suggesting that these two patients were infected by TAHV. In total, five cases of TAHV infection were detected in this study.

Clinical symptoms and outcomes of TAHV infection

All five confirmed cases showed similar clinical symptoms, of which fever was the main symptom (Table 2). Five patients had temperatures above 37.0°C, with one case having a temperature of 37.8°C. Among all other symptoms, the one with the highest frequency was pharyngitis, followed by malaise, inappetence, arthralgia, headache, and drowsiness (Table 2). Because all symptoms were mild, clinical doctors diagnosed cases as acute fever and pharyngeal swelling. The follow-up survey revealed that fever and other symptoms disappeared within 2–5 days. All five patients had good prognoses, and no cases of exacerbated symptoms or death were observed. The results suggest that TAHV infection is generally a self-limiting disease. None of the five patients had traveled outside of Geermu within the 1 month before onset of symptoms, and all cases had a history of mosquito bites.

TABLE 1. TAHYNA VIRUS INFECTION CASES IN GEERMU, CHINA, IN 2009

Cases	No. of Patients	Sex/age	Occupation	Date of onset	Interval after onset (days)	IFA	PRNT	PCR
1	GEM09049	M/25	Farmer	July 27, 2009	1	+	10	–
					14	ND	40	ND
2	GEM09080	M/55	Farmer	July 31, 2009	0	+	80	–
					9	ND	640	ND
3	GEM09091	M/10	Student	August 7, 2009	0	+	160	–
					18	ND	1280	ND
4	GEM09112	F/16	Student	August 10, 2009	0	+	<10	+
5	GEM09121	F/52	Farmer	August 11, 2009	0	+	10	+
					13	ND	40	ND

IFA, indirect immunofluorescence assay; PRNT, plaque reduction neutralization test; PCR, polymerase chain reaction; M, male; F, female; ND, not done.

FIG. 2. Phylogenetic analysis of the nucleotide sequence of Tahyna virus S segments from patients in Geermu, China.

Discussion

There are several kinds of mosquito-borne viral diseases known to occur in Mainland China, in which Japanese encephalitis (JE) and dengue fever (DF) are the two principal arboviral diseases of public health importance at present (Gao et al. 2010, Zheng et al. 2012). Of these two diseases, JE is highly epidemic in Mainland China, because 28 provinces have reported JE cases except Xinjiang, Tibet, and Qinghai (Zheng et al. 2012). In addition, JE is a serious viral encephalitis disease at present in China. DF is mainly found in the coastal areas of southeastern China, and most DF cases are introduced from abroad (Gao et al. 2010). These two diseases were classified as Statutory Notified Infectious Diseases by the Chinese government in 1950 and 1993, respectively (Gao et al. 2010). Qinghai is located in northwest China. The health department has not found any endemic presence of Japanese encephalitis virus and dengue fever virus, or any other mosquito-borne arboviruses in Qinghai since 1950 (Liu et al. 2011). TAHV, however, was isolated from mosquitoes collected in

the rural areas of Geermu, Qinghai, in 2007, and TAHV antibodies were found in the sera of local animals, suggesting that TAHV has circulated naturally in this region (Li et al. 2010). Tahyna virus was first isolated from *Culex* mosquitoes in Xinjiang, China (Lv et al. 2009). In addition, Gu found the high level of neutralizing antibody against THAV from healthy people in Xinjiang, China (Gu and Artsob. 1987). These previous researches indicated that TAHV infection might occur in China, but no confirmed cases were identified. This paper is the first report of confirmed TAHV cases not only in the rural areas of Geermu, Qinghai, where TAHV has previously been isolated, but also in Mainland China. Furthermore, TAHV infection is another known mosquito-borne arboviral disease in China.

Qinghai Province is located in the Qinghai-Tibet Plateau, which is 2800 meters above sea level; its climate is arid. The rural areas of Geermu cover a wealth of salt marshes and swamps, and *Ae. detritus* is one of the dominant mosquitoes (Li et al. 2010). During the local annual rainy season, from June to August, the mosquito density is relatively high, and villagers are more likely to be bitten and infected with the virus during this time. Also, the local cases with TAHV infection identified in this study indicate that human infection with TAHV and related disease epidemics are occurring in the local population. This is the first report of TAHV infection in such a high-latitude region (2800 meters above sea level).

Most patients with TAHV infection only present with flu-like symptoms, although TAHV can also cause atypical pneumonia, myocarditis, and meningitis (Hannoun et al. 1966, Sluka et al. 1969). In the present study, the symptoms of TAHV cases were mild and included fever, headache, sore throat, and fatigue. Most patients suffered primarily from fever, although body temperatures were not high. Because of the mild clinical symptoms caused by TAHV infection and its

TABLE 2. CLINICAL SYMPTOMS OF CASES WITH TAHYNA VIRUS INFECTION IN GEERMU, QINGHAI

Signs or symptoms	No. of cases
Fever	5 (5/5)
Pharyngitis	4 (4/5)
Malaises	3 (3/5)
Inappetence	3 (3/5)
Arthralgia	2 (2/5)
Headache	2 (2/5)
Drowsiness	2 (2/5)
Nausea, vomiting	1 (1/5)
Cough	1 (1/5)

typically good prognosis, many infected people may not go to the clinic for medical treatment or may be ignored by the doctors.

TAHV has been isolated from mosquitoes collected in Xinjiang, Qinghai, and Inner Mongolia, China (Lv et al. 2009, Li et al. 2010, Cao et al. 2011). This suggests that this virus is widespread in Mainland China. Therefore, to understand the disease burden caused by this virus, health departments should strengthen surveillance for it and determine whether it circulates in mosquitoes and/or its infection occurs in local people in other provinces. Studies have shown that TAHV infection is mainly epidemic in Europe, and its epidemicity in Asia has not been reported (Bárdos et al. 1975, Gratz 2004, Hubálek et al. 2008). However, the isolation of the virus from three provinces in China suggests that this virus is widely distributed along the Asian continent. Therefore, surveillance of its further spread to the eastern region of Asia is necessary to prevent and control TAHV infection.

Acknowledgments

We thank Dr. Roger S. Nasci (Centers for Disease Control and Prevention, Fort Collins, CO) for his careful review of the manuscript and valuable suggestions.

This work was supported by grants from National Natural Science Foundation of China (81290342); the Ministry of Science and Technology, People's Republic of China (no. 2011CB504702); Development Grant of State Key Laboratory for Infectious Disease Prevention and Control (2008SKLID105); and China-US CDC Cooperative Agreement (U19-GH000004).

Author Disclosure Statements

No competing financial interests exist.

References

- Bardos V, Danielova V. The Tahyna virus—a virus isolated from mosquitoes in Czechoslovakia. *J Hyg Epidemiol Microbiol Immunol* 1959; 3:264–276.
- Bárdos V, Medek M, Kania V, Hubálek Z. Isolation of Tahyna virus from the blood of sick children. *Acta Virol* 1975; 19:447.
- Bennett RS, Gresko AK, Murphy BR, Whitehead SS. Tahyna virus genetics, infectivity, and immunogenicity in mice and monkeys. *Virology* 2011; 8:135.
- Cao YX, Fu SH, Tian ZF, Lu Z, et al. Distribution of mosquitoes and mosquito-borne arboviruses in Inner Mongolia, China. *Vector-Borne Zoonotic Dis* 2011; 11:1577–1581.
- Duffy MR, Chen TH, Hancock WT, Powers AM, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009; 11:2536–2543.
- Gao XY, Nasci R, Liang GD. The neglected arboviral infections in mainland China. *PLoS Negl Trop Dis* 2010; 4:e624.
- Gratz N. (2004). The vector-borne human infections of Europe—their distribution and burden on public health. WHO Regional Office for Europe, Copenhagen, 2004:144.
- Gu HX, Artsob H. The possible presence of Tahyna (Bunyaviridae, California serogroup) virus in the People's Republic of China. *Trans R Soc Trop Med Hyg* 1987; 81:693.
- Hannoun C, Panthier R, Corniou B. Isolation of Tahyna virus in the south of France. *Acta Virol* 1966; 10:362–364.
- Hubálek Z. Mosquito-borne viruses in Europe. *Parasitol Res* 2008; 103:S29–S43.
- Hubálek Z, Zeman P, Halouzka J, Juricová Z, et al. Antibodies against mosquito-borne viruses in human population of an area of Central Bohemia affected by the flood of 2002. *Epidemiol Mikrobiol Immunol* 2004; 53:112–120.
- Li WJ, Wang JL, Li MH, Fu SH, et al. Mosquitoes and mosquito-borne arboviruses in the Qinghai-Tibet plateau—focused on the Qinghai Area, China. *Am J Trop Med Hyg* 2010; 82:705–711.
- Liu H, Gao XY, Liang GD. Newly recognized mosquito-associated viruses in mainland China, in the last two decades. *Virology* 2011; 8:68.
- Lundstrom JO. Mosquito-borne viruses in Western Europe: A review. *J Vector Ecol* 1999; 24:1–39.
- Lv Z, Lv XJ, Fu SH, Zhang S, et al. Tahyna virus and human infection, China. *Emerg Infect Dis* 2009; 15:306–309.
- Kay MK, Gibney K, Riedo F, Kosoy OL, et al. Toscana virus infection in American traveler returning from Sicily, 2009. *Emerg Infect Dis* 2010; 16:1498–1500.
- Korobeinikova AS, Nafeev AA, Skvortsova TM. Specific markers for the detection of circulation of Tahyna, Inko and Batai viruses (Bunyaviridae, Bunyavirus) in humans, mosquitoes, ticks and cattle of the Uliyanovsk region. *Vopr Virusol* 2003; 48:45–46.
- Mayerova A, Hruzik J, Mayer V. A significant increase of Tahyna virus neutralizing antibodies in patients with the clinical diagnosis of “acute febrile disease”. *Cesk Epidemiol Mikrobiol Immunol* 1966; 15:48–53.
- Simková A, Sluka F. Isolation of Tahyna virus from the blood of a case of influenza-like disease. *Acta Virol* 1973; 17:94.
- Sluka F. Recognition of clinical forms of Valtice fever, a new arbovirus infection. *Wien Med Wochenschr* 1969; 119:765–769.
- Zheng YY, Li MH, Wang HY, Liang GD. Japanese encephalitis and Japanese encephalitis virus in mainland China. *Rev Med Virol* 2012; 22:301–322.

Address correspondence to:

Guodong Liang

State Key Laboratory for Infectious

Disease Prevention and Control

Institute for Viral Disease Control and Prevention

155 Changbai Road, Changping District

Beijing 102206

People's Republic of China

E-mail: gdliang@hotmail.com