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Orientia tsutsugamushi infection in rodents in Anhui Province of China

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ABSTRACT

We conducted an investigation of *Orientia tsutsugamushi* infection among rodents in non-endemic areas in Anhui Province. Fifty-six (56) rodents including 44 *Apodemus agrarius* and 12 *Niviventer niviventer* were trapped and captured in autumn in the fields of three counties in Anhui Province. DNA samples were amplified and sequenced for the 56 kDa protein gene of *Orientia tsutsugamushi*. The overall infection rate in the rodents was 23.2%(13/56). The rate of detection of *O. tsutsugamushi* in *Apodemus agrarius* and *Niviventer niviventer* were 27.3% and 8.3% respectively. Moreover, we identified two genotypes (Karp and Gilliam strains) of *Orientia tsutsugamushi* in rodents. Our study demonstrated that *Apodemus agrarius* is the main host for *O. tsutsugamushi* pathogen and this is the first report of *Karp* and *Gilliam* strains in these non-endemic areas in Anhui Province.

1. Background

Orientia tsutsugamushi is an obligate intracellular bacterium, the causative agent of mite borne disease known as scrub typhus. It is transmitted to humans and small animals through the bites of infected chiggers (trombiculid mite larvae) (Tamura et al., 1995); (Traub et al., 1975). The clinical manifestation of this disease presents a spectrum of severity ranging from mild to fatal (Lai et al., 2009; Kelly et al., 2002; Basnyat et al., 2006). Scrub typhus is endemic to several countries in the Asia-Pacific region, including China and causes an estimated 1 million cases per year (Kelly et al., 2009; Watt and Parola, 2003). To date, the epidemic foci of scrub typhus is still expanding in China (Zhang et al., 2010; Meng and Wang Xian-jun, 2011). Scrub typhus outbreak has been frequently reported in many areas in the northern provinces of China since its emergence in the south (Liu et al., 2013a); (Liu et al., 2009); (Lyu et al., 2013); (Hu et al., 2015). Studies within the north especially in the endemic areas have demonstrated that wild mouse, *Apodemus agrarius* and chigger vector, *L. scutellare* are the main reservoir of this bacterium (Meng and Wang Xian-jun, 2011). Scrub typhus has been an emerging zoonotic disease in Anhui Province of northern China. Several cases have been reported from different areas within the province since its first case in 1982 in Xiuning County (Cao et al., 2016). In 2007 and 2008, there was an epidemic outbreak of scrub typhus in northeast and west part of the province (Zhang et al., 2010); (Al. et al., 2009). A Recent follow up study revealed that scrub typhus is still present in one of the epidemic area (Cao et al., 2016). Although scrub typhus is still endemic in Anhui Province, the epidemiologic investigation of this disease is still essential to monitor its spread. Evaluation of small mammals for infection with *O. tsutsugamushi* provides key information on the focality of scrub typhus. Thus in this present study, we conducted an investigation of *Orientia tsutsugamushi* infection in rodents from Anhui Province.

2. Materials and methods

2.1. Study area

The study was conducted in Anhui Province in three different counties (Huaiyuan, Fengtai and Qingyang). Both Huaiyuan and Fengtai counties are plain farming areas in northern Anhui Province and Qingyang County is a mountainous area in the south. Ethical clearance was obtained from Institutional Review Board (IRB) of Anhui CDC and Anhui Medical University.

2.2. Sample collection and DNA extraction

Fifty-six (56) rodents were trapped and captured in autumn in the fields of Huaiyuan County; Fengtai County and Qingyang County in

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Anhui Province. The rodent species were identified as *Apodemus agrarius* and *Niviventer niviventer*. Both spleen and ear tissue samples were collected from each rodent and stored at − 20 °C until use. Spleen samples from the mice were homogenized using a tissue lyser (QIAGEN). Genomic DNA was extracted from each specimen by using the Qiagen DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and then stored at − 20 °C.

2.3. PCR amplification and sequencing

Nested PCR was used to amplify the gene sequence of the 56 kDa outer membrane protein of *O. tsutsugamushi*. Two sets of primers used were as follows: a pair of outer primers

P34 (TCAAGCTTATTGCTAGTG CAATGTCTGC) and

P55 (AGGGATCCCTGCTGCTGTGCTTGC TGCG) and a pair of inner primers:

P10 (GATCAAGCTTCCTCAGCCTACTATAAT) and

P11 (CTAGGGATCCCGACAGATGCACTATTAGGC) (Yoshida et al., 1994).

The first round of PCR was performed in a 20 μL volume containing 3 μL template DNA, 0.6 μL each 10 pmol/mL primer (forward and reverse primers), 10 μL premix Taq (TaKaRa Taq HS, dNTP Mixture, PCR Buffer) and 5.8 μL sterile distilled water. The PCR conditions were as follows: 5 min at 95 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at 56 °C, and 1 min at 72 °C and a final extension at 72 °C for 10 min. The second round of PCR was conducted using 0.6 μL first-round PCR product as the template under the following conditions: 5 min at 95 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at 57 °C, and 1 min at 72 °C and a final extension at 72 °C for 10 min. The nested PCR products were electrophoresed in 1.2% agarose gel containing ethidium bromide and visualized under ultraviolet light. Positive amplicons with a length of 481–507 bp were purified using QIAGEN gel extraction kit (QIAGEN, Hilden, Germany). To avoid contamination, DNA extraction, reagent setup, initial and nested PCR, and electrophoresis were all performed in separate rooms. Amplified and purified DNA were sequenced directly by a dideoxy chain termination method using an automated DNA sequencer (ABI 3730; Applied Biosystems, Foster, CA). Sangon Biotech, Shanghai.

2.4. Phylogenetic analysis

Sequences obtained in the present study were compared with the corresponding sequences deposited in GenBank by using the BLAST program of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov./Blast.cgi). Sequences were edited and trimmed by lasergene software (DNASTAR Inc., Madison, WI, USA). Complete or partial sequences encoding 56-kDa TSA of *O. tsutsugamushi* reference strains were retrieved from GenBank. Phylogenetic analysis was performed by Mega 7.0 software, Phylogenetic trees were constructed by a neighbor-joining method after 1000 bootstrapped replicates (Tamura et al., 2011).

2.5. Nucleotide sequence accession numbers

DNA sequences of *O. tsutsugamushi* obtained in this study were deposited in GenBank under accession numbers (KY320034 - KY320045 and KY484161).

3. Results

Fifty-six (56) rodents consisting of 44 *Apodemus agrarius* and 12 *Niviventer niviventer* were captured in autumn from three counties; Huaiyuan (10 *Apodemus agrarius*), Fengtai (20 *Apodemus agrarius*) and Qingyang (14 *Apodemus agrarius* and 12 *Niviventer niviventer*) in Anhui Province (Fig. 1). *Orientia tsutsugamushi* DNA was detected by nested PCR in 13(23.2%) mice from the three counties. The rate of infection of *O. tsutsugamushi* in rodents from Huaiyuan, Fengtai and Qingyang counties were 20%, 40% and 11.5%, respectively. Also the rate of detection of *O. tsutsugamushi* in *Apodemus agrarius* and *Niviventer niviventer* were 27.3% and 8.3%, respectively(Table 1).

Fig. 1. Map of Anhui Province indicating the study and endemic areas.
The names of the counties including Huaiyuan, Fengtai and Qingyang marked in black are the studied areas. The names of cities Fuyang, Mingguang and Xiuning marked in red are endemic areas. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Infection rate of *Orientia tsutsugamushi* in rodents.

	No of mice captured	No of positive
County		
Huaiyuan	10	2(20.0%)
Fengtai	20	8(40.0%)
Qingyang	26	3(11.5%)
Total	56	13(23.2%)
Rodents		
Apodemus agrarius	44	12(27.3%)
Niviventer niviventer	12	1(8.3%)
Total	56	13(23.2%)

Thirteen sequences were determined from the rodents captured. The sequences showed 99–100% identity to *O. tsutsugamushi* strains. Phylogenetic analysis showed that 12 of the sequences were clustered with Karp genotypes and one sequence clustered with Gilliam genotype (Fig. 2). The twelve sequences shared 99% homolog with Karp strains whiles one sequence also shared 100% homolog with *O. tsutsugamushi* Sanjie3 strain, Hefei strain and Neimeng 65 strain, which are Gilliam