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Molecular epidemiological survey of bacterial and parasitic pathogens in hard ticks from eastern China

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ABSTRACT

Ticks are able to transmit various pathogens—viruses, bacteria, and parasites—to their host during feeding. Several molecular epidemiological surveys have been performed to evaluate the risk of tick-borne pathogens in China, but little is known about pathogens circulating in ticks from eastern China. Therefore, this study aimed to investigate the presence of bacteria and parasites in ticks collected from Xuzhou, a 11 258 km^2 region in eastern China. In the present study, ticks were collected from domestic goats and grasses in urban districts of Xuzhou region from June 2015 to July 2016. After tick species identification, the presence of tick-borne bacterial and parasitic pathogens, including *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Rickettsia* sp., *Bartonella* sp., *Babesia* sp., and *Theileria* sp., was established via conventional or nested polymerase chain reaction assays (PCR) and sequence analysis. Finally, a total of 500 questing adult ticks, identified as *Haemaphysalis longicornis*, were investigated. Among them, 28/500 tick samples (5.6%) were infected with *A. phagocytophilum*, and 23/500 (4.6%) with *Theileria luwenshuni*, whereas co-infection with these pathogens was detected in only 1/51 (2%) of all infected ticks. In conclusion, *H. longicornis* is the dominant tick species in the Xuzhou region and plays an important role in zoonotic pathogen transmission. Both local residents and animals are at a significant risk of exposure to anaplasmosis and theileriosis, due to the high rates of *A. phagocytophilum* and *T. luwenshuni* tick infection.

1. Introduction

As haematophagous ectoparasites, ticks are able to transmit pathogens such as viruses, bacteria and parasites to their host during feeding (Dantas-Torres et al., 2012). Historically, ticks have been considered the second-most common arthropod vector behind mosquitoes, in their ability to transmit disease agents (Dantas-Torres et al., 2012). Recently, tick-borne diseases have garnered greater attention due to their increasingly harmful impact on the worldwide health of both humans and livestock (Norman et al., 2016). Determining the prevalence of pathogens relevant to public health in wild ticks, is extremely important in the development of appropriate tick-borne disease control and prevention strategies. Nowadays, a vast range of tick-borne pathogens could be detected by molecular diagnosis methods, resulting in the production of detailed epidemiological data (Sparagano et al., 1999).

Since the early 1980s, several molecular epidemiological studies have been performed in China to evaluate the risk of tick-borne pathogens (Fang et al., 2015). So far, 19 emerging tick-borne pathogens have been identified, which are transmitted by approximately 30 different tick species (Fang et al., 2015). Among them, bacteria and parasites *e.g. Anaplasma* sp., *Borrelia* sp., *Rickettsia* sp., *Bartonella* sp., *Babesia* sp., *Theileria* sp., are the most commonly distributed pathogens (Hao et al., 2011; Liu et al., 2012; Yang et al., 2016; Yin et al., 2007; Zhang et al., 2014; Zhou et al., 2014). Furthermore, many of these bacteria and parasites have significant impact as zoonotic agents for both humans and animals. More recently, outbreaks of tick-borne diseases have been reported in almost all regions of China, especially northern and northeastern

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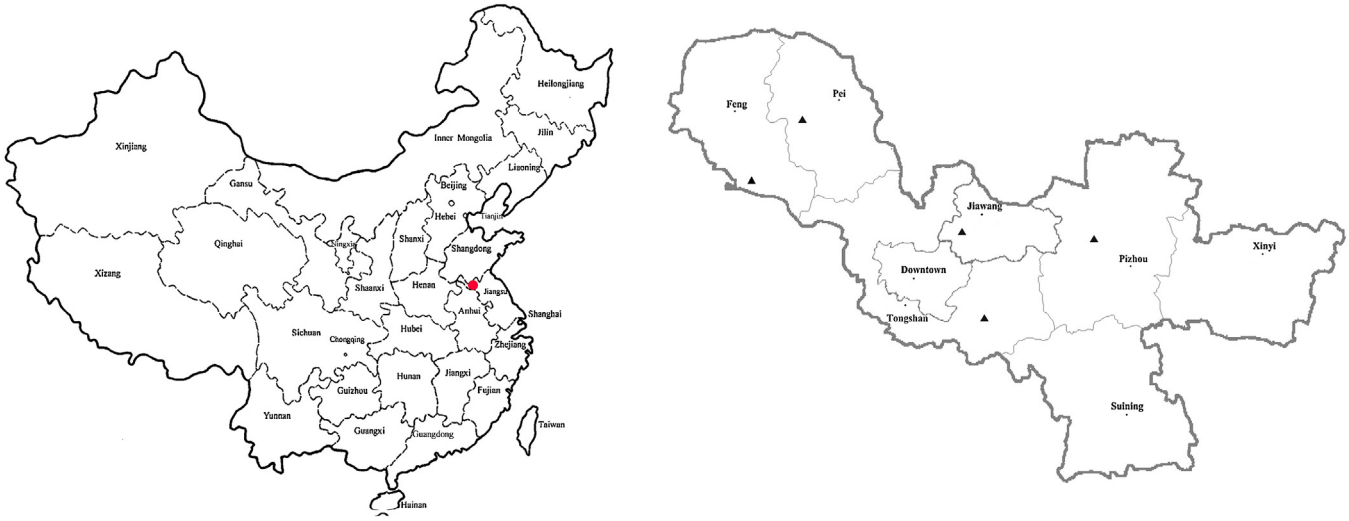

Fig. 1. Geographical location of screened tick habitats in eastern China. Maps indicate Xuzhou city location within China (left, shown in red circle) and distribution of sampling sites throughout the Xuzhou region (right, indicated by black triangles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

China (Wu et al., 2013). However, there is currently very little information about which pathogens are circulating in ticks from eastern China. Therefore, the purpose of this study was to identify the presence and prevalence of bacteria and parasites in ticks collated from eastern China.

2. Materials and methods

2.1. Study site, and tick sample collection

The study was performed between June 2015 to July 2016 in rural areas of Xuzhou located at latitudes 33°43′–34°58′ north and longitudes 116°22′–118°40′ east (Fig. 1). This region was characterized with an annual average temperature of 14 °C and an average precipitation of 800 mm. Sampling sites were identified by a random sampling method as described previously (Chen et al., 2014). Finally, samplings in five different villages located in three counties (Feng, Pei, and Pizhou) and two districts (Jiawang and Tongshan) in Xuzhou were carried out (Fig. 1). Using forceps, ticks were directly removed from domestic goats bred in forests, or collected by dragging vegetation from grasses within urban districts. Tick species were identified based on morphological characteristics (Barker and Walker, 2014), and then confirmed with specific primers 16S + 1 (5′-CCGGTCTGAACTCAGATCAAGT-3′) and 16S-1 PCR (5′-CTGCTCAATGA TTTTTTAAATTGCTGTGG-3′), and direct sequencing (Krakowetz et al., 2011). Each adult tick was individually stored in 1.5 ml tubes at −20 °C before DNA extraction.

2.2. DNA extraction

All collected ticks were washed for 30 min with 75% ethanol, and were then washed three times with sterile deionized water. Each tick was placed into individual sterilized tubes with 200 μl PBS, and homogenized with FasterPrep-24™ 5G Homogenizer (MP Biomedical Life Sciences, CA, USA). Genomic DNA was extracted from 100 μl of each crushed tick solution using the QIAamp Tissue Kit (GIAGEN, Hilede, Germany) according to manufacturer's instructions. Purified DNA was eluted into 60 μl DNase-free water and stored at −20 °C until use in PCR amplifications.

2.3. Molecular detection of tick-borne pathogens

Conventional PCR was performed on tick samples with a MyCycler™ Thermal Cycler (Bio-Rad, CA, USA) using specific primers for each pathogen as described in Table 1. *Anaplasma phagocytophilum* and *Babesia* sp., were amplified with nested PCR as described previously (Ramos et al., 2010; Zhang et al., 2012). Each reaction was carried out in a volume of 25 μl, containing 1 μl DNA, 400 nM of each primer, 200 nM of each deoxyribonucleotide triphosphate (dNTP), 2.5 μl 10 × PCR buffer, and 1 U of *Taq* DNA polymerase (Takara Biomedical Group, Dalian, China). Furthermore, negative control (distilled water) and positive control samples were included in each run. Obtained amplicons were analyzed on 1.0% agarose gels with 0.1 mg/ml ethidium bromide and observed under UV light.

2.4. Sequencing and sequence analysis

PCR products from positive samples were sequenced by the GENEWIZ Biotech Company (Suzhou, China). Nucleotide sequences were compared to those available in the GenBank nucleotide sequence databases using the BLAST (Basic Alignment Search Tool) tool on the NCBI (National Center for Biotechnology Information) website (www.ncbi.nlm.nih.gov/BLAST).

2.5. Statistical analysis

Prevalence rates were compared between females and males using the χ^2 test with Yates' correction. All calculations were performed with Prism 5.0 (GraphPad Software, Inc. USA).

3. Results

3.1. Tick samples

In this study, a total of 500 adult hard ticks (309 females and 191 males) were collected from five different sites (Table 2). All were identified as *Heamaphysalis longicornis*, the most common species in eastern China, and which is widely distributed throughout China. Extracted genomic tick DNA was used to detect the presence of bacterial and parasitic DNA.