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Seroprevalence survey of *Babesia gibsoni* infection and tick species in dogs in East China

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ABSTRACT

A seroprevalence survey of *Babesia gibsoni* infection in dogs in East China was conducted using an ELISA with recombinant *B. gibsoni* thrombospondin-related adhesive protein (BgTRAP). A total of 1170 dogs from East China were examined and the seroprevalence was 9.23%. The proportion of samples was 81.2%, 7.86% and 10.94% from pet, working and fighting dogs, respectively. The fighting dogs showed highest seroprevalence (39.8%) compared with working dogs (26.1%) and pet dogs (3.47%). These results indicate that *B. gibsoni* infection of dogs has a widespread geographic distribution throughout East China. The dominant ticks collected from the dogs were identified as *Rhipicephalus sanguineus* (65.57%), *Haemaphysalis longicornis* (21.58%) and *Rhipicephalus hemaphysaloides* (10.7%). Besides adult, larval and nymph stages of ticks were also recorded on dogs. This is the first report of seroprevalence of canine *B. gibsoni* infection and tick species in dogs in China.

1. 1. Introduction

Babesia gibsoni is a tick-transmitted protozoan parasite. It is a causative agent of canine babesiosis of wild and domestic canids. It is found in almost all parts of Asia, Europe, Africa, America and Australia (Conrad et al., 1991; Casapulla et al., 1998; Macintire et al., 2002). Intraerythrocytic *B. gibsoni* piroplasms are polymorphic, with the predominant shapes being annular, oval, pyriform and ring-shaped. Acute infections are typically associated with remittent fever, progressive anemia, lethargy, thrombocytopenia, hemoglobinuria, marked splenomegaly and hepatomegaly (Wozniak et al., 1997; Goo et al., 2008). Chronic infections are more common and infected dogs remain as carriers without any obvious clinical signs (Conrad et al., 1991). Serology can indicate a past or present persistent infection, therefore, seroprevalence survey of *B. gibsoni* infection will provide an important epidemiological dataIn China, canine babesiosis is caused mostly by *B. gibsoni* infection and is recorded primarily in local reports by the observation of blood smears and PCR detection (Chen et al., 2006; Wang et al., 2007; Shang et al., 2014), and there has been no seroepidemiological investigation of *B. gibsoni* infection in dogs in China. East China is a geographical and loosely defined cultural region that covers the eastern coastal area of China and includes the provinces of Anhui, Fujian, Jiangsu, Jiangxi, Shandong, and Zhejiang, as well as the municipality of Shanghai. In the present study, an epidemiological survey of *B. gibsoni* infection in dogs was carried out in East China using an improved ELISA with recombinant *B. gibsoni* thrombospondin-related adhesive protein (BgTRAP) antigen (Konishi et al., 2008). BgTRAP is a new member of the TRAP family that was identified from the merozoites of *B. gibsoni* (Zhou et al., 2006a). The sensitivity and specificity of recombinant BgTRAP ELISA made this method to be the most promising diagnostic test for the detection of *B. gibsoni* antibodies in dogs (Goo et al., 2008). Apart from a few sporadic reports on the occurrence of ticks in dogs (Dong et al., 2001; Jin et al., 2005; Wang et al., 2013; Shang et al., 2014), no attempt has been made to characterize the dominant tick species of dogs in china. Here, we also attempted to characterize the dominant tick species of dogs in East China.

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Fig. 1. The location of East China in Chinese Map. East China includes six provinces and the municipality of Shanghai. Every area was marked in different number.

2. Materials and methods

2.1. Sampling

Sera and ticks were collected from domestic dogs that were examined or vaccinated in animal hospitals located within East China (Anhui, Fujian, Jiangsu, Jiangxi, Shandong, Shanghai and Zhejiang; Fig. 1) from April to November of 2013 and 2014. Normal dogs and clinical suspicion of the dogs (presence of ticks, presence of scar, clinical symptoms such as fever and anemia) were sampled respectively, about 200 μl serum was collected and sent to laboratory. The dogs were divided into pet dogs, working dogs and fighting dogs. There were 3–5 veterinary clinics in each select geographic area, with 20–30 pet dogs for each clinic, and no limitation on the number of working and fighting dogs. The profile of the dogs, such as tick infestation and clinical histories, was determined by the veterinarians treating these animals. Veterinary clinics also collected ticks from the dogs and a maximum of 20 were collected per animal. Ticks were preserved in 70% ethanol and placed in 50-ml sampling containers for identification.

2.2. Seroprevalence survey of B. gibsoni infection in dogs by ELISA

ELISA with BgTRAP was carried out as described by Goo et al. (2008). Briefly, 96-well microplates were coated with the antigen, glutathione *S*-transferase (GST)-BgTRAP and GST (negative control) at a concentration of 250 ng/well. Canine sera diluted at 1:100 with PBS containing 0.05% Tween 20 were used as the source of primary antibody and HRPO-conjugated goat anti-dog IgG antibody was used as the second antibody. After addition of substrate [2,20-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], OD_{415} of each well was measured. The ELISA titer was expressed as the difference between the absorbance of the antigen (GST-BgTRAP)-containing well and that of the control antigen (GST)-containing well. An OD_{415} value 0.25 was calculated by obtaining the mean OD value plus three standard deviations of 30 specific pathogen-free dog sera.

2.3. Identification of tick species and developmental stages

The collected ticks were counted, and speciation was performed by using the criteria key (Teng and Jiang, 1991; Sonenshine, 1993).

Table 1
The seroprevalence of *B. gibsoni* infection in different types of dogs in East China.

Groups	Number of samples	Number of positive samples	Seroprevalence (%)
Pet dogs	950	33	3.47
Working dogs	92	24	26.1
Fighting dogs	128	51	39.8
Total	1170	108	9.23

Table 2
The geographic distribution of the infected pet dogs in East China.

Location	Number of samples	Number of positive samples	Seroprevalence (%)
Shandong	130	5	3.70
Jiangsu	141	11	7.80
Anhui	135	4	2.96
Shanghai	140	3	2.14
Zhejiang	138	1	0.72
Jiangxi	132	3	2.27
Fujian	134	6	4.48
Total	950	33	3.47

2.4. Statistical analysis

SPSS statistical software was used for the statistical analysis, *p*-value (chi-square) < 0.05 was regarded as the significant difference.

3. Results

3.1. Seroprevalence of B. gibsoni infection in dogs in East China

One hundred and eight (9.23%) of the 1170 dogs were tested positive by *B. gibsoni*-specific ELISA. The proportion of samples was 81.2%, 7.86% and 10.94% from pet, working and fighting dogs, respectively. The pet dog samples were collected from all seven areas in East China; however, the samples from working and fighting dogs were only collected in some areas of East China. The different types of dogs were all infected according to the serological survey and showed different prevalence (Table 1). The fighting dogs showed highest seroprevalence (39.8%) compared with working (26.1%) and pet (3.47%) dogs. The statistical analysis indicated seroprevalence of *B. gibsoni* infection between different types of dogs are significantly different.

3.2. Geographic distribution of the infected pet dogs

Samples were obtained from 130 to 140 pet dogs from all seven areas of East China and their seroprevalence data could be used to determine the geographic distribution of *B. gibsoni* infection. Thirty-three (3.47%) of the 950 dogs were tested positive by *B. gibsoni*-specific ELISA. *B. gibsoni* infection in East China showed a wide distribution.Table 2 shows the geographic distribution of the infected pet dogs in East China. Seroprevalence of *B. gibsoni* infection among different location of dogs are significantly different (chi-square). We found a significantly high seroprevalence in Jangsu province (7.8%) and a significantly low seroprevalence in Zhejiang province (0.72%) compared with seroprevalence of total pet dogs.

3.3. Profile of ELISA-positive dogs

In this study, both normal dogs and clinical suspicion of the dogs were sampled for investigation. The sample number of normal dogs and clinical suspicion of the dogs were 556, 614 respectively. The clinical suspicions of the dogs were recorded as presence of ticks, presence of scar, clinical symptoms (anemia and fever), clinical symptoms and ticks, and clinical symptoms and scar. The profile