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Serological investigation of vector-borne disease in dogs from rural areas of China

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ABSTRACT

Objective: To evaluate the *Anaplasma phagocytophilum* (*A. phagocytophilum*), *Ehrlichia canis* (*E. canis*), *Dirofilaria immitis* (*D. immitis*) (canine heartworm), *Borrelia burgdorferi* (*B. burgdorferi*) infections in countryside dogs from Yunnan, Hainan and Anhui provinces. **Methods:** Serum samples were collected from 26 dogs in Yunnan, Hainan and Anhui provinces. The samples were tested using a commercial ELISA rapid diagnostic assay kit (SNAP® 4Dx®; IDEXX Laboratories, Inc. U.S.A.). Meanwhile, indirect immunofluorescence assay (IFA) recommended by WHO was conducted to detect IgG to *A. phagocytophilum*. Two methods were analyzed and compared. **Results:** The number of serologically positive dogs for IgG to *A. phagocytophilum* was only 2 which was from Hainan province and none of the 26 dogs responded positive for *E. canis*, *D. immitis* (canine heartworm), and *B. burgdorferi* by ELISA rapid diagnostic method. The number of serologically positive dogs for IgG to *A. phagocytophilum* was 13 (50%) by IFA method. Data of the two methods were analyzed by statistical software and the difference was statistically significant (P=0.002). **Conclusions:** It can be concluded that IFA method was more sensitive than ELISA rapid diagnostic method. However, we need conduct further and intensive epidemiology survey on tick-born diseases pathogens including *A. phagocytophilum*, *E. canis*, *D. immitis* (canine heartworm), and *B. burgdorferi* which have public health significance.

1. Introduction

Zoonotic vector-borne diseases are important public health concerns in the world. Many pathogens can be transmitted by dogs[1]. In rural areas of China, nearly all farmer families keep dogs for guarding their belongings. In urban areas, more and more people keep pet dogs as a friend. Because dogs and people share the same environment and are exposed to the same vectors, dogs can serve as sentinels for certain vector-borne diseases affecting humans. For example, a human outbreak of Rocky Mountain spotted fever was reported in the White Mountain region of eastern Arizona in 2004. During this outbreak investigation, *Rhipicephalus sanguineus* was implicated as a vector for *Rickettsia rickettsii*, and the role of local dogs as short-term reservoirs and primary hosts for the vector tick was suggested because high prevalence of tick-borne pathogens in dogs from an Indian reservation in northeastern Arizona was reported[2,3]. Dogs are known to be susceptible to a wide range of emerging human infections. In this study, a serological survey on *Anaplasma phagocytophilum* (*A. phagocytophilum*), *Ehrlichia canis* (*E. canis*), *Dirofilaria immitis* (*D. immitis*) (canine heartworm), *Borrelia burgdorferi* (*B. burgdorferi*) infections in rural dogs from Yunnan, Hainan and Anhui provinces was conducted from 2008 to 2009.

2. Materials and methods

2.1. Sampling blood of dog and IgG detection

Twenty six blood samples were collected from rural dogs in Yunnan (14), Hainan (8) and Anhui (4) provinces of People's Republic of China. Sera were separated from clots and were used to test IgG specific antibodies to *A. phagocytophilum*, *E. canis* and *B. burgdorferi* and specific antigen of *D. immitis* by using a commercial ELISA assay kit (SNAP® 4Dx®; IDEXX Laboratories, Inc. U.S.A.) according to the manufacturers' instruction. In the commercial ELISA assay kit, the specific IgM and IgG antibodies were used to test the specific antigen of *D. immitis*. The synthetic peptide from the major surface protein (p44/MSP2) was used for detecting the specific antibodies of *A. phagocytophilum*. The P30 and P30-1 outer membrane proteins of *E. canis* were used for demonstrating the specific antibodies of *E. canis*,

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and the C6 peptide of *B. burgdorferi* was used for assaying its specific antibodies. Any color development in the sample spots indicated the presence of heartworm antigen, *A. phagocytophilum* antibody, *B. burgdorferi* antibody or *E. canis* antibody in the sample.

The sera samples were also tested IgG antibody against *A. phagocytophilum* by using indirect immunofluorescence assay (IFA) recommended by WHO[4] and the sensitivity of both methods, *i.e.* the IFA and the rapid ELISA assay, was compared.

2.2. Statistical analysis

Statistical analysis was conducted using SAS software (version 9.1). Comparison of the sensitivity of both methods *i.e.* the IFA and the rapid ELISA assay was performed using the χ^2 test. The significance level for the analysis was defined as a P level of 0.05.

3. Results

Only 2 dogs from Hainan province were serologically positive for *A. phagocytophilum* by ELISA assay and none of the 26 sera responded positive for *E. canis*, *D. immitis*, and *B. burgdorferi*. For IFA, 13 (50%) sera were positive for the IgG antibody against *A. phagocytophilum* and the positive cases were distributed in all of 3 provinces. Our studies showed that the results obtained by both methods were not in good agreement with each other. The number of positive detected by ELISA was lower than that detected by IFA ($P<0.05$). The major reason caused the different sensitivity of the two methods might be that these recombinant antigens used in the tests originated from different isolates of zoonotic bacteria. Because of limited samples in the study, it was not possible to obviate the likelihood of the sampling error, so it was necessary to increase the number of case.

4. Discussion

From 2007 to 2009, a serological survey on *D. immitis*, *A. phagocytophilum*, *E. canis*, and *B. burgdorferi* infections in rural hunting and urban shelter dogs mainly from southwestern regions of the Republic of Korea was conducted and results indicated that the number of serologically positive dogs for any of the four pathogens was 93 (40.6%) when 229 wild boar or pheasant hunting dogs were investigated. The highest prevalence observed was *D. immitis* (22.3%), followed by *A. phagocytophilum* (18.8%), *E. canis* (6.1%) and the lowest prevalence was *B. burgdorferi* (2.2%). In contrast, stray dogs found within the city limits of Gwangju showed seropositivity only to *D. immitis* (14.6%), and none of the 692 dogs responded positive for *A. phagocytophilum*, *E. canis* or *B. burgdorferi* antibodies[5]. The study indicates that the risk of exposure to vector-borne diseases in rural hunting dogs can be quite high in Korea, while the urban environment may not be suitable for tick infestation on dogs, as evidenced by the low infection status of tick-borne pathogens in stray dogs. In the United States, the researchers evaluated a comprehensive national database that documents canine infection with, or exposure to, these four vector-borne disease agents, in order to assess geographic trends in rates of positive tests[6]. The percent of positive test results varied by agent in different regions of the United States. *D. immitis* antigen and antibodies to *E. canis* were more commonly identified in dogs from the South (3.9% and 1.3%, respectively), and antibodies to *B. burgdorferi* and *A. phagocytophilum* were more frequently found in dogs from the upper Midwest and Northeast (4.0%-6.7% and 5.5%-11.6%, respectively). Evidence of at least one agent was found in dogs from every state considered. Furthermore, each organism also appeared to occur in endemic foci within larger areas of relatively low prevalence. Relocation of infected or previously exposed dogs from endemic regions likely accounts for some of the unexpected geographic distribution seen, although local transmission in previously underrecognized areas of endemicity could also be occurring.

A recent study of spatial distribution of seroprevalence for four vector-borne pathogens, *i.e. A. phagocytophilum*, *B. burgdorferi*, *E. canis*, and *D. immitis*, across the 3 western coastal states of the contiguous United States that extend from the northern Mexican to the southern Canadian border was conducted and the results showed that the highest overall seroprevalence was for *A. phagocytophilum* (2.4%), followed by *B. burgdorferi* (1.2%), and *E. canis* (0.7%). The prevalence of infection with *D. immitis* was 0.7%[7].

Further surveillance and study of these zoonotic vector-borne diseases are needed to investigate their distribution and local potential vectors as well as their role in the transmission of these agents. Such information would help to understand human infection risk and to make differential diagnosis of febrile illnesses among people residing and working in these areas.

Conflict of interest statement

We declare that we have no conflict of interest.

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