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The comparison of spatial variation and risk factors between mosquito-borne and tick-borne diseases: Seroepidemiology of *Ehrlichia canis*, *Anaplasma* species, and *Dirofilaria immitis* in dogs

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ABSTRACT

Canine ehrlichiosis/anaplasmosis and heartworm diseases are vector-borne and zoonotic infections. To compare epidemiology of these vector-borne diseases, a community-based study was conducted to determine the seroprevalence and risk factors of *Ehrlichia canis*, *Anaplasma* sp. and *Dirofilaria immitis* infections among healthy pet dogs. Prevalence distribution patterns were geographically contrasting between tick-borne *E. canis*/*Anaplasma* sp. infections and mosquito-borne *D. immitis* infection. Although highly enzootic communities of ehrlichiosis/anaplasmosis scattered in mountainous environment at elevations between 100 m and 1000 m, those of heartworm disease mainly distributed in urbanized plains. After multiple logistic regression analysis, it further showed that older age group and outdoor housing were associated with higher risk of heartworm infection; being male and having tick infestation associated with higher risk of *E. canis* infection whereas being male and free-roaming associated with higher risk of *Anaplasma* infection. These findings may reflect different vectors for disease transmission, and different kinetics of environment–pathogen–host interaction.

1. Introduction

Vector-borne diseases that infect domestic dogs have gained a new perspective in their significance not only in veterinary medicine but also in human public health, due to the growing concern of emerging zoonoses [1–3]. As the impact of canine vector-borne diseases is increasing, studies have been conducted to determine prevalences of these infections geographically [3,4].

Tick-borne ehrlichiosis/anaplasmosis and mosquito-borne heartworm disease are important canine infectious diseases with a wide range of symptoms from subclinical to severe [5]. These infections may also pose a potential zoonotic risk in enzootic regions [6,7]. In subtropical regions, *Ehrlichia canis*, *Anaplasma platys*, and *Dirofilaria immitis* (heartworm) are of common veterinary concerns [7]. *E. canis* and *A. platys*, transmitted by or presumably transmitted by *Rhipicephalus sanguineus* (the brown dog tick) [8,9], are both gram-negative intracellular organisms but with distinct cellular tropism in canine peripheral mononuclear cells and platelets, respectively [5]. *D. immitis* transmitted by susceptible mosquitoes is a nematode in the family *Onchocercidae*, which causes canine heartworm disease. Although the transmission and pathogenesis of these

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infections are quite different, it is similar that the infected dog enters a subclinical infectious status, and the etiologic agent could persist in the dog without apparent clinical manifestations for a prolonged period [5,10].

Higher seroprevalence of *E. canis* tends to be observed in lower latitudes, suggesting that the likelihood of the infection could be associated with the geographic distribution of the common tick species, *R. sanguineus* [11]: 3.9% in Arizona, USA [12], 19.8% in Brazil [13], 43.8% in Grenada [14], and 44.1% in Mexico [15]. The risk factors associated with seropositivity to *E. canis* include age greater than 2 year-old and tick infestation [15]. Human *E. canis* infection has been reported from endemic regions [16]. *A. platys* causes cyclic thrombocytopenia that has been confirmed only in dogs [5]. *A. platys* infection is more frequently seen in sub-tropical to tropical regions where *E. canis* is endemic [14,17]. Up to date, very limited studies have addressed factors contributing to seropositivity to *A. platys* infection, partly due to lack of specific serological test and unavailability of culture isolation [18]. The prevalence of canine heartworm disease varies significantly by sampling regions [12,19] and population [20], with decrease trend in previously enzootic areas [21] and expansion to new areas [10]. It has been suggested that high tick infestation and subsequent high prevalence of *E. canis* infections [13] prompts local veterinary practitioners the indiscriminate use of tetracycline to treat suspected *Ehrlichia* infection, which may contribute to the reduction of adult worms and its reproduction capability by destroying a filarial endosymbiont *Wolbachia* sp. [21].

In Taiwan, the subtropical to tropic climate that favors the abundance of *R. sanguineus* [22], *E. canis* and *A. platys* are considered to be enzootic in dogs [23]. Since the first description of heartworm disease in 1935, the seroprevalence of *D. immitis* in Taiwan had steadily increased up to the end of the last century [24–26]. The zoonotic potential of these infections becomes particularly higher in enzootic areas with high density of stray dogs. The proximity of dogs to humans makes asymptomatic dogs as perfect sentinels to evaluate the risk of human exposure to these infections through common vectors. Further identification of factors associated with these infections and understanding their spatial distributions with regard to disease prevalence are essential to improve disease control in either dogs or humans. Therefore, the main objective of this study was to compare the risk factors and geographical distributions of *E. canis*, *Anaplasma* sp., and *D. immitis* infection among healthy pet dog populations in an endemic region in Taiwan.

2. Materials and methods

2.1. Study area

Taiwan is an island located in the western Pacific monsoon region (20–24°E, 120–122°N). With the influence of the Asian winter monsoon and the presence of the Central Mountain Range (mean elevation 3000 m, up to 3952 m), it has a subtropical climate in the north and a tropical climate in the south. This study was conducted at Nanto County located in central Taiwan (23°58′N, 120°58′E) with an area of 4109 km^2 (Fig. 1). This county is the only inland county in Taiwan, situated between the western plain and the Central Mountain Range. More than 90% of the county area is covered by mountains (>1000 m) and hills/terraces (100–1000 m) and with scattering small basins mostly less than 1 km^2, while a small portion of alluvial plain (<100 m) spreads in the western edge of the county. Although the Tropic of Cancer crosses the southern border of the county, the vertical stratification of the terrain produces diverse climatic conditions ranging from typical subtropical to temperate climate in this small area. Therefore, it is the perfect region to study vector-borne diseases. According to demographic information of Nanto County in 2010, human population was 531,733 and mainly distributed in western townships, and the estimated number of pet dogs was 42,882 (http://animal.coa.gov.tw).

2.2. Sample collection

This study was conducted during the annual rabies vaccination campaign in the study area. Pregnant females and puppies less than 3 months were excluded. Whole blood samples were collected by convenient sampling in healthy dogs during two periods (March 15–April 30 in spring and September 15–October 31 in fall, 2010). A total of 35 communities in the 12 townships and the Nantou city were visited once during the study period. A total of 344 dog blood specimens were obtained, including 266 specimens collected in spring and 78 specimens collected in fall. The health condition of each dog was evaluated by a veterinarian prior to rabies vaccination. After using 23G needle-syringe to bleed animals, blood samples were collected in EDTA sterile vacutainer tubes, and were transported at 4 °C for further serological examinations.

2.3. Serologic testing

The whole blood was tested for antibodies against *E. canis* and *A. phagocytophilum*, and antigens of *D. immitis* adults using a commercial dot-ELISA assay SNAP® 4Dx® (IDEXX Laboratories, Westbrook, ME) following the manufacturer's instructions. SNAP® 4Dx® is a point-of-care ELISA designed for detection of antibody to *E. canis, A. phagocytophilum*, and *Borrelia burgdorferi*, and of adult worm antigen of *D. immitis*. According to the manufacturer's instructions, antibody cross-reactivity could occur between *A. platys* and *A. phagocytophilum*. Based on previous studies [12,20], positive results to *A. phagocytophilum* tend to be regarded as the positive reaction to *A. platys* in areas where *R. sanguineus* is dominant. Therefore, in this study, positive test results to *A. phagocytophilum* were recorded as seropositive status of *Anaplasma* sp. due to the indistinguishable nature of the test.

2.4. Data collection

Demographic information was collected after interviewing the dog owner through a structured questionnaire. The information collected included sex, age, breed, and housing conditions (indoor, indoor and outside, outside,