



## Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: Results of a national clinic-based serologic survey

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### ABSTRACT

We evaluated a comprehensive national database that documents canine infection with, or exposure to, four vector-borne disease agents, *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in order to assess geographic trends in rates of positive tests. While the percent positive test results varied by agent in different regions of the United States, with *D. immitis* antigen and antibodies to *E. canis* more commonly identified in dogs from the South (3.9% and 1.3%, respectively), and antibody to *B. burgdorferi* and *A. phagocytophilum* found more frequently 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 unrecognized areas of endemicity could also be occurring. Although data were only available from the 48 contiguous states (Alaska and Hawaii were not included), taken together, our results suggest that these disease agents may be present over a wider geographic area, and thus pose greater animal and public health risks, than is currently recognized. Dogs can serve as sentinels to identify the presence of vector-borne disease agents of both veterinary and public health significance.

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### 1. Introduction

Concern over vector-borne disease in domestic dogs is evidenced by the common use of tick, mosquito, and

heartworm preventatives in small animal practice; just over half of all pet owners report administering parasite control products to the pets in their care (APPMA National Pet Owners Survey, 2003–2004). The vector-borne canine disease agents of most common concern to the U.S. veterinary community are *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia* spp., and *Anaplasma* spp. While infection with these agents may be prevented to some extent through vector avoidance or other control measures, morbidity and mortality due to these diseases continue to occur in domestic dogs. Indeed, the use of

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acaricides and insecticides alone is an ineffective means of breaking the enzootic transmission cycles of these pathogens. As the roles that these agents play in animal and human health have become elucidated over the last few decades, the need for further data on the natural history and prevalence of these infections has become apparent.

Heartworm, a disease of canids caused by infection with the nematode *D. immitis*, is perhaps the most important helminthic disease of dogs in North America. Microfilariae circulate in the blood, where they may be ingested by a feeding mosquito. Development into infective third-stage larvae occurs in the malpighian tubules of the vector, and the parasites can then infect a new host (or reinfect the same host) upon subsequent feeding by the mosquito. Ultimately, the immature heartworms migrate to the dog's pulmonary arteries, and within 6–9 months begin producing microfilariae. It is this presence of the adults in the pulmonary arteries that causes right heart and/or pulmonary disease. Although dogs are the natural hosts, infection can also occur in coyotes and ferrets. Infections in cats and people often undergo truncated development, but these infections are sometimes associated with pathogenic manifestation, sometimes severe (Bowman, 2003; Theis, 2005).

Lyme borreliosis is a bacterial disease caused by infection with the spirochete *B. burgdorferi*; in dogs, disease is commonly characterized by lameness, fever, anorexia, lethargy, and lymphadenopathy (Kahn, 2005). The most important vector in the eastern U.S. is *Ixodes scapularis*, commonly known as the black-legged tick or deer tick. On the West Coast, *I. pacificus* serves as the main vector for *B. burgdorferi*. The *Ixodes* spp. involved in *B. burgdorferi* transmission are three-host ticks that acquire spirochetes when feeding on rodents as larvae or nymphs, and then can transmit infection as nymphs or adults. The most important reservoir host is thought to be the white-footed mouse (*Peromyscus leucopus*), although other rodents may also serve as a source of spirochetes to infect ticks (Schmidt and Ostfeld, 2001; LoGiudice et al., 2003; Brisson et al., 2008). Mean infection rates in *I. scapularis* nymphs in endemic areas ranges from about 20% to 40% (Daniels et al., 1998; Tsao et al., 2004; Wang et al., 2003). Infection of the nymphal western vector, *I. pacificus*, is lower, in the range of 0–14% (Eisen et al., 2004).

Lyme disease is the most common tick-borne infection among people in North America and Europe (Wormser et al., 2006), with approximately 20,000 cases reported in the United States each year (CDC, 2007a). Cases in the southern U.S., however, are notably low (CDC, 2007a). Infection of *I. scapularis* nymphs with *B. burgdorferi* has not been documented in the South (Wormser et al., 2006), and infection rates in adult *I. scapularis* are much lower than those in the northeastern U.S., at 1.4–4.6% (Clark, 2004; Oliver et al., 2000). Confirmed human infection with *B. burgdorferi* is considered rare, if it occurs at all, in states south of Maryland and Virginia (Wormser et al., 2006; Dennis, 2005). Disease is characterized in people by an early set of skin-related and flu-like symptoms, and, in the absence of treatment, may be followed by arthritic or neurologic complications (Wormser et al., 2006; Steere et al., 2004).

The rickettsial organisms *Ehrlichia canis* and *Anaplasma phagocytophilum* (the latter formerly known as *E. phagocytophila* and *E. equi*) are both tick-borne obligate intracellular bacteria with a tropism for leukocytes (Rikihisa, 1991). Disease caused by infection with these pathogens is typically characterized by fever, depression, myalgia, anorexia, and thrombocytopenia. Domestic and wild dogs are the natural hosts of *E. canis*, which has a worldwide distribution. The primary means of transmission is through the bite of *Rhipicephalus sanguineus*, the brown dog tick, although *Dermacentor variabilis*, the American dog tick, has also been shown to be a capable vector (Groves et al., 1975; Lewis et al., 1977; Johnson et al., 1998). The host range of *A. phagocytophilum* is significantly wider; rodents are considered the primary reservoir host, but infections also occur in dogs, sheep, cows, horses, and various species of wildlife (Rikihisa, 1991; Bown et al., 2003). Transmitted by *I. scapularis* in the Northeast and upper Midwest, and *I. pacificus* in the Western states, *A. phagocytophilum* is also responsible for human granulocytic anaplasmosis (HGA, formerly human granulocytic ehrlichiosis) (Rikihisa, 2006). Because *A. phagocytophilum* shares a vector and reservoir host system with *B. burgdorferi*, the geographic distribution of cases of HGA parallels that of Lyme borreliosis, and co-infections with the two agents may be seen (Daniels et al., 1998).

Evidence of infection with or exposure to the causative agents of all four of these diseases can be tested for via a single-use, in-house diagnostic known as the SNAP<sup>®</sup> 4Dx<sup>®</sup> Test (IDEXX Laboratories, Westbrook, ME). Many veterinarians are already familiar with this approach as the SNAP<sup>®</sup> 3Dx<sup>®</sup> and SNAP<sup>®</sup> 4Dx<sup>®</sup> test are widely used throughout the United States as an annual heartworm plus tick-borne disease screening tool. A portion of these results are captured through a central reporting system, which has assembled data from tests performed on several million dogs since 2001. Access to this comprehensive dataset provided an excellent opportunity to assess prevalence and distribution of these four organisms in well-cared for dogs throughout the United States. These results were then compared with previous assessments of geographic range and prevalence. In addition, because multiple agents were tested for simultaneously, the frequency of co-infection was evaluated.

## 2. Materials and methods

### 2.1. Source of data

The SNAP<sup>®</sup> 3Dx<sup>®</sup> test (IDEXX Laboratories, Westbrook, ME) is an in-clinic ELISA for simultaneous qualitative detection of canine antibodies to *E. canis* and *B. burgdorferi*, and to *D. immitis* antigen, in canine serum, plasma, or whole blood. In 2001 the test became available for commercial use as a replacement option for in-clinic "heartworm only" screening protocols. Starting in 2001, IDEXX began offering practice rebates toward the cost of the SNAP<sup>®</sup> 3Dx<sup>®</sup> assay in exchange for practices submitting a log of all test results. The offer was extended to veterinary practices across the United States; therefore,

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