



The risk of vector-borne infections in sled dogs associated with existing and new endemic areas in Poland

Part 1: A population study on sled dogs during the racing season



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ABSTRACT

The achievements of sled dogs in competitions depend both on their training and on their health. Vector-borne infections may lead to anaemia, affect joints or heart muscle or even cause death. Between December 2009 and October 2010, one hundred and twenty six individual blood samples were collected from 26 sled dog kennels situated in different regions of Poland. The majority of samples were taken during the racing season (winter 2009/10). The prevalences of 3 vector-borne infections- including 2 'old pathogens' *Anaplasma phagocytophilum* and *Babesia canis*, and 'new pathogen' *Hepatozoon canis*—were estimated in sled dogs using PCR and nested PCR. Additionally, 25 serum samples originating from a subset of 3 kennels situated in a tick-borne encephalitis (TBE) endemic area (Mazowiecki region), were tested for antibodies against the tick-borne encephalitis virus (TBEV). Because of the recently reported occurrence of *Dirofilaria repens* in Central Poland and that of fatal cases of unknown aetiology in two of the kennels, blood samples collected from dogs at these kennels in 2010 and in February–May 2013 and from two unaffected kennels were checked for evidence of presence of this parasite.

Babesia canis DNA was detected in 11 sled dogs (4 with clinical babesiosis, 7 asymptomatic; 8.7% inhabiting mainly endemic regions of Poland (9/11 cases). Three serum samples originating from one location tested positive for TBEV antibodies (total seroprevalence: 3/25 = 12%, local seroprevalence: 3/12 = 25%). The risk of TBEV infection was associated with previous *B. canis* infections. *Dirofilaria repens* DNA was detected in 15 dogs (44%). Prevalence was especially high in two sled dog kennels situated near Grodzisk Mazowiecki (50–57%). No blood samples tested positive for *A. phagocytophilum* or *H. canis* DNA. The present study has established that the prevalence of vector-borne pathogens in working sled dogs is significant in the endemic regions and has justified the important role of surveillance of reservoir hosts in the epidemiology of TBE. Our results emphasize the need for regular monitoring for the presence of *D. repens*.

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1. Introduction

Vector-borne infections constitute serious health problems for dogs worldwide (Baneth et al., 1998; Dantas-Torres, 2008; Matjila et al., 2008; Solano-Gallego et al.,

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2006; Hamel et al., 2012), including Poland (Welc-Faleciak et al., 2009; Adaszek and Winiarczyk, 2008a,b; Zygnier et al., 2007). Dogs can become infected with a variety of vector-borne parasites including filarial species (i.e. *Dirofilaria immitis*, *D. repens*), protozoa (*Babesia canis*, *Hepatozoon canis*), bacteria (*Anaplasma phagocytophilum*, *Borrelia burgdorferii* s.l.) and viruses such as the tick-borne encephalitis virus (TBEV) (Pfeffer and Dobler, 2011; Leschnik et al., 2002; Kirtz, 1999). These pathogens are mostly transmitted by the two most common tick species in Central Europe, *Ixodes ricinus* and *Dermacentor reticulatus* and by mosquitoes (in the case of *Dirofilaria* spp.).

Following our recent demonstration of the surprisingly high prevalence and abundance of intestinal parasites in a population of sled dogs in Poland (Bajer et al., 2011a), we carried out a parallel study on vector-borne infections in the same canine population. A preliminary study showed that vector-borne infections may adversely affect the health of working dogs, including sled dogs (Welc-Faleciak et al., 2009). The common symptoms of infections, such as loss of appetite, anemia, weakening, muscle and joint disorders, liver, kidney and heart disorders are likely to dramatically decrease the fitness of working dogs and even lead to their death (Bajer et al., 2008, 2013; Baneth, 2011). Sled dogs are particularly exposed to vector-borne infections because the majority of training sessions and racing sites are situated in forest areas where ticks and mosquitoes are abundant. Moreover, the dogs are usually housed in kennels where there may be an additional high risk of tick and flea infestation. There are still few studies on the extent of vector-borne diseases among working dogs in Europe, especially among sled dogs. In Poland, only one such study, based in Central Poland (Welc-Faleciak et al., 2009), has been published and there are no population based studies from other regions of the country.

The racing season lasts from autumn to spring, with 12–15 weekend races/year in different areas of Poland and Europe (www.mushing.pl). Musers and their dogs travel extensively between race meetings in different parts of Europe, often into endemic regions where there may be a high risk of acquiring vector-borne infections. It is believed that about half of Poland, to the west of the Vistula river, is free of canine babesiosis but participating in race meetings in the eastern part of the country, where babesiosis is endemic clearly risks exposure to babesiosis. Additionally, new foci of *Hepatozoon canis* infections have been detected recently in foxes and dogs in Slovakia and Hungary, in close proximity to the southern border of Poland (Hornok et al., 2013; Majlathova et al., 2007). Among new emerging vector-borne diseases of dogs in Poland are infections with *Dirofilaria repens* (Demiaszkiewicz et al., 2009, 2012; Masny et al., 2011). Central Poland is already well recognized as a recently established endemic region for this species, with a high prevalence having been reported in dogs (prevalence = 26–53%; Demiaszkiewicz et al., 2009; Demiaszkiewicz, 2013; Masny et al., 2011), then in mosquitoes (Masny unpublished) and even as autochthonous zoonotic invasions in humans (Cielecka et al., 2012; Masny et al., 2013). As neither the veterinarians nor dog owners are yet generally aware of the risk of dirofilariosis, no prophylaxis measures are being

undertaken to prevent or control this disease in Poland. Although infections with *D. repens* are believed to have a lesser impact on dog performance and health than *D. immitis* (Tarello, 2011), there have been some recent reports of fatal outcomes, including liver, renal and heart failure due to prominent microfilariemia especially in the fine capillaries (Demiaszkiewicz, 2013). Given several fatal cases among dogs in two sled dog kennels near Grodzisk Mazowiecki between 2009 and 2012, the causes of which were never identified, the current study also incorporated examination of DNA extracted from the blood of dogs from these two kennels sampled in 2010 and 2013 for evidence of dirofilarial infections, and, as controls, from two additional sled dog kennels without such cases (Fig. 1; Kury and Nieporet).

Vector-borne infections, particularly viral or bacterial tick-borne infections, may be asymptomatic in dogs but nevertheless constitute a health risk for humans. As the risk of tick infestation is estimated to be much higher for dogs than for humans living in the same localities, monitoring of seroprevalence in dogs may provide important epidemiological data of public health importance. Therefore, we carried out also the first study of the occurrence of TBEV antibodies in dogs from the Mazowiecki endemic region in Central Poland, including animals from the kennels where the first confirmed case of canine tick-borne encephalitis (TBE) in Poland was reported (Bajer et al., 2013).

The aims of the present work were: (1) to estimate the prevalence of four vector-borne infections in sled dogs during the racing season using PCR and nested PCR for diagnosis, (2) to determine the seroprevalence of TBEV antibodies in dogs in an endemic region and (3) to analyze the factors affecting the occurrence of vector-borne infections.

2. Materials and methods

2.1. Sampling

From December 2009 to October 2010, one hundred and twenty six individual blood samples were collected from sled dogs in different regions of Poland, randomly sampling about 25% of the Polish racing dog population. The majority of samples ($n = 109$) were collected in the middle of the 2009/10 racing season, between the ESDRA European Championships held in February 2010 in Kubalonka, Istebna (Poland) and the Polish Championships, held in March 2010 in Tri Studne (Czech Republic). Samples were collected from 26 sled dog kennels situated throughout Poland (Fig. 1a–c). The number of dogs sampled from each of the kennels ranged from 2 to 30. Additionally, in Spring 2012, 25 serum samples were taken from three sled dog kennels situated in the Mazowiecki region (Warsaw, Kury and Nieporet; Fig. 1) and from 3 dogs presenting with neurological symptoms at a veterinary clinic in the town Tłuszcz, near the Kury kennels. For these three dogs, sera were collected at both the onset of neurological symptoms and 2 weeks later, (in total 6 samples). Sera were sent to In Vitro Labor (Vienna, Austria) for the determination of the TBEV IgG antibody titer (ELISA test).

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