Prevalence of *Hepatozoon canis* infection in dogs from the area of Lublin Voivodship

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Introduction

Canine hepatozoonosis is a tick-borne protozoal disease caused by two species: *H. americanum* and *H. canis. H. canis* infections are widespread. In contrast, *H. americanum* infections are exclusive to North America.

Transmission of *Hepatozoon* occurs when dogs ingest the oocysts of the parasite from the definitive host, *R. sanguineus*. Predation of infected vertebrates also serves as a possible route for *H. canis* infection, along with vertical transmission.

Recently, clinical cases of *H. canis* infection in dogs were reported for the first time in Poland. The source of infection, however, remains unidentified. This raises questions about whether these infections were isolated incidents or if *H. canis* infections in Polish dogs are more common but underdiagnosed. To clarify this, molecular monitoring for *H. canis* infections is essential.

Objective

The aim of the study discussed was to detect *Hepatozoon* genetic material in blood samples from dogs suspected of tickborne diseases, shedding light on the prevalence and clinical implications of these infections.

Materials and Methods

Blood samples were obtained from 107 dogs diagnosed with anemia. Among these, 99 samples were collected from dogs that had never left Polish territory (group 1), while 8 samples were from dogs that had traveled with their owners to Turkey for holidays (group 2).

Using Bionote Vcheck M analyzer, the samples were subjected to real-time PCR to detect DNA from various pathogens, including *Leishmania* spp., *Babesia* spp., *Mycoplasma haemocanis, Hepatozoon* spp., *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia rickettsii*, and *Bartonella* spp.. Samples that tested positive for *Hepatozoon* underwent further amplification and sequencing using conventional PCR.

Results

Using PCR, we detected Babesia in 26 samples, *Anaplasma phagocytophilum* in 6 samples, and *H. canis* in 3 samples. Among these, *H. canis* was found in one dog from group 1, which had an infestation of *Ixodes ricinus* ticks, and in two dogs from group 2, which were infested with *Rhipicephalus sanguineus* ticks. These dogs exhibited symptoms such as severe apathy, anorexia, and muscle weakness.

The Vcheck M analyzer provided Ct values ranging from 25 to 28 for these *H. canis* positive samples. Subsequently, conventional PCR and sequencing confirmed the presence of *H. canis* in all three dogs (Figure 1).



Figure 1. PCR amplification of a partial sequence of *H. canis*

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(M) molecular weight marker = 100 bp; (1) negative control; (2-4) positive samples obtained in own study.

Conclusion

In Poland, *H. canis* has been sporadically diagnosed, and its primary host, *R. sanguineus* ticks, are only occasionally found in the country. However, These findings emphasize the importance of including *H. canis* in the differential diagnosis of vector-borne diseases, especially in dogs with anemia, in Poland. Moreover, the study underscores the necessity for further research on the roles of different tick species in the transmission of hepatozoonosis. This research is particularly critical given the increasing pet travel and climate changes that could potentially expand the geographic distribution of the disease.

Due to increased travel and climate change, unusual pathogens may be discovered. This study highlights the need for comprehensive screening tests, such as the Vcheck M CV8, to identify various potential threats.

