Polish Journal of Veterinary Sciences Vol. 23, No. 3 (2020), 469–471

DOI 10.24425/pjvs.2020.134694

Short communication

Babesia gibsoni infection in dogs in Poland

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Abstract

Canine babesiosis is a tickborne, protozoal, haemoparasitic disease. *Babesia* organisms are frequently classified as either large (*B. canis*) or small (*B. gibsoni*). The aim of this study was an attempt to detect *B. gibsoni* DNA in blood samples taken from dogs suspected of suffering from tick-borne diseases. 216 samples were tested using PCR, of which, in 99 of them *B. canis* DNA was detected, whereas in 3 of them *B. gibsoni* was detected. Positive PCR results for *B. gibsoni* were confirmed using a Qube MDx real-time analyzer. The results indicate that infections with this *B. gibsoni* should be taken into account and included in the differential diagnosis of vector-borne diseases in dogs in Poland, and that the accurate identification of the species of parasite causing the infection is crucial for developing the correct treatment regimen and prognosis.

Key words: *Babesia gibsoni*, dogs, PCR, vector-borne diseases

Introduction

Canine babesiosis is a disease caused by infection with parasites of the genus *Babesia* (Vial and Gorenflot 2006). Numerous species of *Babesia* exist worldwide. Two groups of these parasites were recognized, leading to the naming of the larger form (3-5 µm) - *B. canis*, and the smaller (1-3 µm) - *B. gibsoni.*

B. canis is the main piroplasma species found in dogs in Europe, including Poland (Łyp et al. 2015). Clinical cases associated with infection by *B. gibsoni* have been so far described in Germany (Hartelt et al. 2007), Croatia (Beck et al. 2009), Italy (Trotta et al. 2009), Serbia (Davitkov et al. 2015) and Spain (Criado-Fornelio et al. 2003, Tabar et al. 2009). In Poland, the only case of *B. gibsoni* infection was confirmed by molecular tests in 2018 by Adaszek et al. (2018) in a dog from the Gdynia region (Baltic Sea coast). This raises the question whether the infection was accidental or *B. gibsoni* infections in dogs

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in Poland occur but are not diagnosed. To answer this question it is necessary to perform molecular monitoring of *B. gibsoni* infections. Confirmation of infections due to this parasite species is also important from a clinical point of view, because they need a different scheme of therapy than *B. canis* infections (Vial and Gorenflot 2006).

The aim of this study was an attempt to detect genetic material of *B. gibsoni* in DNA samples isolated from the blood of dogs suspected of suffering from tick-borne diseases.

Materials and Methods

The sample material contained total DNA isolated from 216 whole blood samples submitted to the Clinic of Infectious Diseases of the Faculty of Veterinary Medicine of the University of Life Sciences in order to conduct an analysis for selected tick-borne diseases (babesiosis, anaplasmosis, ehrlichiosis, Lyme borreliosis). Blood samples were sent from private veterinary clinics and veterinary diagnostic laboratories between March 2018 and March 2020. Each sample was labelled with a number without providing details about the owner or the dog. The examined samples were collected from dogs which have never left the territory of Poland.

The PCR blood test for *Babesia/Theileria spp.* was carried out using a pair of primers i.e. GR2 and GF2, which amplify a fragment of the conserved 18S rRNA gene with a length of 559 bp (Adaszek and Winiarczyk 2008). The PCR products were purified using QIAquick spin columns (Qiagen) and eluted in 50 µl of Tris buffer 10 mM pH 7.6. The DNA sequence was determined on both strands using the same primers employed for PCR at a DNA sequencing core facility (Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw, Poland). DNA sequences were assembled and edited using SeqMan (DNAstar, Lasergene, Madison, USA), and ClustalV alignments with the published 18S rRNA gene sequences in the NCIB GeneBank for *B. gisoni* LC012808.1, and *B. canis* EU622792, and EU622793.

All DNA samples positive for *B. gibsoni* were additionally amplified in a Qube MDx real-time analyzer (Credo Biomedical Pte Ltd, Singapore) using a *Babesia gibsoni* detection Kit (BioinGentech Biotechnology, Chile), enabling qualitative demonstration of the genetic material of this protozoa species in the examined sample.

Results and Discussion

Out of 216 samples tested with the PCR method, the DNA of *Babesia spp.* was detected in the blood of the 102 dogs. The sequences of 99 *Babesia spp*. products showed a high similarity (98.0-100%) with *B. canis* EU622792, and EU622793 sequences. Three remaining samples showed high homology (99.8%-100%) to the sequence *B. gibsoni* LC012808.1. All of the three samples positive *for B. gibsoni* DNA also yielded a positive signal for *B. gibsoni* in the Qube MDx analyzer.

Babesia gibsoni is transmitted by *R. sanguineus* and *Haemaphysalis longicornis* ticks (Hatta et al. 2013, Iwakami et al. 2014), of which the first is found only occasionally in Poland, while the second occurs in Asia. The transfers of tick species out of their areas of natural distribution are divided into natural transfers (e.g., migration of ticks on hosts) and accidental transfers (e.g., resulting from the transport of livestock animals, trade in exotic animals, and transfers on animals during travel). It is important to monitor the occurrence of unknown tick species on hosts in Poland. Failure to establish how (and with which vector) the patient contracted the disease means that the reported case of babesiosis in terms of the epidemiology of the disease still lacks critical information.

Our results indicate that infections with *B. gibsoni* should be taken into account and included in the differential diagnosis of vector-borne diseases. The diagnosis, as our own observations show, cannot be arrived at in a straightforward manner by examining stained blood smears. It is hard to distinguish small *Babesia* species from the dye, and they also do not have a characteristic shape such as *B. canis*, so the identification of infection via light microscopy is unreliable, so in the case of suspicion of the disease it is advisable to perform a PCR assay with complementary primers for *B. gibsoni* genes (Miró et al. 2015).

The accurate identification of the species of parasite causing the infection is crucial for developing the correct treatment regimen and prognosis. The observations presented above indicate that babesiosis caused by small piroplasms should be considered in the differential diagnosis of vector diseases, and that these cases, due to climate changes in many parts of the world, including Poland, will most likely be noted more often.

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