First detection of small babesiae in two dogs in Hungary

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BABESIOSIS is a well-recognised tickborne protozoal disease of veterinary importance in domesticated animals including dogs. It was originally thought that only two species of *Babesia* could infect dogs, namely *Babesia canis* and *Babesia gibsoni*. *B canis* belongs to the group known as the large babesiae because it is one of the largest *Babesia* species, measuring up to 5 µm in length and 2·5 to 3 µm wide. This species has three subspecies or genotypes, namely *Babesia canis canis*, *Babesia canis vogeli* and *Babesia canis rossi* (Uilenberg and others 1989). *B gibsoni*, which is smaller than *B canis* (3 to 4 µm × 1 to 2 µm), was thought to be the only small species of *Babesia* known to infect dogs, but recently new piroplasms, designated as *Babesia microti*-like and *B gibsoni*-like, were detected in dogs (Kjemtrup and others 2000, Zahler and others 2000, Kocan and others 2001). These findings have resulted in a major change in the concepts of epidemiology and diagnosis of babesiosis in dogs.

Canine babesiosis caused by *B canis* has been reported in several European countries, particularly in the Mediterranean region, where *Rhipicephalus sanguineus* Latreille and *Dermacentor reticulatus* Fabricius are its vectors. The presence of small babesiae in dogs in Europe was uncertain until the end of the 1980s. Although some cases have been described recently, knowledge of the veterinary and zoonotic importance of these parasites is still very limited (Casapulla and others 1988, Zahler and others 2000, Camacho and others 2001, 2002, Suarez and others 2001). Babesiosis of dogs caused by *B canis* is endemic in Hungary, where it is transmitted by *D reticulatus* (Horváth and Papp 1996). However, to the authors' knowledge, confirmed cases of *B gibsoni* or other small babesiae in dogs have not been reported in the country. This short communication describes two confirmed cases of canine babesiosis in Hungary caused by small babesiae.

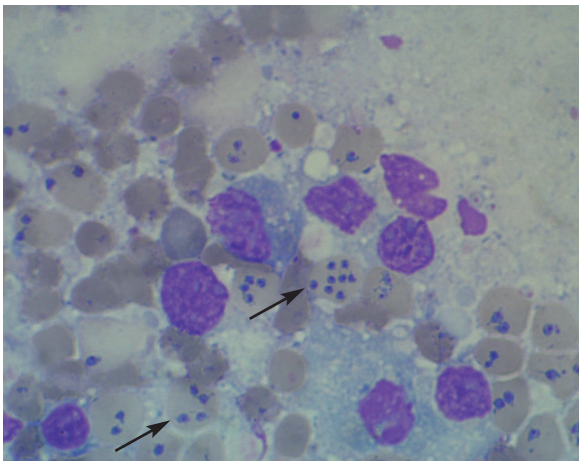

FIG 1: Splenic impression smear of dog 1 demonstrating severe parasitaemia. Multiple infected erythrocytes with small, round to ring-shaped, oval or comma-like parasites can be seen (arrows). Wright's stain. × 1000

In February 2002, a six-month-old male Scottish terrier was taken to a local small animal clinic because of weakness, lethargy and anorexia. According to the owner, the animal had never left Budapest. The owner had walked the dog in two different parks in Budapest about a week previously. On clinical examination, the dog had pale mucous membranes and a rectal temperature of 39·5°C. The animal was treated with antibiotics. It was also examined the following day when no recovery was observed and abdominal pain was detected. The veterinarian suspected that the clinical signs might be due to ileus; therefore, the animal was referred to the Department and Clinic of Surgery and Ophthalmology, Faculty of Veterinary Science, Szent István University.

After ultrasonography, a laparotomy was performed. Severe internal haemorrhage in the abdominal cavity due to the rupture of the spleen was observed. The dog was splenectomised and a diagnosis was made by examination of splenic impression smears obtained from the ruptured spleen after the operation. The thin smears were stained with modified Wright's stain (Diff-Quik) and examined. Erythrocytes were infected with many small parasites which appeared to be round to ring-shaped, oval or comma-like. The infected red cells contained two, four or eight organisms, but the parasites were not connected to each other (Fig 1). It was not known whether multiple infection had resulted from one parasite subsequently dividing. Morphometric studies were performed on the intracytoplasmic parasites. The mean (sd) diameter of the parasites measured at random was 1·81 (0·34) µm (n=35). One tick specimen was removed from the animal and identified as a partly engorged female *D reticulatus*.

After surgery, the dog was treated with antibiotics and 5 mg/kg imidocarb diproprionate (Imizol; Schering-Plough Animal Health) subcutaneously, and then sent home. Two weeks later the dog had recovered; there were no clinical signs of the protozoal disease. Four months after surgery the dog was healthy.

The second case involved a three-year-old male collie from Budapest, which was kept in the garden of a detached house and walked regularly in a neighbouring forest. In March 2002, the dog was found to be healthy during a clinical examination. A blood sample was taken for routine haematological and biochemical profiles. Haematological values (Table 1) showed slight erythropenia and leucopenia and mild throm-

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TABLE 1: Results of haematological and biochemical profiles of a male collie with babesiosis

Parameter	Value	Normal range
Haematocrit (PCV) (litre/litre)	0·33	0·35-0·55
Haemoglobin (g/litre)	120	120-180
Erythrocytes (RBC) ($\times10^{12}$/litre)	4·81	5·5-8·5
Leucocytes (WBC) ($\times10^{9}$/litre)	4·5	6·0-12
Neutrophils ($\times10^{9}$/litre)	3·3	3·0-11·5
Lymphocytes ($\times10^{9}$/litre)	1·0	1·0-4·5
Eosinophils ($\times10^{9}$/litre)	0·2	0·1-1·3
Monocytes ($\times10^{9}$/litre)	0·1	0·1-1·3
Platelets ($\times10^{9}$/litre)	116	200-800
AST (U/litre)	16	<30
ALT (U/litre)	20	<40
GGT (U/litre)	3	<10
Lipase (U/litre)	279	<800
Glucose (mmol/litre)	7·4	3·5-5·5
Total cholesterol (mmol/litre)	11·5	2·5-8·0
Urea (mmol/litre)	5·4	4·0-9·0
Creatinine (µmol/litre)	61	40-140
Phosphorous (mmol/litre)	1·0	0·8-1·8
Calcium (mmol/litre)	2·52	2·0-3·0
Sodium (mmol/litre)	139·6	135-155
Potassium (mmol/litre)	4·1	3·5-5·5
Iron (µmol/litre)	11·1	15-40

PCV Packed-cell volume, RBC Red blood cells, WBC White blood cells, AST Aspartate aminotransferase, ALT Alanine aminotransferase, GGT Gamma-glutamyl transferase

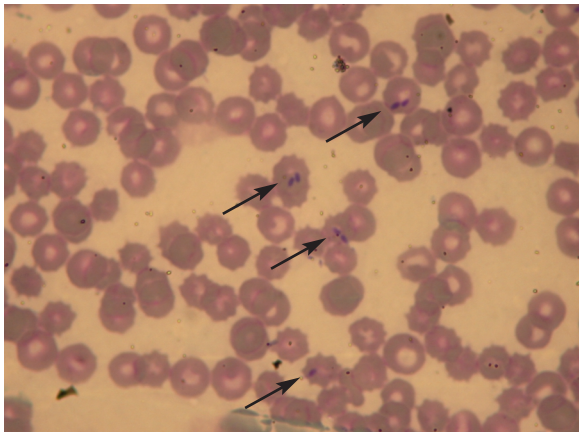

FIG 2: Thin blood smear of dog 2. Some erythrocytes contain small parasites which occur singly or in pairs in a single cell (arrows). Wright's stain. x 1000

bocytopenia. The biochemical parameters were normal except for glucose, cholesterol and iron (Table 1). Thin blood smears were prepared and stained with modified Wright's stain (Diff-Quik) and examined. Some erythrocytes contained small parasites which appeared to be comma-like in shape and which occurred singly or in pairs in a single cell (Fig 2). The mean (sd) diameter of the parasites measured at random was 1·72 (0·39) µm (n=35) which suggested that they were small babesiae. Therefore, the dog was treated once with 5 mg/kg imidocarb diproprionate (Imizol; Schering-Plough Animal Health) subcutaneously, and sent home. No parasites were found in stained blood smears from the animal one month later.

On the basis of the size of the intracellular parasites observed in both cases, it was assumed that the dogs were infected with small babesiae; this is the first time that they have been isolated from dogs in Hungary. The most frequently reported clinical signs of canine babesiosis, such as fever, anaemia, icterus and haemoglobinuria, were not observed in the infected animals. Babesiosis without a characteristic clinical picture is, however, not unusual, because the clinical signs can vary greatly depending upon the species and strain of *Babesia* species and its virulence, the age of the animal, the stage of the disease and the complications caused by other pathogens (Kontos and Koutinas 1997). It can be assumed that the immune response of these animals to these haemoprotozoa might also influence the clinical picture. This hypothesis is supported by the observation of rapid proliferation and high (10 to 13 per cent) parasitaemia of *B gibsoni* in a severe combined immune deficiency mouse model (Fukumoto and others 2000).

Many infected erythrocytes were found in splenic impression smears obtained from the ruptured spleen of dog 1. These results are consistent with the observation by Schetters and others (1998) who reported that the localisation of babesiae was in the spleen of experimentally infected dogs because this internal organ increased considerably in size and was packed with erythrocytes. Multiple infections of many erythrocytes suggested the proliferation of localised parasites.

Based on the identification of intraerythrocytic babesial piroplasms on thin blood smears under oil immersion, it could not be confirmed whether *B gibsoni* or other small babesiae caused the infection of the dogs. Canine babesiosis caused by *B gibsoni* has been reported from some European countries (Casapulla and others 1988). However, the clinical picture in the present cases was not consistent with infection caused by *B gibsoni* (Yamane and others 1993, Zahler and others 2000, Kocan and others 2001). It is likely that another species caused the infection in these dogs because *B gibsoni* is considerably more pathogenic and difficult to treat than the infections reported here. If dogs infected with *B gibsoni* do not receive prompt, effective treatment, they generally die (Yamane and others 1993). It might be possible that these two animals were infected by other small babesiae which have been reported in dogs as being *B gibsoni*-like or *B microti*-like in Europe and overseas (Zahler and others 2000, Camacho and others 2001, 2002, Kocan and others 2001), but different species or strains of small babesiae cannot be excluded. Because these parasites are too small to be identified definitively by light microscopy, a more sensitive diagnostic method is needed, for example, DNA amplification by PCR.

Further research is required to determine the origin of the small babesiae occurring in Hungary. They might be introduced into the country by asymptomatic carrier dogs, which have been imported from abroad or are returning from holidays in endemic countries. The dogs described here had not travelled abroad.

Although a tick was found on one dog, there was no demonstration of a causal relationship between tick species and *Babesia* infection. Consideration should be given to assessing the vector competence of the local tick species, notably *D reticulatus* and *I ricinus*, that feed on dogs in areas where canine babesiosis occurs (Farkas and Földvári 2001). There is a great risk that infections caused by small babesiae can easily be spread in Hungary because of the abundance of ticks in the country and the easy transportation of asymptomatic chronic carrier dogs.

Babesiosis caused by small babesiae may pose a serious threat to dogs in Hungary because the clinical disease resulting from infection with either 'large' or 'small' *Babesia* species is often indistinguishable, serological diagnosis of the two forms is hampered by cross-reactivity (Yamane and others 1993, 1994) and not all the anti-*Babesia* drugs are effective against small babesiae. For these reasons it is crucial to diagnose canine babesiosis properly. The gathering of data on the epidemiology of canine babesiosis and the education of veterinarians about the risks and methods of controlling small babesiae infections are, therefore, vital for the development of effective control programmes. Further research would also be required to determine whether these parasites could be zoonotic, especially for non-immunocompetent or splenectomised patients.

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