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POSTMORTEM SMALL BABESIA-LIKE MORPHOLOGY OF *BABESIA CANIS* – SHORT COMMUNICATION

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Here we report a case of canine babesiosis with unusual morphology of the causative agent. A male, seven-week-old Labrador retriever puppy, exhibiting severe anaemia and haemoglobinuria, was presented at the Clinic of Internal Medicine in February 2011. The puppy was euthanised. The most relevant pathological changes were icterus, severe splenomegaly, generalised lymphadenopathy and haemoglobin nephrosis. Samples were collected from various organs for histology within one hour post mortem. Impression smears were also prepared from the spleen after overnight storage at 4 °C. Tissue sections and smears showed the presence of multiple, coccoid intraerythrocytic bodies that measured 1–2 μm and resembled small babesiae. No large piroplasms were seen. DNA was extracted from the spleen, and a conventional PCR was performed for the amplification of a 450-bp region of the 18S rRNA gene of piroplasms. The causative agent was identified as *Babesia canis canis*, with 99% sequence identity to other European isolates. Sequence identity to *B. gibsoni* was only 91%. This is the first account to verify that the morphology of the large canine piroplasm, *B. canis*, can be uniformly small babesia-like post mortem or following the storage of tissue samples.

Key words: *Babesia canis*, *Babesia gibsoni*, small babesia, morphology, viscera

Piroplasms (Apicomplexa: Piroplasmea) are obligate intracellular, haemotropic parasites of vertebrates, with a worldwide occurrence. They belong to three genera (*Babesia*, *Theileria* and *Cytauxzoon*) and have ixodid ticks as biological vectors. Babesiae can be found in red blood cells of their hosts (Homer et al., 2000). Dogs have at least three genetically distinct large, and further three small *Babesia* species or subspecies (Uilenberg et al., 1989; Kjemtrup et al., 2000). Although the originally described small canine piroplasm, *B. gibsoni* has a size range (0.5–3.5 μm) overlapping with that of the large type-species, *B. canis* (2–7 μm) (Kjemtrup et al., 2000), most authors consider small and large

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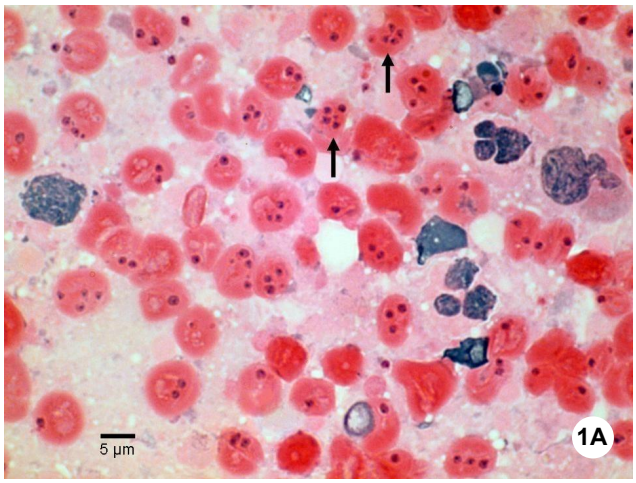
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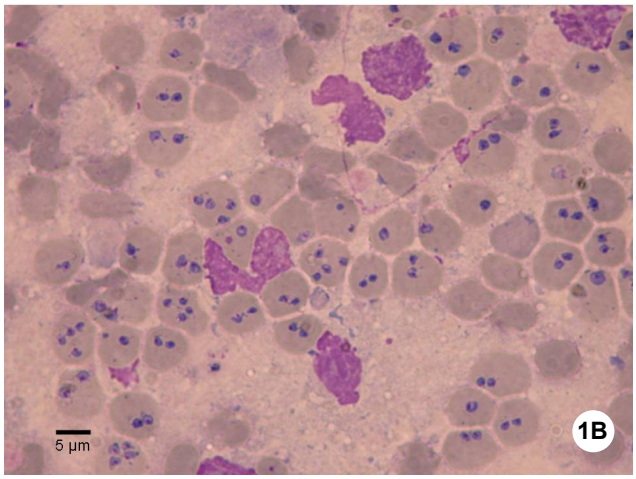
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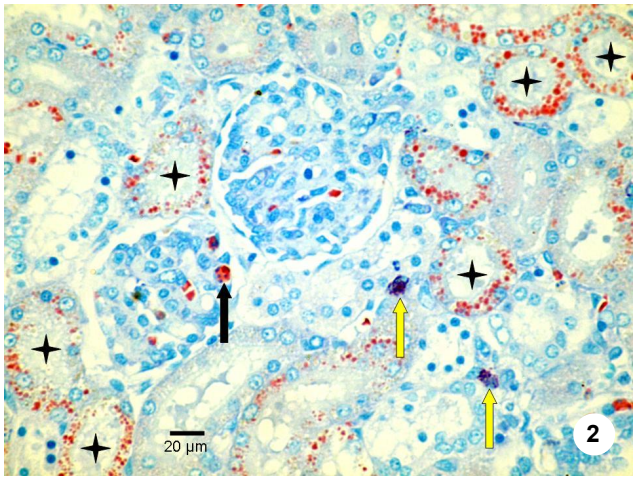
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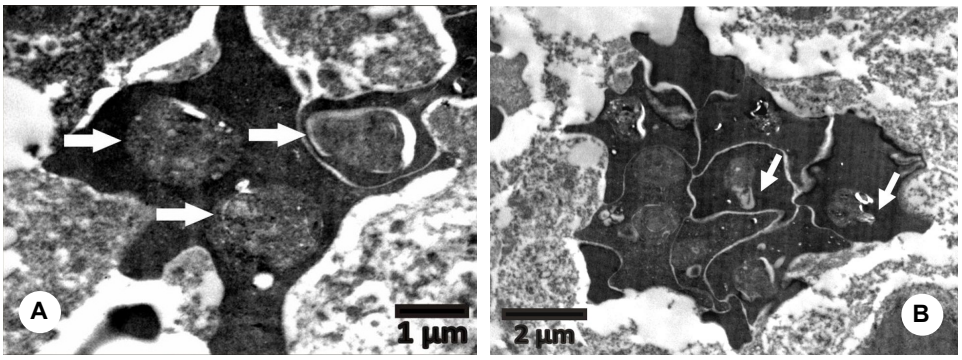

Fig. 3. Electron micrograph of (A) *B. canis* trophozoites in extravascular erythrocytes (white arrows). (B) Some trophozoites showed protruding merozoite buds (white arrows)

babesiae of dogs distinguishable according to morphological criteria. However, here we report a case of canine babesiosis with uniformly unusual appearance of the causative agent. As concluded from the findings presented below, accounts on small babesiae recognised as such only in impression smears or tissue sections, but not confirmed with molecular identification (e.g. in Hungary: Farkas et al., 2004), may include misdiagnosed cases of *B. canis* infection.

A post-weaned, seven-week-old male Labrador Retriever puppy was brought to the Clinic of Internal Medicine in February 2011. Upon clinical evaluation, severe anaemia and haemoglobinuria were seen. Careful inspection of the fur coat revealed mild flea infestation, but ticks were not found. Since the puppy was presented in a moribund state, no treatments (neither curative nor palliative) were attempted. Euthanasia was done with 0.3 ml/kg body weight intravenous T-61 solution.

Immediate macroscopic examination of the carcass revealed icterus, severe splenomegaly, moderate enlargement of most lymphoid tissues (generalised lymphadenopathy), and haemoglobin nephropathy. The urinary bladder was filled with dark-red urine. Tissue samples from the spleen, kidneys, liver, lungs, lymph

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Fig. 1.A. Impression smear from the spleen prepared during autopsy, after overnight storage at 4 °C (present case). Multiple, small (1–2 µm) coccoid bodies are visible in red blood cells. Arrows indicate post-division forms that are still connected by cytoplasmic processes. Haematoxylin-eosin (HE) staining

Fig. 1.B. For comparison, impression smear from the spleen prepared one hour following splenectomy during the first unsequenced, suspected case of small babesia-infection in Hungary (previously unpublished picture, case description in Farkas et al., 2004). Wright's staining

Fig. 2. Babesia-infected red blood cell (black arrow) in a renal glomerulus. Sample taken one hour post mortem (present case). Signs of haemoglobin nephrosis can be seen in most of the epithelial cells of the renal tubules (marked by black stars). Yellow arrows indicate mast cells. Giemsa staining

nodes and the small intestine were collected within one hour post mortem and were processed for routine histopathological evaluation. Additionally, spleen fragments were prepared for transmission electron microscopy (TEM; JEM 1011, JEOL Ltd., Japan) with osmium tetroxide fixation, alcohol dehydration and permeation with epoxy resin. After overnight storage at 4 °C, impression smears were made from the spleen, fixed with methanol and stained with haematoxylin and eosin.

Evaluation of splenic impression smears and tissue sections showed the presence of multiple (one to four) intraerythrocytic, coccoid bodies measuring approximately 1 μm and resembling small babesiae (Figs 1. A and 2). Most of the red blood cells were infected. These piroplasms had a distinct nucleus. Still connected post-division forms were also detected (Fig. 1). Histopathological evaluation of the tissue samples indicated extramedullary erythropoiesis and haemoglobin nephropathy (Fig. 2) suggestive of an intense, acute haemolytic process, as well as a moderate mast cell infiltration of most organs (Fig. 2). Ultrastructural examination by TEM confirmed the presence of numerous intraerythrocytic babesia trophozoites with diameters up to 1.5 μm (Figs 3. A and 3. B). In summary, the clinicopathological findings noted in the present study were consistent with those known for canine babesiosis (Máthé et al., 2006).

DNA was extracted from the spleen by QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Contamination of the sample was monitored by the use of an extraction control. Conventional PCR amplification of a 450 bp long region of the 18S rRNA gene, modified from Muhlnickel et al. (2002), was performed with extracted DNA. PCR products and appropriate test controls were visualised in a 1.5% agarose gel prestained with ethidium-bromide (Promega, Madison, WI, USA). Purification and sequencing was done by Macrogen Inc. (Seoul, South Korea), then the results were submitted to the GenBank. Molecular analysis of the causative agent (accession number JF750671) revealed 99% sequence similarity to former Hungarian and other European isolates of *B. canis canis* (e.g. DQ174284, AY072926). Sequence identity to *B. gibsoni* was only 91%.

Since small and large babesiae of the same host species may show overlapping size regions, if merozoites are evaluated individually, seldom they may be difficult to distinguish. Concerning canine piroplasms, the small species, *B. gibsoni* was demonstrated to have large stages easily mistaken for the merozoites of *B. canis* (Fukumoto et al., 2000). On the other hand, the unanimous small babesia-like morphology of *B. canis* in smears and tissue sections was hitherto unreported. Interestingly, connected post-division forms in the present study showed high resemblance to stages of *B. gibsoni* grown in culture (Fukumoto et al., 2000).

Intraspecific morphological differences of *B. canis* as revealed between formerly reported infections and the present one were most likely influenced by postmortem demonstration of the causative agent in the latter case. A similar

phenomenon was observed in cattle, in which host the large piroplasm, *B. bigemina* may round up and contract to resemble the small *B. bovis* one to eight hours after death (Johnston et al., 1977). In this study, *B. canis* was also small babesia-like (in tissue sections) as soon as one hour post mortem, which may be relevant to all situations when samples cannot be taken freshly (e.g. during regular pathological examinations) or processed and evaluated immediately (if diagnosis is made from stored organ or tissue samples, e.g. removed spleen: Fig. 1. B). At the same time, unlike what has been reported for *B. bigemina* in cattle (Johnston et al., 1977), here all the evaluated *B. canis* trophozoites showed morphological changes, and these unusual piroplasms persisted and remained recognisable (in impression smears) for at least 12 h post mortem.

In conclusion, if molecular biological methods or species-specific immunoassays are unavailable, large and small babesiae of dogs can only be distinguished in freshly obtained samples. In the light of the present findings, previous reports on unsequenced small canine piroplasms should also be carefully interpreted.

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