doi: 10.17221/8881-VETMED

Possible vertical transmission of *Babesia canis canis* from a bitch to her puppies: a case report

L. Adaszek[1], J. Obara-Galek[2], T. Piech[1], M. Winiarczyk[3], M. Kalinowski[1], S. Winiarczyk[1]

[1]Faculty of Veterinary Medicine, Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences, Lublin, Poland
[2]Dogs and Cats Ambulatory "Vera", Sandomierz, Poland
[3]Department of Vitreoretinal Surgery, Medical University of Lublin, Lublin, Poland

ABSTRACT: The present study reports the possible vertical transmission of *Babesia canis canis* from an infected bitch to her puppies. The study concerns a bitch that had developed babesiosis in week seven of pregnancy and her litter, three puppies that exhibited symptoms of the disease in Weeks 8–9 post-partum. In all animals, the infection with protozoa was confirmed by the results of a PCR blood test. The identity of the nucleotide sequences of the amplified fragment of the gene (18S RNA) isolated from the blood of the puppies and the bitch was 100%, which indicates that all the dogs were infected with the same strain of protozoa. This result, together with the exclusion of other possible routes of babesiosis transmission in puppies, suggests that they were infected with *Babesia canis canis in utero*.

Keywords: *Babesia canis canis*; dogs; vertical transmission

Canine babesiosis is a common and clinically significant tick-borne disease caused by haematozoan parasites of the genus *Babesia* (Adaszek and Winiarczyk 2008). The classification of *Babesia* spp. places them in the order Piroplasmida within the phylum Apicomplexa. Two morphologically distinct forms of the erythrocytic stage in the canine host were recognized in early studies that led to the naming of the larger form, measuring approximately 3–5 µm as *B. canis*, and the smaller (1–3 µm) as *B. gibsoni*. On the basis of cross-immunity, serological testing, vector specificity and molecular phylogeny *Babesia canis* was reclassified into three sub-species: *B. canis canis, B. canis rossi,* and *B. canis vogeli*. All of them are now considered to be separate species (Zahler et al. 1998; Matijatko et al. 2012). So far, only *B. canis* has been found in dogs in Poland (Zygner et al. 2007; Adaszek et al. 2011). These parasites are also the most common etiologic factor of babesiosis in dogs in other parts of Europe (Cardoso et al. 2008; Solano-Gallego et al. 2008; Kubelova et al. 2011). Clinically, these pathogens cause remittent fever, progressive anaemia, haemoglobinuria, and marked splenomegaly and hepatomegaly in dogs and, in some cases, the death of infected animals (Milczak et al. 2004; Adaszek et al. 2009). The most common mode of babesiosis transmission is by tick bite, but also other routes of transmission such as direct transmission via blood transfer from dog bites or blood transfusion are possible. Transplacental transmission has also been described (Fukamoto *e*t al. 2005).

The present study reports the possible vertical transmission of *Babesia canis canis* from an infected bitch to her puppies.

Case description

In September 2013, a four-year-old Russian Terrier bitch was admitted to the Department of Infectious Diseases, Faculty of Veterinary Medicine in Lublin, Poland. The bitch was in Week 7 of pregnancy, showing the following symptoms: apathy,

doi: 10.17221/8881-VETMED

lack of appetite, fever (40.2 °C), pale mucus membranes and brown-coloured urine. About four days earlier, the owner had removed three ticks from the animal's skin. Blood tests revealed anaemia (RBC = 3.24 × 10^{12}/l; reference ranges 5.5–8.0 × 10^{12}/l), leukopenia (WBC = 5.1 × 10^{9}/l; reference ranges 6–16.5 × 10^{9}/l), and thrombocytopenia (PLT = 62 × 10^{9}/l; reference ranges 200–580 × 10^{9}/l). Biochemical serum tests demonstrated elevated levels of AST (62 IU; reference ranges 1–37 IU), ALT (88 IU; reference ranges 3–50 IU), urea (11.14 mmol/l; reference range 3.32 to 7.47 mmol/l), total bilirubin (2.2 mg/dl; reference ranges < 0.6 mg/dl) and creatinine (187.2 µmol/l; reference ranges 88.4–150.3 µmol/l). The Giemsa-stained blood smear demonstrated the presence of *Babesia* protozoa within the erythrocytes. Based on the results of the PCR test performed by Adaszek and Winiarczyk (2008) and sequencing of the obtained amplification products, the protozoa were classified as the 18S RNA-B strain. The abdominal ultrasound in the bitch revealed enlargement of the spleen; all foetuses (n = 3) were alive. The animal was administered a single subcutaneous injection of imidocarb dipropionate (5 mg/kg), which resulted in a significant improvement 24 h following administration of the drug. The bitch became more vivid and regained appetite. Control haematological and biochemical tests performed one week after administration of imidocarb only demonstrated persistent mild thrombocytopenia (PLT = 154 × 10^{9}/l). On the 59th day after mating, the bitch gave birth to three healthy puppies (one female No. 1, and two males No. 2 and No. 3). In Week 7 after birth, the puppies were weaned and sold. In Week 8, in the female (No. 1) and in one male (No. 2), and in Week 9 in the other male (No. 3), the owners observed symptoms typical of babesiosis (apathy, lack of appetite, brown urine (males No. 2 and No. 3), pallor (male No. 2), yellowing of the mucous membranes (female No. 1 and male No. 3) and vomiting and diarrhoea (female No. 1).

In all animals, the blood tests revealed anaemia, thrombocytopenia and leukopenia (Table 1). The microscopic examination of blood smears demonstrated the presence of *Babesia* protozoa in the erythrocytes of both males (No. 2 and No. 3), whereas PCR testing showed the presence of *B. canis canis* 18S RNA-B genetic material in the blood of all three puppies. The identity of the nucleotide sequences of the amplified gene fragment isolated from the blood of the puppies and the bitch was 100%, which indicates that all animals were infected with the same strain of protozoa.

A single subcutaneous injection of imidocarb dipropionate (5 mg/kg) administered to all three puppies resulted in a significant improvement in their health status 24–72 h after drug administration. Control examination (PCR) of the blood samples collected two weeks after therapy did not reveal the presence of genetic material of *Babesia canis canis* in any of the studied puppies.

DISCUSSION AND CONCLUSIONS

As in all the puppies fipronil (spray) was applied to prevent ticks and none of them showed the presence of ticks on the skin, the animals did not receive (except for a prophylactic vaccination and anti-parasitic prophylaxis) any medical-veterinary treatment (iatrogenic infection could be excluded). Thus, it is assumed that the probable route of *Babesia* transmission was *in utero* infection. This is also supported by the 100% identity of DNA fragments of the 18S RNA B gene of *B. canis canis* isolated from the mother and puppies, suggesting that all four dogs were infected with the same strain of the parasite.

However, other routes of transmission of *Babesia* should be considered, such as congenital transmission in the birth canal or through colostrum. So far there are no data about these modes of transmission of canine babesiosis. It is documented (Perez

Table 1. The results of haematological examination of the puppies infected with *Babesia canis*

Number of animals	RBC (10^{12}/l)	Ht (%)	WBC (10^{9}/l)	PLT (10^{9}/l)
No. 1 (female)	4.2	33	5.5	88
No. 2 (male)	4.75	38	5.1	81
No. 3 (male)	4.0	33	4.9	100
Reference ranges	5.5–8	37–55	6–16.5	200–580

doi: 10.17221/8881-VETMED

et al. 1996; Allsopp et al. 2007) that seropositive dams are able to transmit *Babesia* antibodies (but not the parasites) to their new-borns through colostrum, but these antibodies will decrease in time after birth, so this way of transmission may be excluded in the present case.

In the available literature there are very little data concerning the possible vertical transmission of *B. canis canis* from a bitch to her puppies. Mierzejewska et al. (2014) presented the case of vertical transmission of *B. canis* in a litter of Central Asian Shepherds. The DNA of the protozoa was detected using PCR in three pups with babesiosis symptoms, and in their asymptomatic mother. The isolates derived from the pups and the female – 520 bp 18S rRNA gene fragments – were compared and analysed. All isolates from the pups and their mother were identical and showed 100% identity with *B. canis* EU622793, supporting a common source of infection.

The transplacental transmission of *B. gibsoni* has been experimentally demonstrated in a bitch that delivered a litter of one stillborn and four live pups (Fukumoto et al. 2005). These four pups died of congenital babesiosis between Days 14 and 39 post-birth.

For *Babesia* infection, there are reports describing the possibility of congenital babesiosis in horses (Neitz 1956; Allsopp et al. 2007), calves (De Vos et al. 1976; Yeruham et al. 2003), and sheep (Neitz 1956).

In horses, once an animal is infected with *T. equi*, it remains a lifelong carrier, since anti-theilerial drugs do not completely eliminate the parasite (Allsopp et al. 2007). Infected mares can transmit *T. equi* piroplasms across the placenta and this might result in abortion or neonatal piroplasmosis. Colostral antibodies to *T. equi* may suppress parasitaemia in new-born foals thereby reducing the incidence of clinical neonatal equine piroplasmosis, which could control parasitaemia during the early months of life (Allsopp et al. 2007). In the present report, an almost two-month time interval between possible infection *in utero* and clinical disease might be explained by a similar protection conferred on the pups by maternal colostrum. Nevertheless, and although they had no detectable ticks, the possibility that the pups were infected after birth by a tick vector cannot be ruled out completely (Simoes et al. 2011).

A similar case of a dog infected with *B. microti*-like piroplasms, probably *in utero*, was reported by Simoes et al. (2011). The disease occurred in a two-month old female German Shepherd bitch. As the pup had never had detectable ticks, did not travel, and came from an area where *B. microti*-like piroplasms are not endemic, it was concluded that the disease was the result of vertical transmission.

From a clinical standpoint, the development of a highly sensitive diagnostic method for congenital canine babesiosis is required. The detection of maternal antibodies is unsuitable because they fall below the detectable level a few weeks after birth (Fukemoto et al. 2005). Also, as shown by the results of our observations, microscopic examination has limited sensitivity: the presence of parasites in Giemsa-stained blood smears was only demonstrated in two out of the three infected pups. Only PCR could detect the parasites in all pups. Therefore, we suggest that a PCR-based test is most suitable for the specific diagnosis of congenital babesiosis.

REFERENCES

Adaszek L, Winiarczyk S (2008): Molecular characterization of Babesia canis canis isolates from naturally infected dogs in Poland. Veterinary Parasitology 152, 235–241.

Adaszek L, Winiarczyk S, Skrzypczak M (2009): The clinical course of babesiosis in 76 dogs infected with protozoa parasites Babesia canis canis. Polish Journal of Veterinary Sciences 12, 81–87.

Adaszek L, Martinez AC, Winiarczyk S (2011): The factors affecting the distribution of babesiosis in dogs in Poland. Veterinary Parasitology 181, 160–165.

Allsopp MTEP, Lewis BD, Penzhorn BL (2007): Molecular evidence for transplacental transmission of Theileria equi from carrier mares to their apparently healthy foals. Veterinary Parasitology 148, 130–136.

Cardoso L, Costa A, Tuna J (2008): Babesia canis canis and Babesia canis vogeli infections in dogs from northern Portugal. Veterinary Parasitology 156, 199–204.

De Vos AJ, Imes GD, Cullen JS (1976): Cerebral babesiosis in a new-born calf. Onderstepoort Journal of Veterinary Research 43, 75–78.

Fukumoto S, Suzuki H, Igarashi I (2005): Fatal experimental transplacental Babesia gibsoni infections in dogs. International Journal for Parasitology 35, 1031–1035.

Kubelova M, Tkadlec E, Bednar M (2011): West-to-east differences of Babesia canis canis prevalence in Dermacentor reticulatus ticks in Slovakia. Veterinary Parasitology 180, 191–196.

Matijatko V, Torti M, Schetters TP (2012): Canine babesiosis in Europe: how many diseases. Trends in Parasitology 28, 99–105.

doi: 10.17221/8881-VETMED

Mierzejewska EJ, Welc-Faleciak R, Bednarska M, Rodo A, Bajer A (2014): The first evidence for vertical transmission of Babesia canis in a litter of Central Asian Shepherd dogs. Annals of Agricultural and Environmental Medicine 21, 500–503.

Milczak A, Riha T, Abramowicz B, Madej E (2004): Hemostatic disorders during the course of canine babesiosis. Medycyna Weterynaryjna 60, 1067–1070.

Neitz WO (1956): Classification, transmission, and biology of piroplasms of domestic animals. Annals of the New York Academy of Sciences 64, 56–111.

Perez E, Jaen M, Herrero MV (1996): Effect of management and inherent factors on birth seropositivity and seroconversion to Babesia bovis in calves in Costa Rica. Annals of the New York Academy of Sciences 23, 100–109.

Simoes PB, Cardoso L, Araujo M (2011): Babesiosis due to the canine Babesia microti-like small piroplasm in dogs-first report from Portugal and possible vertical transmission. Parasites and Vectors 4, 50–54.

Solano-Gallego L, Trotta M, Carli E (2008): Babesia canis canis and Babesia canis vogeli clinicopathological findings and DNA detection by means of PCR-RFLP in blood from Italian dogs suspected of tick-borne disease. Veterinary Parasitology 157, 211–221.

Yeruham I, Avidar Y, Aroch I (2003): Intra-uterine infection with Babesia bovis in a 2-day-old calf. Journal of Veterinary Medicine B: Infectious Diseases and Veterinary Public Health 50, 60–62.

Zahler M, Schein E, Rinder H (1998): Characteristic genotypes discriminate between Babesia canis isolates of differing vector specificity and pathogenicity in dogs. Parasitology Research 84, 544–548.

Zygner W, Rapacka G, Gojska-Zygner O (2007): Biochemical abnormalities observed in serum of dogs infected with large Babesia in Warsaw (Poland). Polish Journal of Veterinary Sciences 10, 245–253.

Received: 2014–07–21
Accepted after corrections: 2016–03–12

Corresponding Author:

Lukasz Adaszek, University of Life Sciences, Faculty of Veterinary Medicine, Department of Epizootiology and Infectious Diseases, 30 Gleboka St. 20-612, Lublin, Poland
E-mail: ukaszek0@wp.pl