

**Szent István University
Postgraduate School of Veterinary Science**

Clinicopathological alterations in canine babesiosis

PhD Thesis

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Full-text articles in referred scientific journals

- Máthé, Á., Vörös, K., Vajdovich, P., Kótai, I. and Soós, P. (1998): Immunohaemolytic anaemia of dogs. Review and case report (in Hungarian). Magyar Állatorv. Lapja 120, 261-267.
- Máthé, Á., Vörös, K., Németh, T., Biksi, I., Hetyey, Cs., Manczur, F. and Tekes, L. (2006): Clinicopathological changes and effect of imidocarb therapy in dogs experimentally infected with *Babesia canis*. Acta Vet. Hung. **54**, 19-33.
- Máthé, Á., Vörös, K., Papp, L. and Reiczigel, J. (2006): Clinical manifestations of canine babesiosis in Hungary (63 cases). Acta Vet. Hung. **54**, 367-385.
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Congress abstracts

- Máthé, Á.: Immunohaemolytic anaemia of dogs (in Hungarian). 7th Annual Congress of the Hungarian Small Animal Veterinary Association. 18th April 1998.
- Máthé, Á., Vörös, K. and Papp, L.: Complications of canine babesiosis (in Hungarian). 11th Annual Congress of the Hungarian Small Animal Veterinary Association. 4th May 2002.

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- Máthé, Á., Biksi, I., Németh, T., Diószegi, Z., Vörös, K., Tekes, L. and Erdélyi, K.: Clinicopathological changes in dogs experimentally infected with *Babesia canis* (in Hungarian). 25th January 2001.
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Award

- Máthé, Á., Vörös, K. and Papp, L.: Therapy of complicated babesiosis (in Hungarian). Main prize of the György Márkus Foundation 2002.

Introduction and objectives

Canine babesiosis is an important tickborne protozoonosis enzootic in many geographical locations all over the world. It is caused by the three subspecies of the large, pyriform-shaped *Babesia canis* and the small, pleomorphic *B. gibsoni*. With the advance of molecular genetic methods, nowadays it has been proven that the small piroplasms of dogs are genotypically different, and belong to at least three species. Various names were suggested by researchers for naming the strains, as *B. gibsoni*(-like), *B. microti*(-like), *B. conradae* and *Theileria annae*. *B. canis canis* is the only subspecies identified so far in Hungary. Recently however, organisms resembling small *Babesia* were found in two dogs that never travelled abroad from our country. Still, the vast majority of babesiosis cases in Hungary is caused by *B. canis*, and this thesis concentrates on the clinical and certain pathological aspects of this large *Babesia* infection.

The clinical picture and complications of canine babesiosis vary with the subspecies affecting the population. *B. canis vogeli* is common in most tropic, subtropic areas and it is also present in South Africa, in the United States and in Australia. Canine infections have recently been described in Slovenia, as well. This subspecies causes a relatively mild disease. The most virulent subspecies is *B. canis rossi* in the southern regions of Africa. *B. canis canis* is mostly detected in Europe, the pathogenicity of the organism is intermediate. The incidence, symptomatology and treatment of *B. canis* infection in Hungary were discussed earlier in Hungarian language. Although there are numerous English publications on the clinical appearance of canine babesiosis in different geographical regions, none of them describes the characteristics of the disease occurring in Central-Eastern Europe.

10. In spite of intravenous fluid therapy, mild transient azotaemia developed in the spleen intact dog and one of the splenectomized animals several days after resolution of haemoglobinuria. This result probably means that it is not only haemoglobinuric nephrosis responsible for babesial nephropathy.
11. Both splenectomized dogs were successfully cured after collection of 400 ml highly parasitized blood. Thereby we provided a new experimental model, proving that large-amount antigen-production is possible with rescuing the infected animals.
12. The spleen intact dog clinically recovered receiving supportive treatment, with no imidocarb therapy, and probably became a subclinical carrier of *B. canis*.
13. **In the third study**, severe acute necrotizing tubulonephrosis was detected with histological and electron microscopic examinations in dogs naturally infected with *B. canis* and treated with imidocarb. The proximal convulated tubuli were more seriously affected. The lesions are probably the result of hypoxic renal injury, on the contrary of the previous hypothesis suggesting that haemoglobinuric nephrosis is the major cause of babesial nephropathy. Systemic hypotension leading to vasoconstriction in the kidneys might be the most important cause of renal hypoxia in *B. canis* infections, but anaemia and alterations of haemoglobin may also contribute to inadequate oxygenation. Imidocarb should be applied with caution in patients having renal involvement, until further data becomes available regarding the potential nephrotoxicity of the drug in dogs.

Therefore our first goal was to report on the clinical picture of canine babesiosis in Hungary, so we retrospectively analysed 63 infections with *B. canis* referred to the Small Animal Clinic of the Veterinary Faculty in Budapest. This should provide a valuable reference for those foreign researchers who would like to compare the characteristics of the epidemic in different regions. The symptoms, additional examination findings, incidence and prognosis of both uncomplicated and complicated babesiosis cases are discussed, along with the treatment of various manifestations. Babesial complications were never described earlier in Central-Eastern Europe.

Reports on clinical signs, diagnosis, and treatment regimens of canine babesiosis have been mainly carried out on naturally infected dogs. These studies are all influenced by the heterogeneity of the patients. Experimental *B. canis* infections so far mostly focused either on the diagnosis or on the prevention of the disease.

Therefore in the second part of this work we examined the clinicopathological alterations and complications of *B. canis* infection during controlled experimental conditions. The efficiency of imidocarb therapy and additional symptomatic treatment were also studied in spleen-intact and splenectomized beagle dogs.

Acute renal failure (ARF) is a severe, often fatal sequel of canine babesiosis. The mechanical and toxic effects of haemoglobin on renal tubuli (i.e. haemoglobinuric nephrosis) were thought to be responsible for the development of ARF in babesiosis for several decades. However, tissue hypoxia due to anaemia, hypovolaemia and renal vasoconstriction might also have a major role in babesiosis related nephropathy. Furthermore, immunologic and inflammatory processes were also suspected in the pathogenesis, as well. A recognised complication of babesiosis,

6. There was significant difference between the mean age of dogs having uncomplicated disease, babesiosis with a single complication and babesiosis with multiple complications (3.4, 4.8 and 8.6 years, respectively, $p < 0.001$) in our study. These new results suggest that older animals are predisposed for babesial complications. Old dogs might have subclinical disorders that deteriorate to organ failure during *Babesia* infection.
7. The recovery rate (78, 68 and 25%, $p = 0.005$) and mortality rate (3, 21 and 67%, $p < 0.001$) of dogs having uncomplicated disease, babesiosis with a single complication and babesiosis with multiple complications, respectively, also showed significant tendency. Complications were associated with increased mortality in this study, especially if multiple organs were affected.
8. DIC was found to predict MODS more sensitively in this study than SIRS: there were 6 animals developing MODS out of 11 identified with DIC, while only 5 dogs developed MODS out of 22 having SIRS. Therefore, in canine babesiosis DIC could be a more important factor resulting in multiple organ failure than SIRS, as shown by our novel results.
9. **In our second study** splenectomized and intact beagles were experimentally infected with *B. canis*. Acute hepatopathy was detected in all dogs with elevated alanine aminotransferase activity that was more seriously altered in the splenectomized dogs. Diffuse changes in the liver structure and hepatomegaly were seen in ultrasonography. Liver biopsy and histology revealed acute, non-purulent hepatitis in splenectomized dogs, a new finding in dogs with *B. canis* infection.

New scientific results

1. **In our retrospective study** of 63 babesiosis cases most patients had babesiosis in the spring and autumn, similarly to French *Babesia canis* infections, and correlating with the seasonal activity of ticks.
2. Male animals (50/63; 79%) and large breed dogs appeared in higher numbers, probably due to an over representation of outdoor dogs, and due to dog keeping habits in our country.
3. There were 31/63 animals demonstrating babesiosis with complications. Dogs that do not improve after imidocarb therapy for babesiosis are frequently referred to the Small Animal Clinic of the Veterinary Faculty from nearly all locations in the country. This fact probably explains the high incidence of complicated babesiosis in our study.
4. Most Rottweilers (7/9) developed complicated disease, suggesting that this breed might have a similar predisposition for babesiosis than it has for parvovirus enteritis.
5. Hepatopathy (41%), pancreatitis (33%), acute renal failure (31%) and DIC (24%) were frequent complications, while immune-mediated haemolytic anaemia (10%), ARDS (6%) and cerebral babesiosis (3%) were rarely observed. Occurrence rates of babesial complications are seldom reported in the English literature, and they are difficult to compare due to different inclusion criteria of the various manifestations. We suggested objective criteria for complications in this study.

disseminated intravascular coagulation (DIC), may also lead to microthrombosis and dysfunction of the kidneys.

Our goal was to provide additional information on babesial nephropathy, as described in the third part of this thesis. Therefore, histopathological and electron microscopic examinations were performed from the kidneys of dogs suffering from naturally acquired *B. canis* infection and ARF.

Clinical manifestations of canine babesiosis in Hungary

Clinical observations of *B. canis* infection in 63 dogs during a 1 year period are summarised demonstrating the pathogenicity of the *Babesia* strain endemic in Hungary.

Most babesiosis cases occurred in the spring and autumn, correlating with the seasonal activity of ticks. Male animals (79%) and large breed dogs appeared in higher numbers, probably due to predominance of outdoor dogs, and due to dog keeping habits in our country.

Imidocarb appeared to be highly effective in eliminating the *Babesia* infection.

Uncomplicated babesiosis was diagnosed in 32 cases. The disease affected dogs of any age in this study. Symptoms were similar to those published in other parts of the world: lethargy, fever, splenomegaly, pallor, icterus, haemoglobinuria and presence of ticks were the most common findings. Thrombocytopenia, lymphopenia, eosinopenia and neutropenia were frequent changes in the haemogram.

There were 31 babesiosis patients with complications. Most Rottweilers (7/9) developed complicated disease, suggesting similar breed predisposition to babesiosis and parvovirus enteritis. Hepatopathy (41%), pancreatitis (33%), ARF (31%) and disseminated intravascular coagulation (24%) were frequent complications, while immune-mediated haemolytic anaemia (10%), acute respiratory distress syndrome (ARDS; 6%) and cerebral babesiosis (3%) were rarely observed. The incidence of babesial complications is seldom mentioned in the literature, and is difficult to compare due to different inclusion criteria of the various manifestations. Hepatopathy was common, while pancreatitis, severe ARF, ARDS and cerebral babesiosis were rare in *B. canis* infected South African dogs.

caused severe tubulonephrosis in horses, cattle and goats, and tubular necrosis was also reported in one dog treated with therapeutic dose of the drug. Therefore imidocarb should be used with caution in patients with possible renal involvement, until further data become available on the potential nephrotoxicity of the drug in dogs.

Histological and ultrastructural studies of renal lesions in dogs with *B. canis* infection and (partly) treated with imidocarb

This study was intended to help the understanding of babesial nephropathy, a frequently fatal complication of the infection. Histological and electron microscopic examinations are presented from the kidneys of 8 dogs suffering from fatal naturally acquired *B. canis* infection and nephropathy. Seven animals were treated with imidocarb dipropionate on average 4.5 days prior to death. Severe anaemia was present only in 2 cases.

Degenerative histological changes observed mostly in proximal convoluted tubuli included vacuolar-hydropic degeneration, necrosis and detachment of renal tubular epithelial (RTE) cells from the basement membrane. Necrotic debris occasionally formed acidophil casts within the tubuli. In some cases, necrosis of the whole tubulus was observed. Haemoglobin casts in the tubuli and haemoglobin droplets in RTE cells seldom appeared. No significant histological alterations were shown in the glomeruli.

Newly described ultrastructural lesions in RTE cells were characterised by nuclear membrane hyperchromatosis, karyopyknosis, and karyolysis, swelling or collapse of mitochondria with fragmentation of cristae and vacuolar-hydropic degeneration in nucleus, endoplasmatic reticulum and microvilli. Many RTE cells exhibiting necrosis collapsed. Vacuolar-hydropic degeneration and necrosis were also observed in glomerular and interstitial capillary endothelium.

The severe acute tubular necrosis described in this study is probably the result of hypoxic renal injury. Systemic hypotension leading to vasoconstriction in the kidneys might be the most important cause of renal hypoxia in *B. canis* infections, but anaemia and alterations of haemoglobin may also contribute to inadequate oxygenation. Application of imidocarb

There was significant difference among the mean age of dogs with uncomplicated disease, babesiosis with a single complication and babesiosis with multiple complications (3.4, 4.8 and 8.6 years, respectively, $p < 0.001$) in our study. The recovery rate (78, 68 and 25%, respectively, $p = 0.005$) and mortality rate (3, 21 and 67%, respectively, $p < 0.001$) also showed significant relationship in these groups. These new findings suggest that older animals are predisposed to babesial complications. Old dogs may have subclinical disorders deteriorating to organ failure during *Babesia* infection. Complications were associated with increased mortality in this study.

Systemic inflammatory response syndrome (SIRS) and DIC are two possible pathways leading to multiple organ dysfunction syndrome (MODS) in babesiosis. In SIRS massive release of inflammatory mediators may cause uncontrolled inflammation in vital organs, while in DIC widespread microthrombosis could damage various tissues. DIC was found to predict MODS more sensitively in this study than SIRS: there were 6 animals developing MODS out of 11 identified with DIC, while only 5 dogs developed MODS out of 22 SIRS cases. Therefore, in canine babesiosis DIC could be a more important factor resulting in multiple organ failure than SIRS, as shown by our novel results.

Clinicopathological changes and effect of imidocarb therapy in splenectomized and intact dogs experimentally infected with *B. canis*

In this study an intact dog (**A**) and two splenectomized dogs (**B_{SE}**, **C_{SE}**) were infected with *B. canis*. Our goals were to study the clinical picture and organ involvement in babesiosis during controlled experimental conditions, to evaluate the efficiency of imidocarb treatment and to produce large-amount of *Babesia* antigen for the development of a serological test.

All animals developed an acute disease characterised by fever, haemoglobinuria and anaemia, the latter being more severe in the splenectomized dogs. Fever and parasitized red blood cells were detected for 3 days after imidocarb treatment in the splenectomized animals. Haematological abnormalities included regenerative anaemia, thrombocytopenia and leukopenia (due to neutropenia and lymphopenia) in the acute phase, followed shortly by leukocytosis, neutrophilia and left shift a few days later. Acute hepatopathy was detected in all dogs with elevated alanine aminotransferase activity that was more seriously altered in the splenectomized dogs. Diffuse changes in the liver structure and hepatomegaly were seen in ultrasonography. Liver biopsy and histology revealed acute, non-purulent hepatitis in splenectomized dogs, a new finding in dogs with *B. canis* infection. In spite of intravenous fluid therapy, mild transient azotaemia developed in dogs **A** and **B_{SE}** several days after resolution of haemoglobinuria. This finding suggests that it is not only haemoglobinuric nephrosis responsible for babesial nephropathy. Pancreatitis was not found in the experimental animals, while mild subclinical DIC was revealed in dog **C_{SE}** on postinfection day 3.

Both splenectomized dogs were successfully cured after collection of 400 ml highly parasitized blood. Thereby we provided a new

experimental model, proving that large-amount antigen-production is possible with rescuing the infected animals. Whole blood transfusion, imidocarb and supportive care with infusions, antipyretics, glucocorticoids and diuretics were applied. The intact dog clinically recovered receiving supportive treatment, with no imidocarb therapy, and probably became a subclinical carrier of *B. canis*.

Microbial infections developed in both splenectomized animals (**B_{SE}**: osteomyelitis caused by *Escherichia coli*, **C_{SE}**: haemobartonellosis), probably as a consequence of immunosuppression after splenectomy and glucocorticoid therapy.