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Állatorvos-tudományi Doktori Iskola

Clinicopathological alterations in canine babesiosis

PhD értekezés

Készítette:

Máthé Ákos

2006

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Állatorvos-tudományi Doktori Iskola

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Készült 8 példányban. Ez az 1. sz. példány.

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List of abbreviations

ALT: alanine aminotransferase

AP: alkaline phosphatase

APTT: activated partial thromboplastin time

ARDS: acute respiratory distress syndrome

ARF: acute renal failure

BUN: blood urea nitrogen

CBC: complete blood count

DIC: disseminated intravascular coagulation

FDP: fibrin degradation products

GGT: γ-glutamyl transferase

GN: glomerulonephritis

HE: haematoxylin-eosin

IMHA: immune-mediated haemolytic anaemia

MODS: multiple organ dysfunction syndrome

PAR: parasitaemia

PCR: polymerase chain reaction

PCV: packed cell volume

PID: postinfection day

PLT: platelet count

PT: prothrombin time

RBC: red blood cell count

RI: reticulocyte index

RTE cells: renal tubular epithelial cells

SIRS: systemic inflammatory response syndrome

WBC: white blood cell count

1. Summary

In the first part of this thesis a retrospective study is provided on natural canine babesiosis cases in Hungary. The clinical features of both uncomplicated and complicated disease are discussed, including the occurrence rates, prognosis and therapy of various manifestations. Although the clinical picture of *Babesia canis* infection varies between geographical locations, detailed investigation of the symptoms and complications have not been performed yet in Central-Eastern Europe.

In the second part, clinicopathological observations acquired during experimental infection of splenectomized and spleen intact young beagle dogs with *B. canis* are presented. Renal and hepatic involvement during the infection was studied, providing new information on the pathology of the disease. The controlled conditions of the experiment ruled out the uncertainty of observations acquired during natural infections arising from the heterogeneity of the patients.

In the third study, renal histopathological and ultrastructural findings are summarised in dogs suffering from acute renal failure (ARF) secondary to naturally acquired *B. canis* infection. The results suggest that hypoxic injury of the renal tissue probably has major role in the development of *B. canis* related nephropathy.

1.1. 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.

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.

1.2. 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 ($\mathbf{B_{SE}}$, $\mathbf{C_{SE}}$) 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 $\mathbf{B_{SE}}$ 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 $\mathbf{C_{SE}}$ 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 ($\mathbf{B_{SE}}$: osteomyelitis caused by *Escherichia coli*, $\mathbf{C_{SE}}$: haemobartonellosis), probably as a consequence of immunosuppression after splenectomy and glucocorticoid therapy.

1.3. 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 diproprionate on average 4.5 days prior to death. Severe anaemia was present only in 2 cases.

Degenerative histological changes observed mostly in proximal convulated 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. Imidocarb should be applied with caution in patients with possible renal involvement, until further data become available on the potential nephrotoxicity of the drug in dogs.

2. Összefoglalás

Klinikopatológiai megfigyelések a kutyák babesiosisa kapcsán

A kutyák babesiosisa világszerte előforduló jelentős protozoonosis. Az ebek fertőződését a nagyobb, körte alakú *Babesia canis* három alfaja (*B. canis vogeli*, *B. canis rossi* és *B. canis canis*), valamint a kisebb pleomorf *B. gibsoni* idézik elő. A molekuláris genetikai vizsgálatok elterjedésével a közelmúltban bebizonyosodott, hogy az ún. *kisbabesiák* is több fajhoz tartoznak. Az eltérő genetikai állományú törzsek elnevezésére a kutatók a *B. gibsoni*, *B. microti*, *B. conradae* és *Theileria annae* fajneveket javasolták. Magyarországon eddig csak a *B. canis canis* alfaj genetikai azonosítására került sor, jóllehet az utóbbi években hazai szerzők már *kisbabesiák* sporadikus előfordulásáról is beszámoltak. A magyarországi kutyababesiosis esetek túlnyomó részét azonban egyértelműen a *B. canis* okozza, és a jelen disszertáció e fertőzés klinikai és egyes patológiai vonatkozásait tárgyalja.

A *B. canis* alfajai és törzsei az egyes földrajzi régiókban különböző patogenitásúak, az általuk kiváltott tünetek és a betegség esetleges szövődményei is különböznek. A *B. canis vogeli* elsősorban trópusi és szubtrópusi területeken elterjedt, de előfordulásáról beszámoltak Dél-Afrikában, az Egyesült Államokban, Ausztráliában, sőt újabban Szlovéniában is. Ez az alfaj okozza a legenyhébb megbetegedést. A legvirulensebb alfaj a *B. canis rossi*, amely Afrika déli területein endémiás. A *B. canis canis* Európában honos, és közepes megbetegítő képességű. A fertőzés hazai előfordulásáról és tüneteiről már több magyar nyelvű közlemény is megjelent. Szintén több angol nyelvű beszámoló részletezi a kutyababesiosis klinikumát Nyugat-Európában és más földrészeken.

Mivel azonban a Közép-Kelet Európában megnyilvánuló *B. canis* fertőzés klinikumáról angol nyelvű tanulmány még nem készült, a jelen disszertáció keretei között először egy retrospektív vizsgálatban mutatom be a hazai kutyababesiosis klinikumát 63 eset kapcsán. Tárgyalom az egyszerű lefolyású és szövődményes esetek tünettanát, kiegészítő vizsgálati leleteit, előfordulási gyakoriságát és kórjóslatát, valamint a gyógykezelés lehetőségeit. A *B. canis* fertőzés szövődményeiről eddig még nem készült részletes hazai felmérés.

A *B. canis* által kiváltott megbetegedés tünettanát és kezelését eddig többnyire természetes fertőződések kapcsán tárgyalták. Az így szerzett tapasztalatokat jelentősen befolyásolják a betegpopuláció heterogenitása és a kutyák esetleges egyéb, korábban szerzett szervi bántalmai. Az eddigi kísérletes munkák inkább diagnosztikai tesztek kifejlesztésére, vagy a fertőzés megelőzésére koncentráltak.

Ezért munkám második részében klinikopatológiai megfigyeléseket teszek közzé intakt lépű és splenectomizált beagle kutyák kísérleti *B. canis* fertőzése kapcsán. Leírom a betegség során kialakult máj- és vesebántalmakat, ezzel – nemzetközi szempontból is figyelemre méltó – adatokat szolgáltatok a hazánkban előforduló *Babesia* törzs patogenitásáról.

Hazánkban *B. canis* fertőzés kapcsán viszonylag gyakran alakul ki heveny veseelégtelenség, ami sokszor végzetes kimenetelű. Korábban a vörösvérsejtek szétesése során kiszabaduló hemoglobin mechanikus és toxikus hatását tették felelőssé a bántalomért. A kutyák babesiosisa kapcsán azonban számos más tényező is okozhat vesekárosodást. Ilyenek a különböző okokra visszavezethető szöveti hypoxia vagy a fokozott immunológiai és gyulladásos válaszreakciók, továbbá a DIC.

Ezért a harmadik vizsgálat során a babesiosis következtében kialakult heveny veseelégtelenség kórszövettani és elektronmikroszkópos elváltozásait tárgyalom. Eredményeink hozzájárulhatnak e gyakran végzetes szövődmény kórfejlődésének jobb megértéséhez.

2.1. A kutyababesiosis klinikuma Magyarországon

Ebben a fejezetben 63 *B. canis* fertőzésben szenvedő kutya ellátása során egy év alatt összegyűjtött klinikai tapasztalatainkat mutatom be.

Az esetek többsége tavasszal és ősszel jelentkezett, a kullancsok szezonális aktivitásának megfelelően. A betegek 79%-a hímivarú volt, valószínűleg azért, mert Magyarországon a kullancscsípésnek gyakrabban kitett kerti kutyák között a kanok többségben vannak. Valószínűleg szintén a kutyatartási szokásokra visszavezethetően a nagytestű kutyafajták domináltak a vizsgált beteganyagban.

Az imidokarb-terápia nagyon hatékonynak bizonyult a *Babesia*-fertőzés kezelésére.

Komplikációmentes kórlefolyást 32 esetben figyeltünk meg. A klinikai tünetek nem különböztek a más földrajzi régiókban leírtaktól, a leggyakoribb megfigyelések a következők voltak: bágyadtság, láz, splenomegalia, haemoglobinuria, sápadtság, icterus és kullancsok a gazdaállaton. A vérképvizsgálat során gyakran tapasztaltunk thrombocytopeniát, lymphopeniát, eosinopeniát, valamint neutropeniát.

Szövődményes babesiosis alakult ki 31 állatban. A rottweilerek többségében (7/9) mutatkoztak komplikációk. Ez a fajta más kutyafajtáknál fogékonyabb lehet babesiosisra, hasonlóképpen, mint a kutyák parvovírusos enteritisére. Gyakran tapasztalt szövődmény volt a hepatopathia (41%), a pancreatitis (33%), a heveny veseelégtelenség (31%) és a

disszeminált intravasalis coagulopathia (DIC; 24%), ugyanakkor ritkán jelentkezett immunhaemolyticus anaemia (10%), akut respirációs distressz-szindróma (ARDS; 6%) és cerebralis babesiosis (3%). A *Babesia*-fertőzés szövődményeinek előfordulási arányairól eddig kevés közlemény számolt be, és az eredményeket az eltérő szempontok miatt nehéz összevetni. Dél-Afrikában a hepatopathia gyakorinak mutatkozott, míg pancreatitis, súlyos veseelégtelenség, ARDS és cerebralis babesiosis ritkán fordult elő kutyák *B. canis* fertőzése során.

Jelen vizsgálatunkban szignifikáns különbség adódott a komplikációmentes, egy szövődménnyel terhelt és a többszörös szövődményben szenvedő betegek életkora között (3,4, 4,8, illetve 8,6 év, $p < 0,001$). Ezekben a csoportokban statisztikailag jelentős eltérés volt tapasztalható a gyógyulási arány (78, 68 és 25%, $p = 0,005$) és a mortalitási ráta (3, 21 és 67%, $p < 0,001$) tekintetében is. Új eredményeink azt mutatják, hogy az idősebb állatokban gyakrabban jelentkeznek szövődmények a kutyababesiosis kapcsán. Ennek az lehet az oka, hogy a korosabb kutyák szubklinikai szervi bántalmakat hordozhatnak, melyek a *B. canis* fertőzés hatására szervi elégtelenséggé súlyosbodnak. Vizsgálatunkban a szövődményes esetek magasabb mortalitással jártak, különösen, ha több szerv volt érintett.

Az ún. általános gyulladásos válaszreakció (systemic inflammatory response syndrome – SIRS) és a DIC két olyan lehetséges patomechanizmus, amely ún. több szervi elégtelenséghez (multiple organ dysfunction syndrome – MODS) vezethet babesiosis során. A jelen vizsgálatban a DIC gyakrabban idézett elő többszörös szervfunkciózavart, mint a SIRS: 11 DIC-ban szenvedő állat közül 6 esetben alakult ki MODS, míg a 22 esetben kimutatható SIRS csak 5 kutyában vezetett MODS jelentkezéséhez. Ezek az új tapasztalatok azt sugallják, hogy a babesiosis kapcsán mutatkozó többszörös szervfunkciózavar kórfejlődésében a DIC valószínűleg fontosabb szerepet játszik, mint a SIRS.

2.2. Klinikopatológiai elváltozások és az imidokarb terápiás hatása kísérletesen előidézett *B. canis* fertőzések során lépirtott és intakt kutyákban

Ebben a vizsgálatban egy intakt lépű (**A**) és két splenectomizált kutyát ($\mathbf{B_{SE}}$, $\mathbf{C_{SE}}$) fertőztünk *B. canis*-szal. A kísérlet céljai a következők voltak.

A babesiosis klinikumának és szervi manifesztációinak tanulmányozása tervezett körülmények között, egészséges, fiatal állatokban.

Az imidokarb terápiás hatékonyságának vizsgálata.

Nagy mennyiségű *Babesia*-antigén előállítása egy szerológiai teszt kifejlesztéséhez.

Mindegyik állatban heveny betegség jelentkezett. A típusos tünetek a láz, a haemoglobinuria és a vérfogyottság voltak, utóbbi súlyosabbnak mutatkozott a lépirtott kutyákban. A splenectomizált állatok imidokarb kezelésben is részesültek, ezután még 3 napig voltak lázasak, szintén 3 napig vérkenetükben még *babesiákkal* fertőzött vörösvérsejtek is láthatóak voltak. A betegség heveny szakaszában regeneratív anaemia, thrombocytopenia, neutropenia és lymphopenia fejlődött ki, majd néhány nap múlva leukocytosis, neutrophilia és a vérkép balra tolódása mutatkozott. Heveny májkárosodás minden állatban tapasztalható volt, melyet a vér emelkedett alanin-aminotranszferáz (ALT) aktivitása jelzett. Az ALT-aktivitás emelkedése jelentősebb volt a lépirtott kutyákban. Hasi ultrahangvizsgálattal a máj diffúz megnagyobbodását tapasztaltuk. A splenectomizált állatok májbioptátumában heveny nem gennyes hepatitis volt látható, ami új tapasztalatnak számít kutyák *B. canis* fertőzésében. A folyamatos intravénás folyadékterápia ellenére átmeneti enyhe azotaemia alakult ki két állatban (**A** és $\mathbf{B_{SE}}$) több nappal a haemoglobinuria megszűnése után. Ez a lelet azt valószínűsíti, hogy a haemoglobinuriás nephrosis valószínűleg nem az egyedüli oka a babesiosis kapcsán kialakuló nephropathiának. Pancreatitis a kísérleti állatokban nem jelentkezett. Egy kutyában ($\mathbf{C_{SE}}$) enyhe szubklinikai DIC volt megállapítható a fertőzést követő 3. napon.

Mindkét lépirtott állatot sikeresen meggyógyítottuk 400 ml parasitaemiás vér lebocsátását követően. Ezzel egy új kísérleti modellt hoztunk létre, bebizonyítva, hogy jelentős mennyiségű *Babesia*-antigént lehet előállítani a kísérleti állatok végleges elaltatása nélkül is. A gyógykezeléshez teljes vér transzfúziót és imidokarb injekciót használtunk, illetve szükség szerint alkalmaztunk tüneti terápiát (infúziók, lázcsillapítók, glükokortikoidok és diuretikumok). Az intakt lépű kutya csak tüneti kezelést kapott, és az állat imidokarb alkalmazása nélkül is spontán meggyógyult. Az állat valószínűleg tünetmentes *Babesia*-hordozó lett.

Mindkét splenectomizált kutyában mikrobiális fertőzések jelentkeztek ($\mathbf{B_{SE}}$: *Escherichia coli* által okozott osteomyelitis, $\mathbf{C_{SE}}$: haemobartonellosis), valószínűleg a lépirtás és a glükokortikoid-terápia következtében kialakult immunszuppresszió miatt.

2.3. A *B. canis* fertőzés során jelentkező nephropathia kórszövettani és elektronmikroszkópos vizsgálata (részben) imidokarbbal kezelt kutyákban

Szövettani és elektronmikroszkópos vizsgálatokat végeztünk 8 heveny veseelégtelenséggel szövődött *B. canis* fertőzésben elpusztult kutyában, új adatokat szolgáltatva a babesiosis e gyakran végzetes szövődményének kórtanához. Imidokarb-kezelést

7 állat esetében végeztünk, átlagosan 4,5 nappal elpusztulásuk előtt. Súlyos vérszegénység csak két kutyában alakult ki.

A legsúlyosabb (regresszív) szövettani elváltozások a vesék elsőrendű kanyarulatos csatornácskáiban alakultak ki. Enyhébb esetekben a csatornácskák vakuolás-hydropicus elfajulása, súlyosabb esetekben a tubulushám elhalása és a hámsejtek alaphártyáról való leválása mutatkozott. Az elhalt szövettörmelék időnként acidophil hengereket képezett a tubulusok üregében. Néhány kutyában a tubulusok teljes elhalása volt látható. Hemoglobinhengereket csak ritkán láttunk, és hemoglobincseppek is csak elvétve mutatkoztak a tubularis hámsejtekben. A glomerulusokban jelentős kóros elváltozás nem alakult ki.

Elektronmikroszkópos vizsgálattal szintén degeneratív eltérések mutatkoztak a vesetubulusokban: a sejtmaghártya hyperchromatosisa, karyopycnosis és karyolysis egyaránt látszottak, továbbá a mitochondriumok zavaros duzzadása, kollapszusa és crystáik feltöredezése is megfigyelhetőek voltak. Vakuolás-hydropicus elfajulás alakult ki a sejtmagokban, az endoplazmatikus reticulumban és a hámsejtek microvillusaiban. Sokszor az epithelsejtek összezsugorodtak, ami elhalásukra utalt. Vízforgalmi zavar a glomerularis és interstitialis kapillárisok endothel sejtjeiben is megfigyelhető volt.

Az általunk *B. canis* fertőzésben tapasztalt súlyos tubularis necrosis valószínűleg a vesék hypoxiás károsodása miatt alakult ki. Mai tudásunk szerint a vesék oxigénhiányos állapota leginkább szisztémás hypotonia és következményes renalis vasoconstrictio miatt alakul ki, de a kórfejlődésben súlyos vérszegénység és a hemoglobin károsodása is szerepet játszhat. Az imidokarb nephrotoxicitását több állatfajban leírták. A gyógyszer magas dózisban súlyos tubulonephrosist okozott lovakban, szarvasmarhákban és kecskékben, egy esetben pedig terápiás adagban alkalmazott imidokarb szintén tubularis necrosist okozott kutyában is. Az imidokarb injekciót ezért célszerű óvatosan alkalmazni olyan esetekben, amikor a vesefunkció zavara felmerül.

2.4. Új tudományos eredmények

1. Első vizsgálatunkban 63 kutyababesiosisban szenvedő beteg adatainak retrospektív elemzése során azt tapasztaltuk, hogy a legtöbb *B. canis* fertőződés tavasszal és ősszel jelentkezett, a franciaországi tapasztalatokhoz hasonlóan.
2. A vizsgált beteganyagban a kan kutyák (50/63; 79%) és a nagytestű fajták túlsúlyban voltak, valószínűleg a hazai kutyatartási szokásokra visszavezethetően.

3. Szövődményes babesiosis 31/63 esetben volt megállapítható. A szövődmények nagy előfordulási aránya az egyetemi klinikára referált bonyolult eseteknek tudható be.
4. A vizsgált beteganyagban sok rottweiler szerepelt, és ezek többségében szövődmények is mutatkoztak (7/9). Ez a fajta babesiosisra való fokozott fogékonyságára utalhat.
5. Gyakran tapasztalt szövődmény volt a hepatopathia (41%), a pancreatitis (33%), a heveny veseelégtelenség (31%) és a DIC (24%), ugyanakkor ritkán jelentkezett immunhaemolyticus anaemia (10%), ARDS (6%) és cerebralis babesiosis (3%). Elsőként tettünk javaslatot a babesiosis szövődményeinek objektív kritériumok alapján történő meghatározására.
6. Szignifikáns különbség adódott a komplikációmentes, egy szövődménnyel terhelt és a többszörös szövődményben szenvedő betegek életkora között (3,4, 4,8, illetve 8,6 év, $p < 0,001$).
7. A fenti csoportokban statisztikailag jelentős eltérés volt tapasztalható a gyógyulási arány (78, 68 és 25%, $p = 0,005$) és a mortalitási ráta (3, 21 és 67%, $p < 0,001$) tekintetében is.
8. A DIC gyakrabban idézett elő többszörös szervfunkciózavart, mint a SIRS: 11 DIC-ban szenvedő állat közül 6 esetben alakult ki MODS, míg a 22 esetben kimutatható SIRS csak 5 kutyában vezetett MODS jelentkezéséhez.

9. Második munkánk során kísérletes *B. canis* fertőzést végeztünk lépirtott és intakt lépű beagle kutyákban. Minden állatban heveny májkárosodás jelentkezett. A lépirtott kutyákban jelentősebb mértékben emelkedett az ALT-aktivitás. Ultrahangvizsgálattal a máj diffúz megnagyobbodását tapasztaltuk. A lépirtott állatok májbiopsziás vizsgálatával heveny nem gennyes májgyulladás volt megállapítható.
10. Néhány nappal a haemoglobinuria megszűnése után enyhe átmeneti azotaemia jelentkezett egy intakt lépű és egy lépirtott kutyában.
11. Mindkét lépirtott állatot sikeresen meggyógyítottuk 400 ml parasitaemiás vér lebocsátása után. Ezzel olyan modellt dolgoztunk ki nagy mennyiségű *Babesia*-antigén előállítására, amely lehetővé teszi a kísérleti állatok túlélését.
12. Az intakt lépű kutya imidokarb-kezelés nélkül is meggyógyult, és valószínűleg krónikus *Babesia*-hordozóvá vált.

13. Harmadik munkánkban szövettani és elektronmikroszkópos vizsgálatokat végeztünk heveny veseelégtelenséggel szövődött *B. canis* fertőzésben elpusztult és imidokarb kezelésben is részesült kutyákban. A mintákban súlyos tubularis necrosis mutatkozott,

ami valószínűleg a vesék hypoxiás károsodása miatt alakult ki. Az imidokarb nephrotoxicitását több állatfajban leírták, ezért hangsúlyozzuk, hogy a gyógyszert célszerű óvatosan alkalmazni olyan esetekben, amikor a vesefunkció zavara felmerül.

3. Introduction and objectives

Canine babesiosis is an important tickborne protozoonosis enzootic in many geographical locations all over the world. The disease affects a significant percentage of the local dog population, and there are sporadic imported cases even in non-endemic areas due to the widespread tourism with companion animals. Babesiosis is caused by the three subspecies of the large (2.4 μm X 5 μm), pyriform-shaped *Babesia canis* (**Figure 3.1.**) and the small (1 μm X 3.2 μm), pleomorphic *B. gibsoni* (Taboada, 1998). With the advance of molecular genetic methods like polymerase chain reaction (PCR), it has been proven recently that the small piroplasms of dogs are genotypically different, and belong to at least three species (Kjemtrup et al., 2000). Various names were suggested by researchers for naming the strains, as *B. gibsoni*(-like), *B. microti*(-like), *B. conradae* and *Theileria annae* (Zahler et al., 2000; Camacho et al., 2001; Kocan et al., 2001; García, 2006; Kjemtrup et al., 2006).

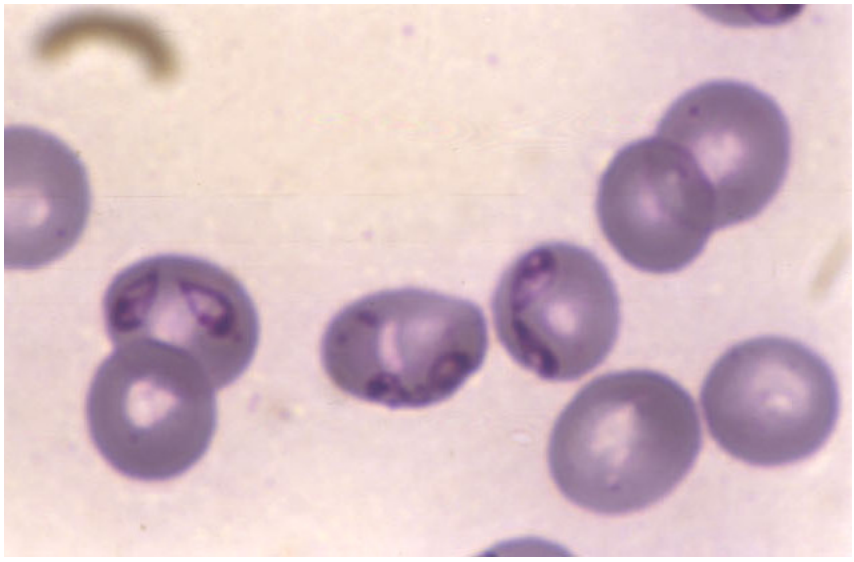

Figure 3.1. *Babesia canis* merozoites in the erythrocytes of a dog

The clinical picture of this parasitic condition varies with the *Babesia* (sub)species infecting the population. *B. canis vogeli* is common in most tropic, subtropic areas (Taboada, 1998) and it is also present in South Africa (Lobetti, 1998), in the United States (Taboada, 1998) and in Australia (Irwin and Hutchinson, 1991). Canine infections have recently been described in Slovenia (Duh et al., 2004), as well. *B. canis vogeli* is spread by the brown dog tick, *Rhipicephalus sanguineus*, and causes a relatively mild disease (Taboada, 1998). The most virulent subspecies is *B. canis rossi* in the southern regions of Africa; its vector tick is *Haemaphysalis leachi* (Taboada, 1998). *B. canis canis* is mostly detected in Europe, the pathogenicity of the organism is intermediate (Taboada, 1998). The carrier tick, *Dermacentor*

reticulatus (**Figure 3.2.**), prefers biotopes close to natural water sites (Janisch, 1986). *B. gibsoni* is transmitted by both *H. bispinosa* and *R. sanguineus* (Kuttler, 1988). This species is enzootic in the Far East and the South of the United States. There are sporadic reports about its presence in Mediterranean countries (Yamane et al., 1993, Taboada, 1998; Casapulla et al., 1998; Suarez et al., 2001). Canine infection with a unique *B. gibsoni*-like parasite was reported from Oklahoma in the United States (Kocan et al., 2001), *B. microti*-like agents and *T. annae* were detected in dogs from Spain (Camacho et al., 2001; García 2006), while *B. conradae* was recently described in California, the United States (Kjemtrup et al., 2006).

Figure 3.2. Engorged *Dermacentor reticulatus* female tick

B. canis canis is the only subspecies identified so far in Hungary (Földvári et al., 2005). According to earlier studies, the only vector tick species found in our country was *D. reticulatus* (Farkas and Földvári, 2001; Földvári and Farkas, 2005a; Földvári and Farkas, 2005b). Newly however, *R. sanguineus* nymphs were collected from 2 dogs kept in a beef cattle farm in northern Hungary. It was suspected, that the ticks were imported from Croatia by a truck used for calf transport. No *Babesia* organisms were discovered in the blood smear of one of these dogs (Hornok and Farkas, 2005). Recently, organisms resembling small *Babesia* were found in two dogs that never travelled abroad from our country (Farkas et al., 2004). These parasites were pleomorphic, and infected red blood cells contained 1-8 organisms. It was not possible to determine the *Babesia* species definitely. However, based on morphometric studies and clinical picture it was suggested that the parasites were similar to *B.*

gibsoni-like or *B. microti*-like organisms described by others (Zahler et al., 2000; Camacho et al., 2001; Kocan et al., 2001).

The incidence, symptomatology and treatment of *B. canis* infection in Hungary were discussed earlier in Hungarian language (Horváth and Papp, 1974; Horváth and Papp, 1996; Csikós et al., 2001). Although there are numerous English language publications on the clinical appearance of canine babesiosis in different geographical regions (Farwell et al., 1982; Irwin and Hutchinson, 1991; Yamane et al., 1993; Wozniak et al., 1997; Taboada 1998; Casapulla et al., 1998; Lobetti, 1998, 2000; Furlanello et al., 2005), none of them describes the characteristics of the disease occurring in Central-Eastern Europe.

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.

Babesiosis also has a growing clinical importance in Hungary, since the epidemic has spread from an isolated western region eastward during the past decades, and nowadays it is diagnosed in most counties of our country (Horváth and Papp, 1996; Földvári and Farkas, 2005b; Hornok et al., 2006). This may be due to a more widespread occurrence of the vector tick, *D. reticulatus* (Földvári and Farkas, 2005a; Földvári and Farkas, 2005b; Sréter et al., 2005). In some Middle European countries, such as Austria, Germany and Switzerland, the disease was suspected to be introduced from Hungary or from the Southern European regions (Gothe et al., 1989).

Infection of canine red blood cells with *Babesia* sporozoites leads to more or less severe haemolytic disorder. Some authors distinguish acute and chronic forms of the disease (Horváth and Papp, 1974; Horváth and Papp, 1996; Taboada, 1998; Csikós et al., 2001). Others mention uncomplicated and complicated babesiosis; the former is further classified into mild and severe forms, depending on the degree of anaemia (Jacobson and Swan, 1995; Lobetti, 1998, 2000). Recently Jacobson (2006) recommended evaluating the patients based on the presence or absence of circulatory and respiratory anomalies, which should better help determining the prognosis of the case.

The various clinical abnormalities appearing in complicated babesiosis are not only due to the direct effect of the parasite. Pathogenesis involves immunologic factors, increased lipid-peroxidation, hypoxic tissue injury and activation of the coagulation cascade. Disseminated intravascular coagulation (DIC) is a long known manifestation of canine babesiosis (Moore and Williams, 1979) that could be fatal due to diffuse microthrombosis in

vital organs. Recently, the role of inflammatory mediators has also been investigated. Tissue hypoxia – which is a common feature in babesiosis – is probably one of the major causes for the release of cytokines, oxygen free radicals, nitric oxide and other inflammatory mediators. These factors may lead to generalised reaction by the host, referred to as systemic inflammatory response syndrome (SIRS) (Jacobson and Clark, 1994; Taboada, 1998). This systemic reaction might be responsible for the involvement of multiple vital organs in complicated babesiosis, as well. Generalised organ failure is called multiple organ dysfunction syndrome (MODS) (Jacobson and Clark, 1994; Taboada, 1998).

Babesial complications were never described in Central-Eastern Europe, so in our retrospective study of 63 babesiosis cases we reported on the occurrence rates and prognosis of different complications. We also investigated the development of certain systemic reactions by the host organism like SIRS and DIC, and how often these reactions may lead to MODS in canine babesiosis. The connection between DIC and multiple organ damage in canine babesiosis based on clinical and laboratory parameters has not been previously studied.

Studies on clinical signs, diagnosis, and treatment regimens of canine babesiosis have been mainly carried out on naturally infected dogs (Farwell et al., 1982.; Irwin and Hutchinson, 1991; Horváth and Papp, 1996; Lobetti, 2000). The nature of parasitaemia and the pathogenesis of *B. canis* infection have also been studied experimentally, although the majority of these studies have focused either on the diagnosis or on the prevention of the disease (Vercammen et al., 1995; Vercammen et al., 1996a; Vercammen et al., 1996b). Haematological and biochemical alterations in experimentally infected dogs have also been reported (Vercammen et al., 1997). Multisystemic organ dysfunction complications affecting the liver, pancreas, lungs and kidneys have been described in natural cases of canine babesiosis (Welzl et al., 2001). However, these alterations have not been investigated experimentally.

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 (Horváth and Papp, 1996; Máthé et al., 2006). The prevalence of this complication seems to be varying with the *Babesia* (sub)species affecting the dogs. Although elevated creatinine levels were frequently found in South African dogs having complicated babesiosis due to *B. canis rossi* infection (Welzl et al., 2001), severe ARF was detected only in 2.2% of the hospitalised cases

(Jacobson and Clark, 1994). Lobetti and Jacobson (2001) also demonstrated, that minimal renal injury manifesting in proteinuria, renal tubular casts and epithelial cells in the urine sediment occurs more often than severe ARF in *Babesia*-infected South African dogs. Biochemical evidence of renal failure was found in 10% of parasitaemic dogs suffering from small *Babesia* infections in northern Spain (Camacho et al., 2001). Later, García (2006) found 36% of 62 dogs having *T. annae* infection to be azotaemic in the same region of Spain, although some of these dogs could have had pre-renal azotaemia. Acute renal failure was diagnosed in 19/61 animals having naturally acquired *B. canis* infection in Hungary (Máthé et al., 2006). The prognosis of ARF was poor in this study: 4/9 patients died or were euthanized if this was the only complication of the disease.

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 (Hildebrandt, 1981; Jacobson and Clark, 1994). However, Lobetti et al. (1996) did not find severe renal pathology, when haemoglobinaemia was experimentally induced in healthy dogs. Tissue hypoxia due to anaemia, hypovolaemia and renal vasoconstriction might also have a major role in babesiosis related nephropathy (Lobetti et al., 1996; Lobetti, 1998; Lobetti and Jacobson, 2001). Furthermore, immunologic and inflammatory processes were also suspected in the pathogenesis, as well (Jacobson and Clark, 1994). Wozniak et al. (1997) demonstrated membranoproliferative glomerulonephritis (GN) in dogs with natural and experimental *B. gibsoni* infections, suspected to be the consequence of chronic antigenic stimulation. A recognised complication of babesiosis, disseminated intravascular coagulation (DIC), may also lead to microthrombosis and dysfunction of the kidneys (Moore and Williams, 1979; Jacobson and Clark, 1994).

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.

4. Clinical manifestations of canine babesiosis in Hungary

Máthé, Á., Vörös, K., Papp, L., Reiczigel, J.: Clinical manifestations of canine babesiosis in Hungary (63 cases). Acta Vet. Hung. 2006. 54. 367-385.

The main goal of this work is to provide additional information about the pathogenicity of *Babesia canis* in Central-Eastern Europe. In the present study the clinical manifestations found in both complicated and uncomplicated babesiosis are summarised, based on the observations of 63 dogs at the Small Animal Clinic of the Veterinary Faculty in Budapest. Detailed information about the prognosis of the different manifestations is also presented, and the prognostic value of certain clinical conditions like systemic inflammatory response syndrome (SIRS) and disseminated intravascular coagulation (DIC) is discussed, as well. Therapeutic protocols used at the authors' clinic are also described.

4.1. Materials and methods

Sixty three cases of babesiosis diagnosed at the Small Animal Clinic of the Veterinary Faculty between 13th of April 1999 and 13th of April 2000 were evaluated retrospectively. The diagnosis and selection of cases were based on detection of *Babesia* organisms in venous blood smears. There are 3 dogs appearing twice in the study, as they acquired repeated infections in the following „tick season". The clinical observations are discussed in two groups: 32 animals had uncomplicated babesiosis, whilst 31 dogs developed complications associated with babesiosis.

History data and physical examination findings were recorded on standardised clinical cards by the veterinarians taking part in the treatments. Complete blood count was carried out by an automatic analyser (Abacus Haematological Analyser, Diatron Kft., Budapest, Hungary), and blood smears stained with Dia-Panoptic (Diagon Kft., Budapest, Hungary) were examined in all cases (n=63). Microscopic evaluation of the smears was used to diagnose babesiosis, and to determine the species of the parasite. Qualitative white blood cell counts were calculated from the total white blood cell numbers, and the percentages of different cell types derived from the blood smears. Various biochemistry parameters like alanine aminotransferase (ALT; n=34), alkaline phosphatase (AP; n=14), γ-glutamyl transferase (GGT; n=5), amylase (n=6), lipase (n=10), blood glucose (n=4), blood urea nitrogen (BUN; n=13) and creatinine (n=61) were determined depending on the health state of the patients, using an automatic spectrophotometer (Dr. Lange 400, Dr. Bruno Lange GmbH,

Berlin, Germany). Activated partial thromboplastin time (APTT; n=45) (Actimat Biomérieux SA, Marcy-l'Etoile, France), prothrombin time (PT; n=42) (Thromborel-S, Schnitger & Gross: Amelung GmbH, Lieme, Germany) and fibrin degradation products (FDP; n=44) (Biomérieux BV Boxtel, The Netherlands) were also evaluated with standard methods in the majority of the dogs. Venous blood gas and acid-base parameters were measured with an ABL 555 blood gas analyser (Radiometer, Denmark). Urine samples of 7 animals had been analysed. Two-dimensional abdominal ultrasonography was done in 8 complicated cases (Panther 2002 ultrasound instrument, Brüel & Kjaer, Denmark) equipped with 3.5-5.0MHz mechanical sector transducers.

Uncomplicated babesiosis was classified as severe (packed cell volume: PCV < 0.16 l/l), moderate (0.16 l/l ≤ PCV < 0.35 l/l), or mild disease (PCV ≥ 0.35 l/l), depending on the severity of (haemolytic) anaemia (Jacobson and Clark, 1994; Lobetti, 1998).

Complicated babesiosis involves clinical manifestations that are unrelated to haemolytic disease (Lobetti, 1998). The criteria used to define babesial complications, SIRS and multiple organ dysfunction syndrome (MODS) are summarised in **Table 4.1.** Indirect demonstration of spherocytes was attempted by an osmotic fragility test (Slappendel, 1986), but the test was not included in the diagnostic criteria of immune-mediated haemolytic anaemia (IMHA) because of poor specificity (43/48 results were positive). Makinde and Bobade (1994) also found, that major subpopulations of the erythrocytes have increased osmotic fragility in *B. canis* infected dogs. The direct antiglobulin test (Coombs' test) was not performed, as this method is also positive in the majority of *Babesia* positive dogs, and is not regarded to be specific for the diagnosis of immune-mediated anaemia (Yamane et al., 1993; Lobetti, 1998).

Table 4.1. Criteria of complications in canine babesiosis

Complication	Criteria
Hepatopathy	**At least** 2 liver enzymes elevated (ALT > 60 U/l, AP > 280 U/l, GGT > 10 U/l) **or** a single enzyme above ALT 120 U/l, AP 560 U/l, GGT 20 U/l **Exclusion**: nodular ultrasonographic structure
Pancreatitis	Both pancreatic enzymes elevated (amylase > 900 U/l, lipase > 800 U/l) **and/or** ultrasound alterations in the pancreatic region (ill-defined hypoechoic to complex mass, or multifocal hypoechoic regions, or cyst-like as well as hyperechoic lesions within the enlarged pancreas)
ARF	Creatinine > 150 µmol/l **and** PCV < 0.55 l/l (no severe dehydration)
DIC	Presence of **at least** three of the following markers: APTT > 25 sec, PT > 12 sec, FDP test positive, PLT < 200 G/l
IMHA	(Spherocytosis **and/or** autoagglutination) **plus** low (< 0.3 l/l) or decreasing PCV. IMHA was ruled out if the patient recovered without immunosuppressive therapy, or follow up of the case was not possible.
ARDS	Clinical signs: dyspnoea, crepitating respiratory sounds, coughing radiography, pathology results
Cerebral babesiosis	Cerebral symptoms, pathology results
SIRS	Presence of **at least** two of the following alterations: respiratory rate > 30/min**,** heart rate **>** 120/min, abnormal rectal temperature (normal 38.0-39.2 ^{o}C), abnormal WBC (normal 6-12 G/l) with left shift (stab neutrophils > 0.3 G/l)
MODS	Presence of **at least** two of the following complications: ARF, hepatopathy, pancreatitis, cerebral babesiosis, ARDS

Legends: ARDS: acute respiratory distress syndrome, ARF: acute renal failure, PLT: platelet count, WBC: white blood cell count
Remarks: biochemical parameters were measured in plasma. SIRS and MODS criteria are based on Jacobson and Clark (1994), Lobetti (1998, 2000).

A single dose of imidocarb injection was given to 60 dogs to eliminate the *Babesia* infection. Three patients died or were euthanized shortly after admission, before imidocarb could be applied. Treatment of the complicated cases was done as suggested by the literature (Jacobson and Swan, 1995; Lobetti, 1998, 2000). Drug dosages are summarised in **Table 4.2.**

Statistical analyses (Fisher's Exact Test, One-way ANOVA analysis) were done with a licensed computer software (S-Plus 2000 Professional Edition for Windows, Release 3, Insightful Corp., Seattle USA).

Table 4.2. Drugs used in the treatment of babesiosis

Drug	Indication	Dose
Aminophylline (Diaphyllin venosum inj.)	ARDS	5-10 mg/kg q8-24h slowly IV
Cimetidine (Histodil inj.)	Uraemic gastritis in ARF	5-10 mg/kg q6-12h IV, SC
Dexamethasone (Dexadreson inj.)	IMHA	0.5-1 mg/kg q24h IV, IM
Dopamine (Dopamin inj.)	ARF, anuria	2-5 µg/kg/minute IV
Famotidine (Quamatel inj.)	Uraemic gastritis in ARF	0.5 mg/kg q12-24h IV
Furosemide (Furosemid inj.)	Diuretic, ARF, ARDS	4 mg/kg q8-12h IV, IM, SC
Heparin (Heparin inj.)	DIC, autoagglutination in IMHA	50-100 IU/kg q8h SC
Imidocarb (Imizol inj.)	Babesiosis	3-6 mg/kg IM, SC
Insulin (Insulin Monotard HMge)	Pancreatitis, hyperglycaemia	0.25-0.5 IU/kg q12h SC
Mannitol (Mannisol inf.)	Diuretic, ARF	0.5-2 g/kg q12h IV
Metamizol (Vetalgina inj.)	Fever	50 mg/kg q12h IV, IM
Methylprednisolone (Medrol tabl., Solu-Medrol inj.)	IMHA, cerebral babesiosis	2-4 mg/kg q24h IV, IM, PO
Metoclopramide (Cerucal inj.)	Vomiting in ARF	0.2-0.5 mg/kg q6-12h IV, IM, SC
Pentobarbitone (Nembutal inj.)	Cerebral babesiosis, seizures	3-15 mg/kg IV
Ringer's solution (with 2.5% glucose)	Intravenous fluid therapy, rehydration, diuresis, hepatopathy	20-50 ml/kg IV
Ringer's solution, lactated	Intravenous fluid therapy, rehydration, diuresis	20-50 ml/kg IV
Silymarin (Silegon drg.)	Hepatopathy	3-5 mg/kg q12h PO
Sodium bicarbonate (Alkaligén inf.)	Metabolic acidosis in ARF (pH < 7.2)	mmol = 0.3 x kg x base excess, IV
Thiethylperazine (Torecan inj.)	Vomiting in pancreatitis, ARF	0.3-0.6 mg/kg q8-12h IV, IM

4.2. Results

Microscopic evaluation of the blood smears demonstrated the presence of large (3-5 µm), pyriform, many times paired parasites, *B. canis* in the erythrocytes of all 63 dogs. Repeated blood smear examination was possible in 19 animals 2-4 days after imidocarb treatment. All the repeated blood smears were negative for *Babesia*.

The majority of the patients were intact or castrated males (50/63, 79%), and only 13/63 were intact or neutered females (21%). The infection was most common in mixed breed dogs (12 cases), followed by Rottweilers (9), Kaukasian shepherds (5) and Irish setters (4). Small breeds were sporadically affected. Most cases were admitted to the clinic during spring and autumn (**Figure 4.1.**). The age distribution, recovery and mortality rates of 63 dogs described in this study are presented in **Table 4.3.** and **Table 4.4.**

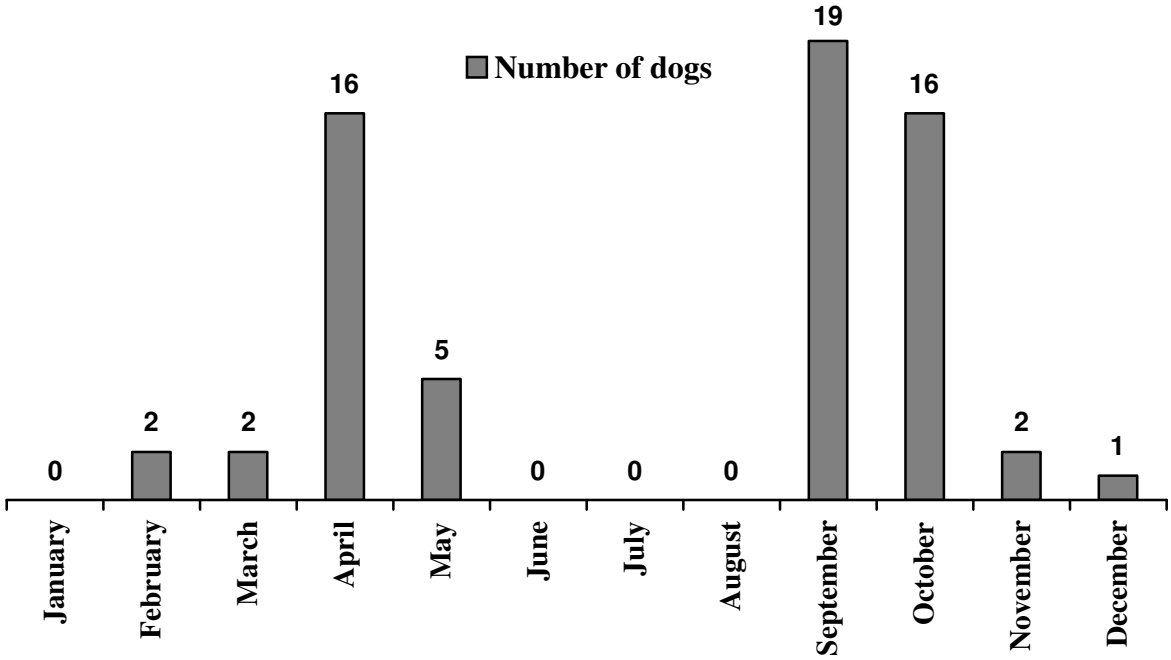

Figure 4.1. Seasonal distribution of 63 babesiosis cases observed within one year at the Clinic of Internal Medicine

Table 4.3. Age of 63 dogs with babesiosis

Group	n	Age (years)			
		Range	SD	Mean	Statistical analysis
Uncomplicated	32	0.3-9.0	2.2	3.4	One-way ANOVA $p < 0.001$
Single complication	19	0.8-12.0	3.2	4.8	
Multiple complications	12	1.2-13.0	3.8	8.6	

Table 4.4. Recovery and mortality rate of 63 dogs with babesiosis

Group	n	Recovery rate			Mortality rate			Unknown outcome
		n	%	Statistical analysis	n	%	Statistical analysis	
Uncomplicated	32	25	78	Fisher's Exact Test $p = 0.005$	1	3	Fisher's Exact Test $p < 0.001$	6
Single complication	19	13	68		4	21		2
Multiple complications	12	3	25		8	67		1

Uncomplicated babesiosis

No complications were found in 32/63 cases (51%). Most uncomplicated cases were treated as ambulatory patients (29/32, 91%), and only 3/32 dogs were hospitalised (9%).

The most frequently mentioned history data and detected examination findings were the following: rectal temperature above 39.2˚C, lethargy, ticks found by the owner or the veterinarian, macroscopic haemoglobinuria or haematuria, anorexia, splenomegaly, paleness of the skin and mucous membranes, jaundice, tachycardia and vomiting. Diarrhoea, abdominal pain, tachypnoea/dyspnoea, tremor, tonsillitis and lymphadenopathy were observed rarely (**Table 4.5.**). Haemoglobinuria was seen in 20/32 dogs, and it was concurrent with fever in 16 dogs. However, in 4/32 animals high fever was the earliest symptom, and the urine yet had yellow colour. There was no information about the colour of the urine in 8/32 cases. Among those 27 dogs that had fever at initial presentation, 8 patients returned to normal temperature within 24 hours. Further 8 dogs had persisting fever for one or two days following imidocarb therapy, the others were not evaluated repeatedly for body temperature.

Table 4.5. History and physical examination findings in dogs with uncomplicated babesiosis (n = 32)

Symptom	n	%
Rectal temperature > 39.2°C	27	84
Lethargy	25	78
Ticks	24	75
Macroscopic haemoglobinuria/haematuria	20	63
Anorexia	18	56
Splenomegaly	12	38
Pallor	10	31
Icterus	9	28
Vomiting	8	25
Tachycardia	8	25
Diarrhoea	4	13
Abdominal pain	4	13
Tachypnoea/dyspnoea	2	6
Tremor	1	3
Tonsillitis	1	3
Lymph node enlargement	1	3

Uncomplicated babesiosis was found to be severe in 2/32 dogs (the 2 youngest puppies in the study; 6%), moderate in 15/32 dogs (47%) and mild in 15/32 cases (47%), according to the severity of anaemia. Thrombocytopenia (platelet count < 200 G/l) was found in all patients suffering from uncomplicated babesiosis. The decrease in circulating thrombocyte numbers was severe (platelet count < 50 G/l) in 25/32 dogs (78%). However,

there were no clinical signs suggesting haemorrhagic diathesis in any of these animals. Repeated complete blood count (CBC) 2-4 days later demonstrated persistent thrombocytopenia in 8 of the 11 animals tested (73%). The white blood cell count (WBC) was determined in 30 uncomplicated cases. Leukopenia was found in 18/30 *Babesia* infected dogs (60%), normal WBC (6-12 G/l) was measured in 12/30 animals (40%). Of the 18 patients having leukopenia, 5 had reduced absolute neutrophil numbers (< 3 G/l), 6 had decreased absolute lymphocyte numbers (< 1 G/l), and 5 had both values abnormally low. However, there were 2 patients with leukopenia having the absolute neutrophil and lymphocyte numbers within the reference range. None of the patients showed absolute eosinophilia (> 0.3 G/l) in the uncomplicated group, in fact, eosinopenia occurred in all dogs except 2. Plasma creatinine concentrations were determined in 30 dogs, all measurements revealed normal values (< 150 μmol/l). It was possible to measure BUN in 6 uncomplicated patients, which was elevated in 3 of them (> 9 mmol/l). Increased BUN concentration was concurrent with macroscopic haemoglobinuria in 2 dogs, however there were also 2 animals having normal BUN values and haemoglobinuria simultaneously.

Laboratory urinalysis was possible in 2 animals. Abnormal findings were haemoglobinuria, proteinuria, bilirubinuria, increased amount of urobilinogen in the urine. Microscopic evaluation of the sediment revealed red blood cells, elevated numbers of white blood cells and tubular epithelial cells.

All but one animal was treated with imidocarb in the uncomplicated group (n = 31). Intravenous fluid therapy was applied in 27 patients (Ringer's solution, lactated Ringer's solution). All dogs showing evidence of intravascular haemolysis (i.e. macroscopic haemoglobinuria/haematuria, n = 20) were given infusions. Mannitol and furosemide were used frequently as well, to stimulate diuresis (n = 24 and n = 17, respectively). Nonsteroidal anti-inflammatory drugs (e.g. metamizol) were given as antipyretics in case of fever (n = 20). Antibiotic treatment with various drugs was started in many febrile dogs before the diagnosis of babesiosis could be obtained (n = 22). Only the two puppies with severe anaemia required blood transfusions.

Complicated babesiosis

Babesiosis with complications were found in 31/63 animals (49%). About half of these animals were treated as outpatients (17/31, 55%), and half of them were hospitalised (14/31, 45%). The majority of Rottweilers appearing in the study developed the complicated form of the disease (7/9, 78%).

The clinical manifestations and treatment of complicated babesiosis varied with the organ system(s) involved. Important observations in each kind of complication are summarised below. Due to technical reasons it was not possible to examine all 63 dogs for every complication. The relative frequencies of babesial complications and associated recovery and mortality rates are presented in **Table 4.6.**

Table 4.6. Relative frequencies, recovery and mortality rates in complicated babesiosis

Animals tested for the given complication		**Positive**		**Outcome**			**Comments**
	n	**N**	**N/n %**	**Recovered**	**Died**	**Unknown**	
Hepatopathy	34	14	41	7	6	1	***when hepatopathy was the only complication***: 5/6 animals recovered
Pancreatitis	12	4	33	1	3	0	***if pancreatitis was concurrent with ARF***: 3/3 animals died
ARF	61	19	31	6	11	2	***when ARF was the only complication***: 4/9 patients recovered, 4/9 patients died
DIC	45	11	24	5	5	1	***when DIC was the only complication***: 3/3 recovered
IMHA	52	5	10	2	3	0	
ARDS	63	4	6	1	3	0	all animals had multiple complications
Cerebral babesiosis	63	2	3	0	2	0	all animals had multiple complications
SIRS	63	22	35	14	5	3	
MODS	63	10	16	3	7	0	

Icterus was visible on the mucous membranes of 9/14 dogs with **hepatopathy**. There were 5/14 dogs without jaundice but with liver involvement. However, 5 animals of the study demonstrated icterus without evidence of hepatopathy, probably due to haemolysis. Abdominal ultrasonography was performed in 5 animals with hepatopathy, and 4 of them exhibited abnormal hepatic morphology. Hepatomegaly was a consistent finding in all 4 dogs. The echogenicity of the liver was increased in 2 dogs. The ultrasound structure was judged as normal in 3 and diffuse in 1 animal. One dog was not included in the hepatopathy group

because of nodular liver structure visualised during ultrasonography. This latter patient had liver cirrhosis confirmed by pathology. Hepatopathy was treated with crystalloid infusions (Ringer's solution) supplemented with 2.5% glucose, vitamin-B complex preparations and silymarin (an extract of the medical herb *Cardui mariae*, which inhibits lipid-peroxidation, and stimulates the regeneration of liver cells).

Pancreatitis was never seen as a single problem; in 3/4 animals it was simultaneously present with ARF. In 1 of these dogs concurrent hyperglycaemia supported the diagnosis, in the second animal ultrasonography demonstrated the pancreatic pathology, and in the third case azotaemia was only moderate (creatinine 169 μmol/l), while pancreatic enzymes were unproportionally elevated (amylase 2950 U/l, lipase 1232 U/l). Altogether it was concluded, that the diagnosis of pancreatitis was not influenced by the concurrent ARF in these cases. Half of the patients had jaundice (2/4); however, these animals also had hepatopathy. There was no indication for cholestasis. Abdominal pain was absent, and interestingly, the WBC was normal in all of the 4 patients. When pancreatitis was diagnosed, the food and water were taken away from the dogs, and they were maintained on fluid therapy. Thiethylperazine was given to control vomiting, and metamizol was used to eliminate fever, when needed. The patient which had moderate hyperglycaemia (17 mmol/l) died shortly after admission, before insulin treatment could be started.

Macroscopic haemoglobinuria/haematuria was observed in 17/19 dogs with **ARF**, in 2/19 cases the urine was yellow, but haemoglobin could be detected during urinalysis. There were 30/63 dogs in the whole population having discoloured urine, with normal plasma creatinine concentrations. The average creatinine concentration at the diagnosis of ARF was 411 μmol/l (range 165-1040 μmol/l). The highest creatinine concentration measured in a dog with favourable outcome was 275 μmol/l. Elevated BUN concentrations (> 9 mmol/l) with normal plasma creatinine levels were found in 5 dogs out of 13 tested. Ultrasonographic examination was performed in 6/19 ARF cases. Increased echogenicity of the kidneys were found in all 6 animals. The cortico-medullary ratio was increased in 3/6 dogs, diffuse ultrasonographic renal alterations were seen in 3/6 dogs, and renomegaly was diagnosed in 1/6 dog. Renal function was supported by intravenous fluid therapy (Ringer's solution, lactated Ringer's solution). Severe metabolic acidosis (pH < 7.2) prompted application of sodium bicarbonate infusions. Furosemide and mannitol were also applied to the rehydrated patients, especially when oliguria was suspected. Anuric patients received dopamine continuous rate infusions. Uraemic gastritis was treated with famotidine. Metoclopramide and thiethylperazine were used as antiemetics.

Whenever it was possible, blood samples were analysed for **DIC**, regardless of whether it was clinically suspected or not. DIC was always subclinical when it was the only complication: there was no evidence of haemorrhage or organ damage. Actually only 4/63 patients had mucous membrane petechiation in the whole study: 3 with DIC and 1 with hepatopathy. This latter dog had normal APTT, PT and FDP values, but the platelet count was very low (10 G/l). Bleeding was not seen in 8/11 patients having DIC. During therapy, special attention was paid to correcting acidosis, shock or hypoxia in the patients suffering from DIC. Fresh frozen plasma was applied in some cases. Heparin therapy was not used.

Spherocytosis and autoagglutination (**Figure 4.2.**) were suggested much more frequently by microscopic evaluation of the blood smears (26/51 dogs and 16/34 dogs, respectively), compared to the occurrence rate of **IMHA** based on complex criteria presented in **Table 4.1.** IMHA was a single complication in 1/5 case (this animal survived), but more typically IMHA occurred together with other manifestations (4/5 dogs). Therapy consisted of immunosuppressive doses of glucocorticoids (dexamethasone, methylprednisolone), protection of the gastric mucous membrane with H_2-receptor blockers (cimetidine, famotidine) and preventive antibiotic treatment.

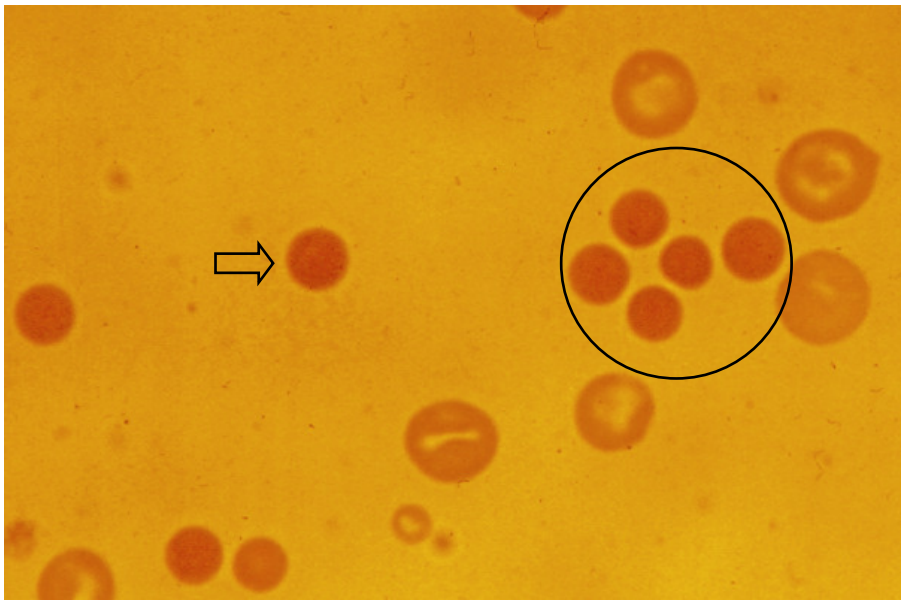

Figure 4.2. Spherocytes (⇨) and autoagglutination (○) in a dog with immune-mediated haemolytic anaemia

All 4 dogs with **ARDS** had clinical symptoms suggestive of pulmonary oedema. In 1 patient infiltration of the lungs was confirmed by radiography, while in 2 other dogs gross pathological examination revealed pulmonary oedema. Fluid therapy was terminated in these animals, and oxygen was supplied. In addition, furosemide and aminophylline injections were given.

One of 2 dogs with **cerebral babesiosis** showed epileptiform seizures. The other dog had ataxia and excitement, and finally it developed opisthotonus and coma. Necropsy was performed only in this latter patient, and findings included multiple intracranial haemorrhages. Seizures were controlled by pentobarbitone and these two patients also received furosemide, mannitol and glucocorticoids.

In this study, SIRS and DIC were considered as two possible pathomechanisms that could result in dysfunction of multiple parenchymal organs. The relationship between SIRS, DIC and MODS is demonstrated in **Figure 4.3.**

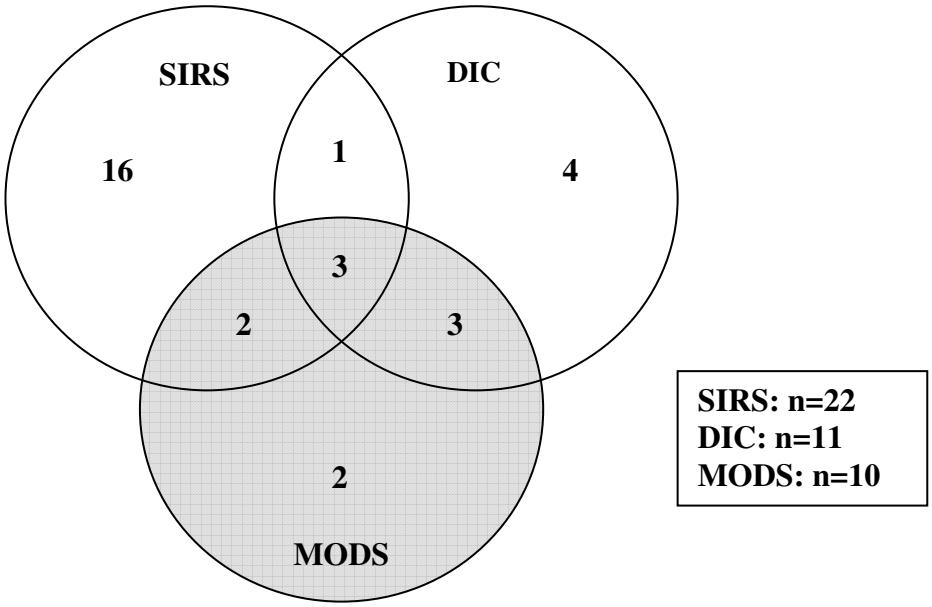

Figure 4.3. Relationship between SIRS, DIC and MODS

Systemic inflammatory response syndrome (SIRS) – that occurs due to the release of inflammatory mediators – was suggested earlier as the pathomechanism responsible for multiple organ dysfunction syndrome (MODS) in canine babesiosis (Jacobson and Clark, 1994; Taboada, 1998). Disseminated intravascular coagulation (DIC) is another possible manifestation of canine babesiosis (Moore and Williams, 1979) that could lead to MODS due to diffuse microthrombosis in vital organs. In this study, patients identified with DIC developed MODS more frequently than patients identified with SIRS.

4.3. Discussion

In line with previous reports from Hungary, there was only *B. canis* detected in the blood of the dogs in this study (Horváth and Papp, 1974; Horváth and Papp, 1996; Csikós et al., 2001). Smaller parasites were not seen in any of our cases, unlike in a recent report from our country, where organisms resembling small babesiae were found in two dogs which never travelled abroad (Farkas et al., 2004).

There was obvious dominance of male dogs and large dog breeds among our patients. These observations can be explained probably by the dog keeping habits, rather than by true gender or breed predisposition (the majority of outdoor dogs are large breed males in Hungary).

As demonstrated in **Figure 4.1.**, babesiosis cases in Hungary are usually seen during spring and autumn. The relatively mild and wet weather of these months is ideal for ticks. Babesiosis is almost never seen in the arid summer, but may appear 1-2 weeks after mild winter days. Similarly to our findings, the infection occurred more frequently in spring and autumn in France (Bourdoiseau, 2006) and in the wet season in Australia (Irwin and Hutchinson, 1991). A recent visit to a natural water site can often be identified in the history of many babesiosis patients in our country, as the reeds and bushes surrounding these places are favourite biotopes of the vector tick *Dermacentor reticulatus* (Janisch, 1986).

Uncomplicated babesiosis

As evidenced by our results, this form of the disease has a favourable prognosis if the correct diagnosis was made, and treatment was initiated in due time (**Table 4.4.**). The major clinical signs do not seem to differ from those of *B. canis* infections in other geographical regions (Farwell et al., 1982; Irwin and Hutchinson, 1991; Taboada, 1998; Lobetti, 1998, 2000) (**Table 4.5.**). Lethargy and fever could be the first manifestations.

According to our clinical observations, early treatment can prevent massive intravascular haemolysis and potential complications. Therefore, in endemic areas, and in the presence of the aforementioned clinical signs, imidocarb therapy is advisable before the diagnosis is confirmed by laboratory examinations, if the results are not expected within a few hours.

Unlike *B. canis vogeli* in Australia or the United States which typically affects puppies (Irwin and Hutchinson, 1991; Taboada, 1998), we have found that babesiae in Hungary (probably *B. canis canis*) can cause clinical disease in dogs of any age. Young animals, however, may develop more severe anaemia than adults, as it is demonstrated in the present work.

Thrombocytopenia is the most consistent and most marked haematological abnormality in uncomplicated cases in our country. However, spontaneous bleeding or petechiation of the mucous membranes due to *Babesia*-induced thrombocytopenia alone are exceptional. In fact, visible bleeding in a dog suffering from babesiosis should raise the suspicion for DIC. The pathomechanism leading to thrombocytopenia is not fully understood. Pooling of platelets in capillaries or in the spleen can be one of the explanations, but removal

of altered or normal platelets by the reticulo-endothelial system may also take place (Moore and Williams, 1979). Leukopenia was common in dogs having uncomplicated babesiosis, confirming an earlier report by Irwin and Hutchinson (1991). It was demonstrated in our work that reduction in both lymphocyte and neutrophil granulocyte numbers can account for the leukopenia. We propose that sequestration of white blood cells in the spleen might be the cause for the decrease in the WBC. Aspiration cytology samples drawn previously from dogs having babesiosis at our clinic revealed increased erythrophagocytosis, extramedullary haematopoiesis and marked infiltration by immune-cells in the spleen. Vercammen et al (1997) described eosinophilia in dogs with experimental *B. canis* infection, however, no animal was found with eosinophilia in our study. Rather, the majority of the patients had eosinopenia. This phenomenon might be explained by the acute infection, or by the stress of severe disease (Latimer, 1995). High BUN concentration is a common finding in babesiosis patients, and the elevation is often unproportional to the creatinine concentrations. Increased degradation of haemoglobin (Lobetti, 2000) and dehydration may both raise BUN concentrations. We had found that severe intravascular haemolysis (haemoglobinuria) is not always associated with elevated BUN.

The presence of red blood cells and tubular epithelial cells in the urinary sediment probably indicates some degree of renal injury, but this is not always associated with ARF.

Treatment of uncomplicated cases consisted of eradication of the parasite with imidocarb, antipyretic injections (metamizol), support of the renal function with infusions (Ringer's solution, lactated Ringer's solution) and diuretic therapy (mannitol, furosemide) in patients with haemoglobinuria. Blood transfusion was only necessary for the 2 puppies with severe anaemia.

Control of Babesia infection

Imidocarb seems to be highly effective against the strain of *B. canis* occurring in this region. Application of a single imidocarb injection is followed by rapid clinical improvement in the majority of cases: fever is usually reduced or absent by the following day, and the colour of the urine returns to normal in haemoglobinuric animals within 48 hours. In our experience, the parasite usually disappears from the blood 2-3 days after the injection, as reported by Penzhorn et al. (1995) too. They actually concluded that a single dose of imidocarb (7.5 mg/kg) could sterilise the infection. This was confirmed by subinoculation of blood from the treated dogs to splenectomized recipients. It was not checked however, whether the imidocarb treated dogs could stay *Babesia* carriers in their spleen, bone marrow or lymphnodes. In the absence of sensitive examination methods (like polymerase chain

reaction: PCR) at the time of our investigation, we could not evaluate the carrier state neither. The recommendation that babesicidal drugs should be avoided in endemic areas (Penzhorn et al., 1995) could be debated. Any illness or immunosuppressive treatment could result in a relapse of babesiosis. In addition, the continuous presence of antigens could lead to IMHA or immune complex deposition in the kidneys and joints.

The manufacturer suggests application of imidocarb injections (6 mg/kg) at 4 week intervals to prevent the disease. This method of prevention is very effective in our experience. However, Vercammen et al. (1996b) found the preventive effect of the drug to be of considerably shorter duration (around 2 weeks) against a European isolate of *B. canis*, using the same dose. Tick control with topical acaricidal preparations can be used simultaneously with imidocarb to reduce parasite exposure. However, secure protection cannot be expected from the spot on products alone. A vaccine produced from cell culture-derived exoantigens of *B. canis* (Pirodog) was available in Hungary, but high costs prevented its widespread use. Variations in strain antigenicity between geographic regions may reduce the protective value of vaccination (Taboada, 1998).

The manufacturer suggests SC application of imidocarb, but this may result in the formation of subcutaneous seromas few weeks after the injection. We usually apply the medication deep IM, thus avoiding local adverse reactions in the vast majority of patients. Wherever imidocarb is applied, the injection causes severe pain for a few seconds. Owners should be warned about this, and also about the transient parasympathomimetic side effects of the drug.

Complicated babesiosis

Most dogs that are treated for babesiosis in field practices recover without complications in Hungary. Dogs that do not improve after imidocarb therapy are frequently referred to our clinic from nearly all locations in the country. This fact probably explains the high incidence of complicated babesiosis in our study.

Seven out of 9 Rottweilers developed complicated babesiosis in this study. It has been demonstrated that Rottweilers are more susceptible to parvovirus enteritis, and develop more severe clinical disease than other breeds (Glickman et al., 1985). It can be speculated, that a similar predisposition in Rottweilers might exist also for *Babesia* infection, resembling the observations of Collett (2000).

Single complications had a fairly good prognosis: 68% of our patients survived. These dogs were only slightly older on average, than the animals of the uncomplicated group (4.8 years and 3.4 years, respectively). The chances of recovery were poor when multiple

complications occurred: 67% of the patients died or were euthanized. Old age is an obvious risk factor for multiple system involvement in babesiosis (mean age of dogs with multiple complications was 8.6 years). Older animals may have various subclinical disorders that deteriorate to organ failure during babesiosis.

In the one year period of this research, hepatopathy, pancreatitis, ARF and DIC were frequent complications of the *Babesia* infection. IMHA, ARDS and cerebral babesiosis were relatively rare observations.

Hepatopathy is usually mild and has a favourable outcome, if this is the only complication. Icterus is a common symptom in dogs with liver damage, but not in every case. Furthermore, jaundice appears in some patients without hepatopathy, because of erythrocyte destruction (haemolytic icterus). The most consistent ultrasonographic observation was hepatomegaly in our patients.

Pancreatitis, a recently described manifestation in babesiosis (Möhr et al., 2000), was a frequent complication in our study. These animals all had multiple organ dysfunctions. The diagnosis should be carefully made in azotaemic patients, as amylase and to a lesser extent lipase could be retained in the plasma during renal failure. In the authors' practice, ultrasonography is considered to be a very useful tool in the detection of pancreatitis, as suggested by others too (Nyland et al., 2002). It can confirm the presence of pancreatic problem in the uraemic dogs, and it also has the potential to demonstrate pancreatitis, when the enzymes are within normal limits.

ARF was a common complication of canine babesiosis in our study. Other investigators detected ARF rarely in *B. canis rossi* infected South African dogs (Jacobson and Clark, 1994). It was also reported that mild renal injury might occur more frequently in babesiosis than severe ARF (Lobetti and Jacobson, 2001). Kidney failure was found to be a dangerous complication in our patients: about half of those patients died, in which ARF was the only organ damage. This might reflect the different pathogenicity of the *B. canis canis* subspecies probably occurring in Hungary (Földvári et al., 2005), compared to the South African strains. The maximum creatinine level associated with recovery was 275 μmol/l in our population. Anuria usually means grave prognosis: restoration of urine production is unsuccessful in most patients, regardless whether furosemide, mannitol and dopamine are used alone or in combination. Although free haemoglobin was not confirmed as a potential cause of ARF in a recent study (Lobetti et al., 1996), the vast majority of our patients having ARF had macroscopic haemoglobinuria (17/19). Histological examination was not performed from the kidneys of dogs suffering from ARF in this work. Earlier experience in the authors' clinic suggests that the most common cause for renal failure in babesiosis is haemoglobinuric

nephrosis (i.e. the mechanical and toxic effects of free haemoglobin). We assume that intravenous fluid therapy can prevent ARF in haemoglobinuric patients: there were 30 patients which had discoloured urine samples, but did not develop ARF. All of them were treated with IV fluids. Actually, the pathogenesis of renal failure appearing in babesiosis is still not clear, and may follow different pathways in individual patients. Anaemic hypoxia, hypovolaemia, intrarenal vasoconstriction and haemoglobinuric nephrosis were all suggested as possible causes (Lobetti and Jacobson, 2001). Systemic reactions of the host, like SIRS and DIC may also lead to dysfunction of the kidneys (Moore and Williams, 1979; Jacobson and Clark, 1994). Frequently observed ultrasonographic abnormalities in our patients were increased echogenicity of the kidneys, diffuse renal structure and increased cortico-medullary ratio.

The manifestation of **DIC** in canine babesiosis had been demonstrated long time ago (Moore and Williams, 1979). Haemolysis, acidosis, hypoxia and shock are all known predisposing factors for DIC, and they all might be present in babesiosis patients (Lobetti, 1998, 2000). In the majority of cases DIC was associated with organ dysfunctions and/or bleeding tendency among our patients, but we detected the subclinical form of DIC as well.

Diagnosing **IMHA** is a challenging task for the practising veterinarian. Multiple data should suggest the diagnosis in each case; a single laboratory result should never be interpreted as evidence of IMHA. Immune-mediated damage of the erythrocytes can be observed in the blood smears (spherocytosis and autoagglutination). The in-saline agglutination test has recently been suggested as a useful method for diagnosing IMHA (Giger, 2005). The Coombs'-test should be avoided in areas where babesial infection is common, because even uncomplicated babesiosis cases have positive test results (Lobetti, 1998, 2000). Previously we suggested the use of an osmotic fragility test for indirect detection of spherocytes (Máthé et al., 1998). However, the results of the present work suggest that most patients suffering from babesiosis have increased osmotic fragility of red blood cells (even without IMHA), similarly to the findings of Makinde and Bobade (1994). This is probably due to *Babesia*-induced damage of the erythrocyte membrane. Clinical aspects should also be considered when IMHA is suspected. Worsening of the anaemia after babesicidal treatment, and/or improvement during immunosuppressive therapy could both confirm the diagnosis. It is possible that some chronic carrier animals develop IMHA as a complication of babesiosis, but the parasite remains undetected by blood smear examinations. In the authors' opinion, treatment with babesicidal preparations could be advisable to every IMHA patient in endemic areas if the *Babesia* infection cannot be ruled out safely. As demonstrated previously, the seasonality of „idiopathic" IMHA cases in Hungary is the same

as the seasonal pattern of babesiosis (Máthé et al., 1998), probably because some IMHA patients are in fact chronic *Babesia* carriers.

ARDS and **cerebral babesiosis** were rare complications in our patients. These cases always had a poor prognosis. Although all of these patients had multiple complications, the involvement of the lungs and brain probably had major role in the death of the dogs.

Multiple complications had poor prognosis in our dogs. The outcome was even worse if multiple parenchymal organs were affected, i.e. if MODS was present (mortality rate was 70%). In a previous study from South Africa the recovery rate was not significantly affected by multiple organ damage (Welzl et al., 2001). Detection of risk factors that could predict the development of MODS would possibly increase the success of therapy. The results of our work indicate that SIRS identified by previously reported criteria (Lobetti, 1998, 2000) has a poor prognostic value for MODS. However, the mechanisms of systemic inflammation do occur in babesiosis, therefore, the definition of SIRS should be specified better. We propose that DIC is another possible prognostic factor for MODS. By the definitions applied in this study, DIC was associated with MODS more frequently than SIRS (**Figure 4.3.**). The two possible pathomechanisms leading to multiple organ damage (SIRS and DIC) can be present alone or simultaneously in individual cases, as demonstrated in this study. The development of MODS was most likely if both SIRS and DIC were present.

The results of this study demonstrate that clinical manifestations of uncomplicated babesiosis in Hungary are similar to those of *B. canis* infections in other geographical locations. Symptoms can be easily controlled with early imidocarb therapy in the majority of cases. Complicated babesiosis, however, is a challenging problem for the veterinarian. Hepatopathy appears to be a benign complication, but other manifestations like pancreatitis, ARF, DIC, IMHA, ARDS and cerebral babesiosis are associated with lower recovery rate and increased mortality.

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

Máthé Á., Vörös K., Németh T., Biksi I., Hetyey Cs., Manczur F., Tekes L.: Clinicopathological changes and effect of imidocarb therapy in dogs experimentally infected with Babesia canis. Acta Vet. Hung. 2006. 54. 19-33.

The major goal of the present work was to document the clinicopathological changes in experimental babesiosis caused by *Babesia canis.* The dogs were also meant to be used for babesial antigen production. The effects of imidocarb therapy and additional symptomatic treatment in the acute phase of the disease were also studied.

5.1. Materials and methods

Experimental animals

Examinations were performed on 3 healthy, naive Beagle dogs originating from isolated kennels free from babesiosis. Each animal was regularly vaccinated against common infectious diseases and dewormed. Flea and tick control was also performed regularly by spot on preparations containing fipronil. The one year old animals were females, and weighed between 9 and 11 kg. Dogs were kept in kennels and fed a commercial dry dog food. Baseline complete blood count (CBC), serum alanine-aminotransferase (ALT) and creatinine examinations were negative in all dogs, just as faecal flotation tests. No *Babesia* or *Haemobartonella* organisms were seen during microscopic evaluation of the stained blood smears.

Maintenance and care of animals were in accordance with the requirements of the National Institute of Health, USA: guide-lines for use of laboratory animals, and the experiment was performed as to the regulations of the Supervising Committee acting in the Faculty of Veterinary Science, Szent István University, Budapest, Hungary.

Experimental infection

Dog **A** was left spleen intact, in dog $\mathbf{B_{SE}}$ and $\mathbf{C_{SE}}$ splenectomy was performed. They recovered uneventfully from the operation. Dog **A** and $\mathbf{B_{SE}}$ were infected by intravenous inoculation of 5 ml whole blood, obtained from a naturally infected dog being in the acute

phase of babesiosis. Inoculation of dog $\mathbf{B_{SE}}$ happened 69 days after splenectomy. Dog $\mathbf{C_{SE}}$ was infected intravenously 230 days after splenectomy, with 5 ml blood drawn from another clinical patient having acute babesiosis.

At the peak of parasitaemia (based on the blood smears), 400 ml of blood was collected from the jugular vein of both dog $\mathbf{B_{SE}}$ and $\mathbf{C_{SE}}$ on postinfection day (PID) 3 for babesial antigen production. The animals were sedated for the procedure with intravenous application of 0.25 mg/kg diazepam and 5 mg/kg ketamin.

Clinical examination

The dogs were examined twice a day for 30 days following the infection. Physical examination included measuring the rectal temperature, pulse rate, capillary refill time, and evaluating the general health state, the colour of mucous membranes, enlargement of the liver/spleen and the colour of the urine. Any other clinical findings were also noted.

Laboratory examinations

Examination of blood samples collected from the saphenous veins of the animals were performed according to the following schedule in all 3 dogs: complete blood count and microscopic blood smear examination daily for 2 weeks, then twice a week for another 2 weeks period after the infection. Reticulocyte count, serum ALT, lipase, creatinine, activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrin degradation products (FDP) were determined on every second day in the first 2 weeks after infection, then these parameters were evaluated twice a week in the following fortnight as well. Blood smears stained with Dia-Panoptic (Diagon Kft., Budapest, Hungary) were examined to detect babesiae in the red blood cells, and to count the percentage of infected erythrocytes (1000 erythrocytes were evaluated in each smear). Qualitative white blood cell counts were calculated from the total white blood cell numbers and the ratio of white blood cells types derived from the blood smears. Reticulocyte percentages were counted in blood smears stained with brillant-krezil-blue. The reticulocyte index (RI) was calculated with the following formula: (haematocrit/0.45) x reticulocyte %. The anaemia was judged to be regenerative, if RI was higher, than 2.5. Complete blood count was carried out by an automatic analyser (Abacus Haematological Analyser, Diatron Kft., Budapest, Hungary).

Biochemistry parameters (ALT, alkaline phosphatase: AP, γ-glutamyl transferase: GGT, lipase, albumin, total bilirubin, bile acids, blood urea nitrogen: BUN and creatinine) were determined using an automatic spectrophotometer (Dr. Lange 400, Dr. Bruno Lange

GmbH, Berlin, Germany). Activated partial thromboplastin time (APTT, Actimat Biomérieux SA, Marcy-l'Etoile, France), PT (Thromborel-S, Schnitger & Gross: Amelung GmbH, Lieme, Germany) and FDP (Biomérieux BV Boxtel, The Netherlands) were also evaluated with standard methods.

Urinalysis was performed in all 3 infected dogs when the urine demonstrated reddish-brownish discolouration, followed up by control examinations a few days later. It consisted of detecting abnormal components and pH by a combined urine dipstick. Microscopic analysis of the urinary sediment was also performed.

Additional examinations

Ultrasonographic examinations were done with a Brüel and Kjaer Panther 2002 ultrasound system, using a 5.0 MHz real-time convex array transducer (Brüel and Kjaer, Naerum, Denmark). Ultrasonographic findings of the liver, gallbladder, spleen, pancreas and kidneys were evaluated according to standard methods (Nyland et al., 2002). The first examinations were performed on PID 4 in all dogs, and follow-up ultrasonography was also carried out on PID 7 in dog $\mathbf{B_{SE}}$ and $\mathbf{C_{SE}}$.

Fine needle aspiration cytology was done on the spleen of dog **A** under ultrasonographic guidance on PID 4. The smears were stained with Dia-Panoptic, and evaluated by light microscopy. In dog $\mathbf{B_{SE}}$ a sample was aspirated from the abdominal effusion on PID 4, stained and examined in the same manner.

The liver was biopsied under ultrasound guidance on PID 7 in both dog $\mathbf{B_{SE}}$ and $\mathbf{C_{SE}}$, applying an automatic needle biopsy technique (Biopty, Radiplast, Uppsala, Sweden) as described earlier (Hager et al. 1985). Liver biopsy samples were stained with haematoxylin-eosin (HE) and Oil-Red-O for histological analysis.

Plain radiography of the left humerus of dog $\mathbf{C_{SE}}$ was performed on PID 13, repeated radiology of the left humerus and both femurs was done on PID 29. A bone biopsy specimen was obtained surgically from the left humerus of the same dog on PID 29. The specimen was prepared for light microscopy using haematoxylin-eosin and Gram's stain. Routine bacteriological and fungal culture with drug sensitivity test was also performed from this bone sample.

Treatment

As a significant amount of blood was taken from the splenectomized dogs, Ringer's infusion was applied through an intravenous catheter at a rate of 500 ml/hour to prevent

hypovolaemic shock during the blood-letting. Epinephrine (0.05 mg/kg), caffeine (10 mg/kg) and 10% sodium chloride (3 ml/kg) injections were given intravenously for the same purpose. Immediately after the blood-letting the same amount of whole blood transfusion was given. Calcium gluconate (50 mg/kg) and methylprednisolone (25 mg/kg) were applied IV to prevent transfusion reactions. Intravenous methylprednisolone was given for a week with gradual reduction of the daily dose to 1 mg/kg. Both dogs were treated with a single dose of imidocarb (6 mg/kg) intramuscularly on PID 3. Dog **A** did not receive imidocarb treatment.

Symptomatic treatment for babesiosis included metamizol injections (50 mg/kg, IV) in case of fever (> 40 ºC), Ringer's infusion with glucose and 10% mannitol infusion (5 ml/kg) if the animals were haemoglobinuric. Furosemide (2 mg/kg twice a day, IV) was also given, if the patients were azotaemic.

H. canis infection was detected in dog $\mathbf{B_{SE}}$, which was treated for 3 weeks twice daily with doxycycline (5 mg/kg orally). In dog $\mathbf{C_{SE}}$ bacterial osteomyelitis was diagnosed, and treated twice daily with norfloxacin (10 mg/kg orally) for 10 days.

5.2. Results

All dogs developed parasitaemia on PID 1 or 2. Evaluation of the blood smears demonstrated the presence of large (3-5 μm), pyriform, paired parasites, *B. canis* in the erythrocytes of all 3 dogs.

Dog A

Fever was measured on PID 2-6 and 9-10, coinciding with the days when parasitized red blood cells were detected. Mild pyrexia occurred on PID 12, 21 and 25. Pallor of mucous membranes was visible between PID 7 and 13. Macroscopic haemoglobinuria was present on PID 3-4. The spleen of this dog was palpable as severely enlarged for two weeks (PID 2-15).

The haematological profile and parasitaemia of the dog are demonstrated in **Figure 5.1.** Serum ALT and creatinine values are shown in **Figure 5.2.** Pathological alterations of haematological and blood chemistry parameters throughout the experiment are summarised in **Table 5.1.**

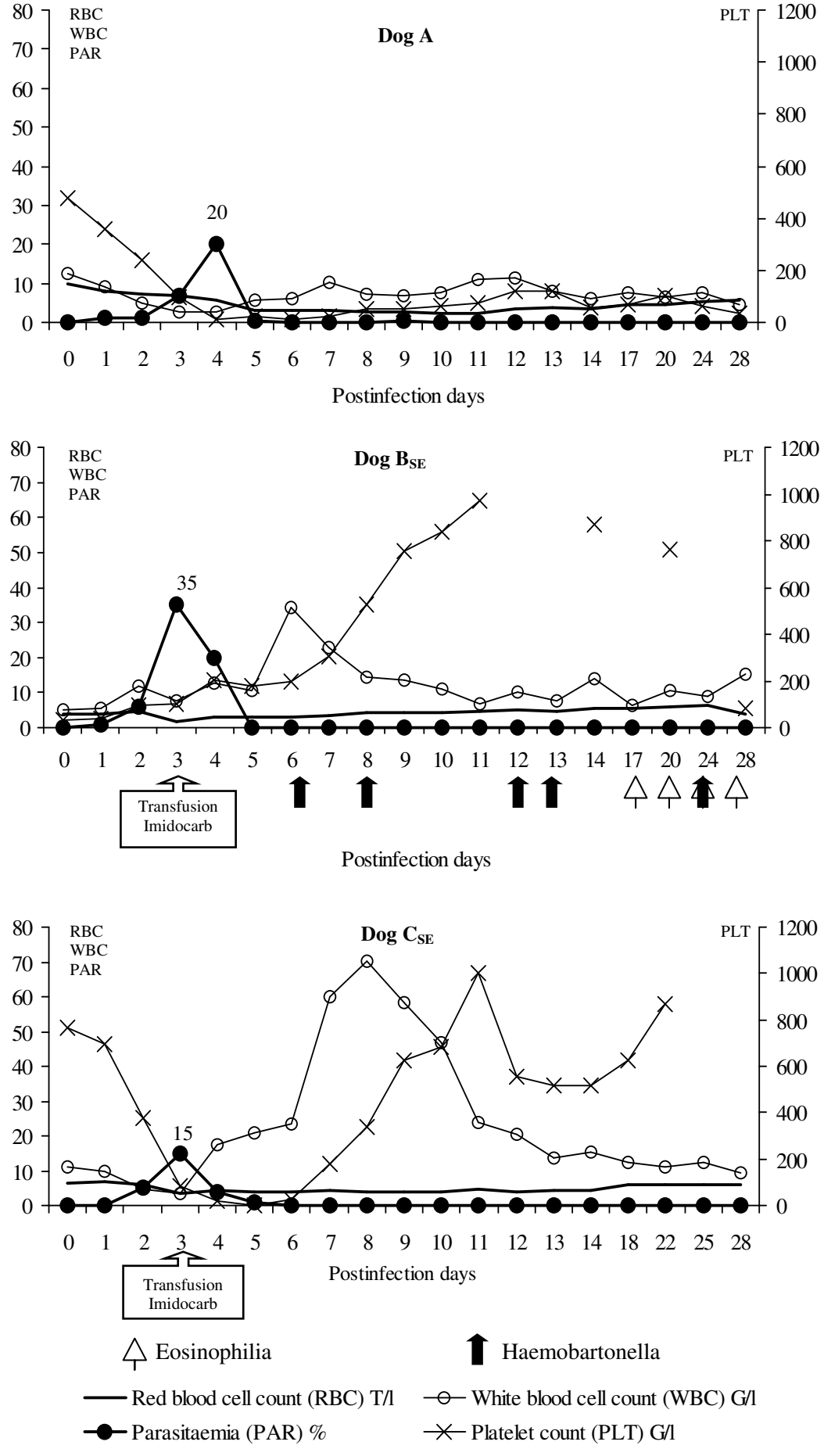

Figure 5.1. Haematological profile of the experimental dogs

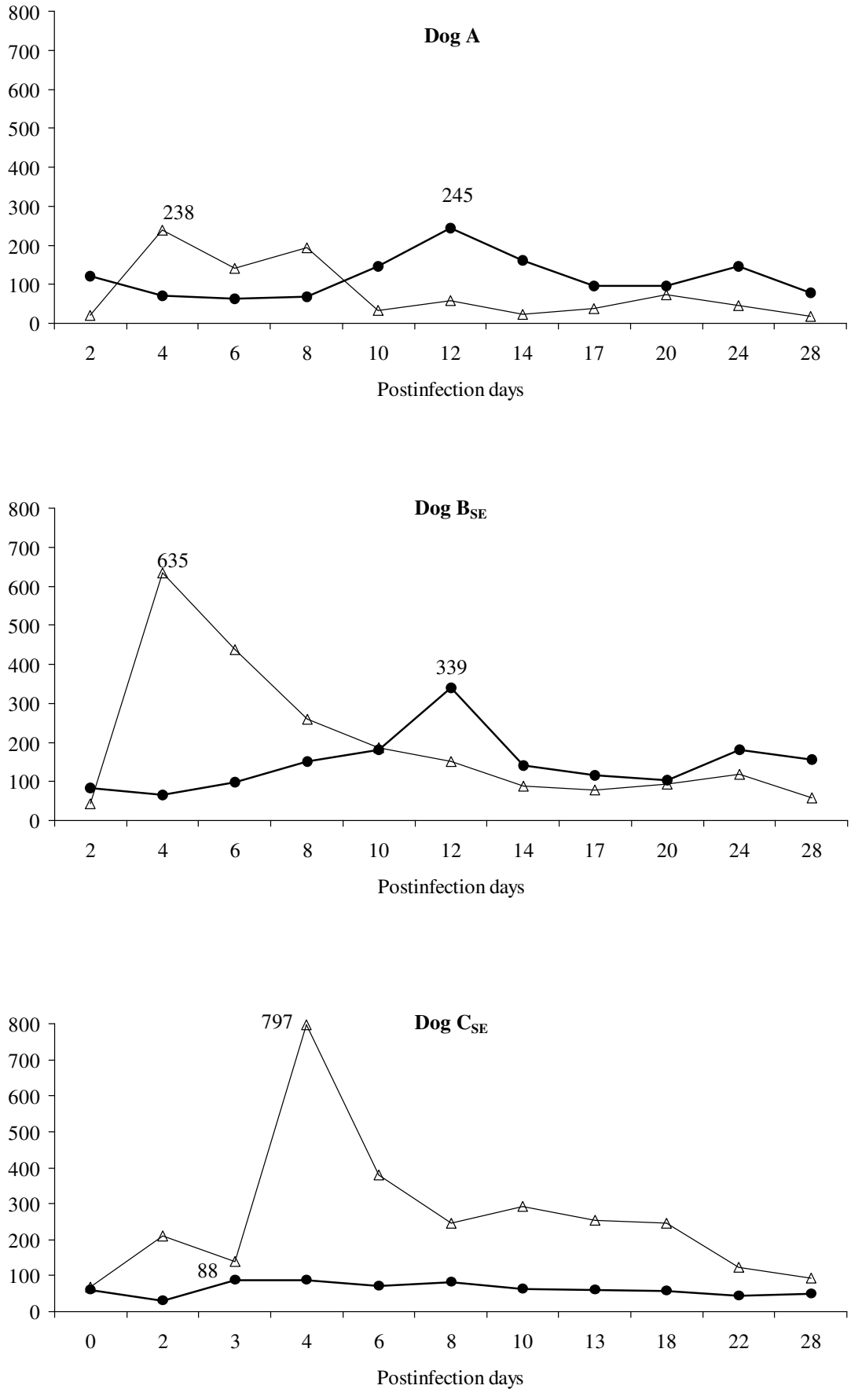

Figure 5.2. ALT activity and creatinine concentrations in the experimental animals

Table 5.1. Alterations of haematological and blood chemistry parameters during the experiment

Parameter	Criterion	Experimental dogs		
		Dog A	Dog B_{SE}	Dog C_{SE}
Parasitized red blood cells		+ PID 1-28	+ PID 1-6	+ PID 2-5
Thrombocytopenia	< 200 G/l	+ PID 3-28	+ PID 0-3	+ PID 3-7
Leukopenia	< 6 G/l	+ PID 2-5	+ PID 0-1	+ PID 2-3
Neutropenia	< 3 G/l	+	+	-
Lymphopenia	< 1 G/l	+	-	+
Leukocytosis	> 15 G/l	-	+ PID 6-7	+ PID 4-12
Neutrophilia	> 11 G/l		+	+
Lymphocytosis	> 5 G/l		-	+
Eosinophilia	> 0.3 G/l	-	+ PID 17-28	-
Anaemia	RBC < 4.5 T/l	+ PID 5-17	+ PID 0-10	+ PID 3-17
Reticulocytosis	RI > 2.5	+ PID 12-17	+ PID 6-10	+ 8-17
Elevated serum ALT activity	> 60 U/l	+ PID 4-8	+ PID 4-24	+ PID 2-28
Elevated serum creatinine	> 140 µmol/l	+ PID 10-14	+ PID 10-28	-
Elevated serum lipase	> 800 U/l	-	-	-
Prolonged APTT	> 45 sec	-	-	-
Prolonged PT	> 15 sec	-	-	PID 3
Presence of FDP		-	-	PID 3,7,10

Legends: RBC: red blood cell count

Urinalysis was performed on PID 3. Pathologic alterations observed were haemoglobinuria and haematuria.

Abdominal ultrasonography on PID 4 demonstrated splenomegaly, hepatomegaly with slight diffuse hepatic lesion and increased echogenicity of the renal cortices.

Lymphoblasts, young plasma cells and reactive macrophages were found in the fine needle aspiration cytology sample of the spleen on PID 4. Erythrophagocytosis was also observed. *B. canis* organisms were visible in red blood cells and extracellularly as well.

Dog B_{SE}

Fever occurred on PID 2-6, when *Babesia* infected red blood cells could be detected. The mucous membranes were pale on the first week (PID 1-7). The urine was discoloured due to haemoglobinuria on PID 3-4.

The haematological profile and parasitaemia of this animal are shown in **Figure 5.1.** *Babesia* organisms were last seen in the stained blood smears 3 days after the imidocarb injection. *H. canis* was visible on the erythrocytes several times between PID 6 and 28. Serum ALT and creatinine values are shown in **Figure 5.2.** Pathological alterations of haematological and blood chemistry parameters throughout the experiment are summarised in **Table 5.1.**

Urinalysis on PID 3 revealed haemoglobinuria and haematuria, which ceased by PID 8.

Results of abdominal ultrasonography on PID 4 included marked hepatomegaly with dilated v. hepatica branches and small amount of free abdominal fluid. Probably due to the free fluid, the pancreas was visible, but showed normal structure. The ultrasonographic appearance of the kidneys was physiologic. A repeated examination on PID 7 demonstrated minimal free fluid being present around the left kidney, but was otherwise negative.

A cytological sample of the abdominal effusion on PID 4 contained very few cells (lymphocytes, neutrophils and mesothelial cells), and was diagnosed as congestive fluid accumulation.

Histology of the liver biopsy specimen on PID 7 revealed acute focal non-purulent interstitial hepatitis and bile stasis, but vacuolization of hepatocytes was also visible (**Figure 5.3.**).

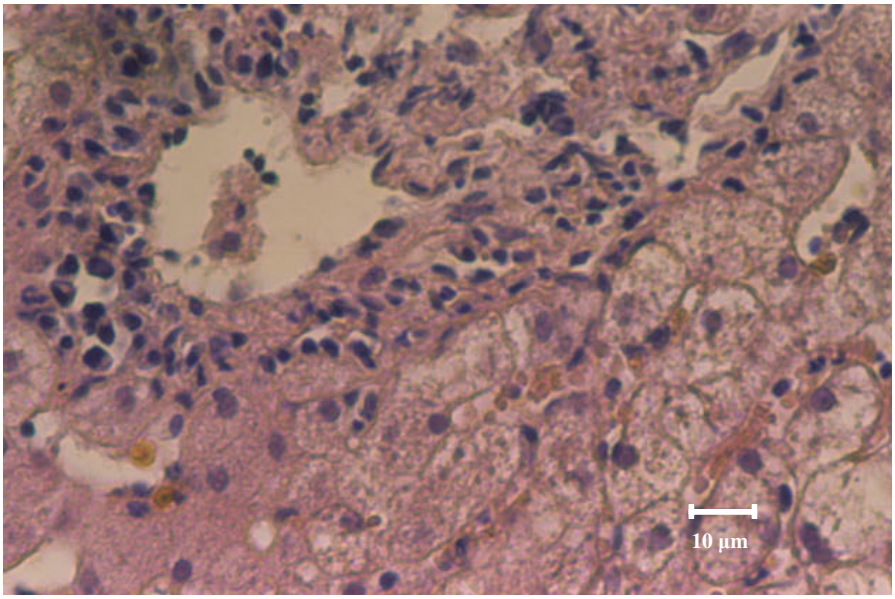

Figure 5.3. Hepatitis and bile stasis in dog B_{SE}

Dog C_{SE}

Intermittent fever was recorded on PID 2-6, coinciding with the detection of parasitized red blood cells. There was a second febrile period on PID 12-20, probably associated with a new clinical sign: the dog became lame in the left front leg, and the left humerus was painful when palpated. Later a painful swelling also developed in both femurs. The mucous membranes of the dog were pale on PID 3-16. Mild jaundice was visible on PID 3-9 and 13-17. Occasional vomiting occurred between PID 2 and 5. The urine was macroscopically haemoglobinuric on PID 2-4.

The haematological results and parasitaemia of this dog are summarised in **Figure 5.1.** *Babesia* infected cells were last detected 2 days after imidocarb treatment. Serum ALT and creatinine values are shown in **Figure 5.2.** Pathological alterations of haematological and blood chemistry parameters throughout the experiment are summarised in **Table 5.1.**

Urinalysis revealed haemoglobinuria and haematuria on PID 3-4. On PID 5 haematuria was reduced, but a small amount of bilirubin granules were seen.

Abdominal ultrasonography performed on PID 4 demonstrated hepatomegaly with a diffuse alteration in the structure of the liver. The renal cortices had increased echogenicity. Swollen lymph nodes were seen around the spleen. The examination was repeated on PID 7. The ultrasonographic appearance of the liver did not change, and the gall bladder was dilated. The renal cortico-medullary ratio was increased, and this time the renal medulla had increased echogenicity, as well.

Histology of the liver biopsy on PID 7 revealed diffuse acute serous hepatitis, accompanied by focal liver cell necrosis, pathologic simple centrolobular fatty infiltration and bile stasis.

Radiological examination of the painful left humerus on PID 13 was negative. Repeated radiology of the painful and swollen left humerus and both femurs showed lesions consistent with periostitis and osteomyelitis on PID 29. Histology of the bone biopsy demonstrated diffuse purulent periostitis and Gram negative phagocytosed bacteria. *Escherichia coli* was cultured in a bacteriological test. Mycological examination was negative.

5.3. Discussion

The experimental infection caused an acute disease in all 3 dogs. Major clinical signs of our infected dogs included fever, anaemia, icterus, vomiting and haemoglobinuria. These symptoms were the same as observed by others in dogs naturally infected with *B. canis*

(Horváth and Papp, 1996; Taboada, 1998; Lobetti 2000). Splenomegaly was found in the spleen intact dog **A**.

Fever could still be measured 3 days after the imidocarb injection in the splenectomized dogs ($\mathbf{B_{SE}}$, $\mathbf{C_{SE}}$). It was typically present on those days, when parasitized red blood cells could be detected by blood smear examination in all dogs. There was a second febrile period in dog $\mathbf{C_{SE}}$ on PID 12-20, probably due to a bacterial complication (osteomyelitis).

Anaemia developed sooner, and became more severe in our splenectomized dogs ($\mathbf{B_{SE}}$, $\mathbf{C_{SE}}$), and the decrease of haematocrit was so severe for PID 3 in both of these animals, that phlebotomy (for antigen production), followed immediately by blood transfusion and imidocarb treatment were applied without delay. Interestingly, Wozniak et al. (1997) found more severe anaemia in spleen intact dogs, than splenectomized ones during an experimental *B. gibsoni* infection. The anaemia in our cases became regenerative 5-7 days after its first detection in all dogs. This is somewhat later, than the generally accepted 3-4 days time gap the bone marrow requires to spin up its red blood cell production. Vercammen et al. (1997) had found that anaemia became regenerative even later, on average 23 days after its onset in dogs having experimental *B. canis* infection.

Dog $\mathbf{B_{SE}}$ actually had mild anaemia, leukopenia and thrombocytopenia on the day of the infection. The exact cause for this is not known, but it can be speculated, that the absence of the spleen (the lack of extramedullary haemopoiesis) could be the reason. If latent haemobartonellosis were responsible for the anaemia, one would expect reticulocytosis with normal leukocyte and thrombocyte numbers. From PID 11 all haematological cell counts of dog $\mathbf{B_{SE}}$ returned to normal. Perhaps worsening anaemia due to *Babesia* infection and phlebotomy were enough stimuli for the bone marrow to increase cell production.

Leukopenia was observed in dogs **A** and $\mathbf{C_{SE}}$ a few days after the experimental infection (PID 2-5). Similarly to the findings of Vercammen et al. (1997), decreased neutrophil and lymphocyte counts both contributed to this phenomenon, which is commonly seen in dogs with natural *Babesia* infections in Hungary, as well (Máthé et al., 2006). As mentioned above, dog $\mathbf{B_{SE}}$ had mild leukopenia due to neutropenia at the time of the infection, which resolved for PID 2. This leukopenia was probably not associated with babesiosis. In the two splenectomized animals ($\mathbf{B_{SE}}$, $\mathbf{C_{SE}}$) severe leukocytosis with neutrophilia and left shift were also observed on PID 4-12. This so called leukaemoid reaction, which is an occasional finding in canine babesiosis (Taboada, 1998), may be an earlier, but not specific indicator of bone marrow activity, than reticulocyte index. In dog $\mathbf{C_{SE}}$ leukocytosis occurred earlier, than the clinical signs of osteomyelitis became obvious, so leukocytosis was probably independent

from the bone infection. Dog **B_{SE}** had an 11 day period with absolute eosinophilia. No intestinal parasites – which might be responsible for eosinophilia – were found during faecal examinations. Eosinophilia in dogs having experimental *B. canis* infection was reported earlier by Vercammen et al. (1997). Recently, Camacho (2005) found eosinopenia in *B. canis* infected dogs, but mild eosinophilia in *Theileria annae* infected dogs. *Haemobartonella* infection was also diagnosed in dog **B_{SE}**, but according to the literature there are no consistent leukogram abnormalities in canine haemobartonellosis (Harvey, 1998).

Thrombocytopenia, the most typical haematological alteration of natural canine babesiosis in our country (Máthé et al., 2006), was discovered in all of our infected animals. In dog **A**, which was not treated with imidocarb, it lasted for several weeks, confirming an earlier report (Vercammen et al., 1997). In dog **B_{SE}** thrombocytopenia was already present on the day of the infection, and it was probably not (only) due to babesiosis.

In the spleen intact dog (**A**), which did not get imidocarb treatment, parasitized red blood cells could be detected until PID 10 in the acute phase of the disease. Thereafter blood smears became negative for *Babesia* until PID 28, which was the last day when the parasite could have been observed. Actually, from PID 26 the dog became clinically healthy.

Parasitized red blood cells were detectable for 2-3 days after the imidocarb injection in the two splenectomized dogs (**B_{SE}**, **C_{SE}**), similarly to the findings of Penzhorn at al. (1995) during experimental *B. canis* infection of spleen intact beagles.

Elevated ALT activity signalled an acute liver injury in all 3 dogs, confirming results of earlier reports (Vercammen et al., 1997; Wozniak et al., 1997; Lobetti, 2000). The peak of the enzyme activity was higher, and its elevation lasted longer in the splenectomized dogs. Both hypoxic liver injury due to pronounced anaemia and glucocorticoid therapy in these animals could have contributed to the more severe liver insult. Histology from the liver of the splenectomized dogs demonstrated acute hepatitis in both animals. Wozniak et al. (1997) described diffuse nonsuppurative periportal and centrilobular hepatitis in dogs suffering from *B. gibsoni* infection. They also found moderate ascites appearing in one of the infected dogs, similarly to the findings of dog **B_{SE}** in our study. In dog **B_{SE}** ballooning degeneration of hepatocytes was also present, resembling glucocorticoid hepatopathy (Badylak and van Vleet, 1980). However, the inflammation in the livers of both splenectomized dogs cannot be attributed to glucocorticoid therapy, more probably it is a consequence of the *Babesia* infection. Hepatopathy was found to be a common complication in natural *B. canis* infections in a recent study in Hungary (Máthé et al., 2006).

Mild azotaemia (elevated creatinine concentration) was detected in dogs **A** and **B_{SE}** between PID 10 and 28, which started several days after resolution of haemoglobinuria.

Several potential pathologies other than haemoglobinuric nephrosis have been suggested in the literature for babesiosis associated nephropathy. These include anaemic hypoxia, hypotonia, systemic inflammatory response syndrome, free radicals, disseminated intravascular coagulation, immune-mediated glomerulonephritis etc. (Jacobson and Clark, 1994; Lobetti and Jacobson, 2001). Further investigations are necessary in this field, especially in Hungary, where acute renal failure appears to be a frequent complication of canine babesiosis (Máthé et al., 2006).

All animals had physiologic serum lipase activity throughout the study. Abdominal ultrasonography was not suggestive of pancreatitis either, so this important complication of babesiosis in dogs (Möhr et al., 2000) could be ruled out in these cases.

DIC is a common consequence of canine babesiosis (Moore and Williams, 1979; Máthé et al., 2006), which was also studied in this research. This condition could be ruled out in dogs **A** and $\mathbf{B_{SE}}$ based on negative APTT, PT and FDP results. In dog $\mathbf{C_{SE}}$ there was a mild suspicion for DIC on PID 3, when the animal had thrombocytopenia, PT was longer than the reference range, and the FDP-test was positive. However, DIC remained subclinical in this animal, as there was no evidence of bleeding tendency or severe multi organ dysfunction.

Brownish or reddish discolouration of the urine (haemoglobinuria) was obvious on PID 2-4 in all dogs, regardless whether imidocarb treatment was applied or not. In the spleen intact dog **A**, the disease appeared to be self-limiting, and haemoglobinuria resolved spontaneously. Urinalysis from the discoloured samples demonstrated the presence of free haemoglobin and red blood cells. Lobetti and Jacobson (2001) studied renal involvement in babesiosis caused by *B. canis rossi*. Haemoglobinuria was the most consistent urinalysis alteration, but it did not predict acute renal failure in their clinical study. Renal tubular epithelial (RTE) cells were present in larger numbers in dogs having severe anaemia or complicated babesiosis. Tubular casts were not demonstrated in their study. Active sediment with RTE cells or casts suggesting acute renal injury were not seen in any of the urine samples during our investigations.

Abdominal ultrasonography on patients with canine babesiosis has not been reported previously, as to our knowledge. Typical findings of our cases included splenomegaly (in dog **A**), hepatomegaly with diffuse changes in the ultrasonographic appearance of the structure of the liver (all animals) and increased echogenicity of the renal cortices (dog **A**, $\mathbf{C_{SE}}$).

Aspiration cytology of the enlarged spleen showed a combination of factors in the background of splenomegaly: a marked immunologic reaction was obvious, and increased breakdown of lysed or infected erythrocytes also took place. Wozniak et al. (1997)

demonstrated expansion of the splenic white pulp and intensive erythrophagocytosis in the red pulp of the spleens of *B. gibsoni* infected dogs, similarly to our findings in *B. canis* infection.

Regarding hepatopathy, both serum ALT activity and morphologic evaluation with ultrasound signalled liver involvement in all animals. Nephropathy was suggested by elevated creatinine concentrations in dogs **A** and $\mathbf{B_{SE}}$, but ultrasound appearance of the kidneys was physiologic in dog $\mathbf{B_{SE}}$. Abdominal ultrasonography suggested abnormal renal morphology in dog $\mathbf{C_{SE}}$, but creatinine remained normal throughout the trial in this animal.

Single dose of imidocarb therapy (6 mg/kg IM) resulted in quick clinical improvement of the two treated infected dogs, although erythrocytes with parasites were visible for up to 3 days after the injection. This confirms the findings of Penzhorn et al. (1995), who found similar parasitaemic period after a single dose of imidocarb (7.5 mg/kg) in dogs experimentally infected with the South African strain of *B. canis*.

Both splenectomized dogs were affected by opportunistic infections, probably due to the absence of the spleen and immunosuppressive glucocorticoid therapy after the blood transfusions. In the blood of dog $\mathbf{B_{SE}}$, *H. canis* was identified several times. However, no clinical signs were related to this infection, and the dog has possibly been carrier of *Haemobartonella* before the experiment. Bacterial osteomyelitis caused by *Escherichia coli* developed in dog $\mathbf{C_{SE}}$, which was successfully treated with norfloxacin.

In conclusion the observed clinical signs and additional findings were rather heterogeneous in this experiment. This could be attributed to the low number of dogs involved in the study, and to the different way the splenectomized and intact animals were treated. The great individual variability of complications occurring in *B. canis* infections should also be considered. Altogether, the clinicopathological alterations reported in this work are probably more typical for the experimental setting, than for babesiosis in general.

However, the described protocol can be suggested for antigen production, enabling the infected dogs to survive.

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

Máthé, Á., Dobos-Kovács, M. and Vörös, K.: Histological and ultrastructural studies of renal lesions in dogs with Babesia canis infection and (partly) treated with imidocarb. Publication in process.

In this study our goal was to provide additional information on babesial nephropathy, therefore we performed histopathological and electron microscopic examinations from the kidneys of dogs suffering from *Babesia canis* infection and acute renal failure (ARF).

6.1. Materials and methods

The kidneys of 8 dogs having naturally acquired *B. canis* infection were examined in this study. Dogs after natural death or euthanasia were selected for this work if they had either biochemical data suggesting renal involvement (i.e. azotaemia), or if gross pathological findings were suspicious for nephropathy.

Seven of these animals were treated as patients of the Small Animal Clinic of the Veterinary Faculty, Budapest. *B. canis* infection was confirmed by visual evaluation of Dia-Panoptic (Diagon Kft., Budapest, Hungary) stained blood smears. Complete blood count was carried out by an automatic analyser (Abacus Haematological Analyser, Diatron Kft., Budapest, Hungary), while serum blood urea nitrogen (BUN) and creatinine concentrations were determined with an automatic spectrophotometer (Dr. Lange 400, Dr. Bruno Lange GmbH, Berlin, Germany). All of the 7 dogs were treated with 3-6 mg/kg imidocarb diproprionate (Imizol inj., Pitman-Moore Ltd., Middlesex, England) to eliminate the *Babesia* infection. Three out of 7 treated dogs were euthanized, 4/7 of the dogs died spontaneously in the small animal hospital.

The 8th dog included in this study was not treated previously. It died spontaneously and arrived for necropsy directly to the Pathology and Forensic Veterinary Medicine Department of the Veterinary Faculty, Budapest. *B. canis* infection was confirmed by impression smear examination of the spleen in this case, which revealed several large *Babesia* organisms within the erythrocytes.

For light microscopic evaluation kidney tissue samples of all 8 dogs were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned to 4-6 µm thick, mounted on glass slides and stained with haematoxylin and eosin.

For electron microscopic examination 1 x 1 x 3 mm blocks were prepared from the renal cortex of the 7 treated dogs as shortly as possible after death (usually within a few hours). Samples were immediately fixed in phosphate-buffered (pH 7.2) 4% paraformaldehyde and 0.2% glutaraldehyde at 4 ºC for 3 hours. The tissue samples were postfixed for 2 hours in phosphate-buffered 1% osmium tetraoxide at room temperature. Then the tissue blocks were dehydrated by passage through graded ethanol rinses and propylene oxide. Samples were imbedded in ACM Durcupan (Fluka AG, Switzerland) for 48 hours on 56 ºC. Ultrathin sections (40-60 nm) were cut (Reichert UM U3 ultramicrotome, Austria), mounted on copper grids, and stained with 2.5% uranyl acetate for 20 minutes and Reynolds' lead citrate for 10 minutes. The stained sections were examined by a JEOL JEM-1011 electron microscope (Japan) and photographed by a Gatan Bioscan 792 Multi.Scan CCD camera system (Germany).

6.2. Results

The mean age of 8 dogs involved in this study was 5,5 years (range 0.3 years to 10.7 years). Regarding the 7 treated animals, the average time elapsed from the onset of clinical signs to death/euthanasia was 7 days (range 2-14 days). From the application of imidocarb 4.5 days had passed on average (range 1-10 days) until death.

Laboratory results

Among the 7 treated animals, the mean red blood cell count was 4.6 T/l (range 2.1-6.8 T/l), the mean packed cell volume (PCV) was 0.28 l/l (range 0.13-0.42 l/l). Severe anaemia (i.e. PCV $\leq$ 0.2 l/l) was only present in two cases. The mean serum creatinine value of 6 animals yielded 582 µmol/l (range 334-871 µmol/l), while in the 7th dog it was not possible to determine the creatinine value due to the strong icterus, so BUN was measured, and found moderately elevated (10.7 mmol/l).

Gross pathologic examination

The blood of the dogs appeared watery with a slightly yellowish colour. A varying degree of pallor and jaundice was visible on mucous and serous membrane surfaces and in the subcutaneous tissue of the animals. Splenomegaly and dark reddish brown pigmentation of renal cortices were obvious in all dogs. Sub-epicardial and oral mucous membrane

petechiation was also present in some of the carcasses; rarely it was visible on other serous surfaces, as well.

Histopathological findings of the kidneys

The most pronounced alteration observed in the glomeruli was lack of red blood cells in the capillaries. Rarely protein rich ultrafiltrate was visible in the lumen of the Bowman's capsule (**Figure 6.1.A.**). In some animals protrusion of tubular epithelial cells was also present within the glomerular capsule. Calcification of the Bowman's capsule was noted in 2 dogs, probably as a consequence of uraemia.

Degenerative changes characterised the histological appearance of the renal tubuli. The proximal convulated tubuli were more seriously affected by either vacuolar-hydropic degeneration or necrosis of renal tubular epithelial (RTE) cells. In affected regions RTE cells showed karyopyknosis, karyolysis or hyperchromatosis of the nuclear membrane. Acidophil cell necrosis, detachment of RTE cells from the basement membrane could also be observed in some areas (**Figure 6.1.B.**). Necrotic debris occasionally formed homogenous acidophil casts within the tubuli. In some cases renal tubuli were homogeneously and poorly stained, suggesting necrosis of the whole tubulus (**Figure 6.1.C.**). Haemoglobin casts in the lumen of the tubuli were visible only in a few dogs. Rarely, haemoglobin panels were present, as well (**Figure 6.1.D.**). However, reabsorbed haemoglobin droplets seldom appeared and in only a few RTE cells. Calcium deposition was observed in the necrotic RTE cells of one dog. Renal tubuli and RTE cells contained bile granules in two dogs. Regeneration of RTE cells was seen in some tubuli. Occasionally focal interstitial oedema appeared in the samples.

Histopathological findings of the untreated animal were similar to the imidocarb treated dogs, and were characterised by massive tubular necrosis (**Figure 6.2.**).

There was no obvious relationship between the severity of lesions and age of dogs, duration of illness, time elapsed from imidocarb treatment or degree of azotaemia in the examined animals. In some cases various stages of degeneration were present in the same kidney.

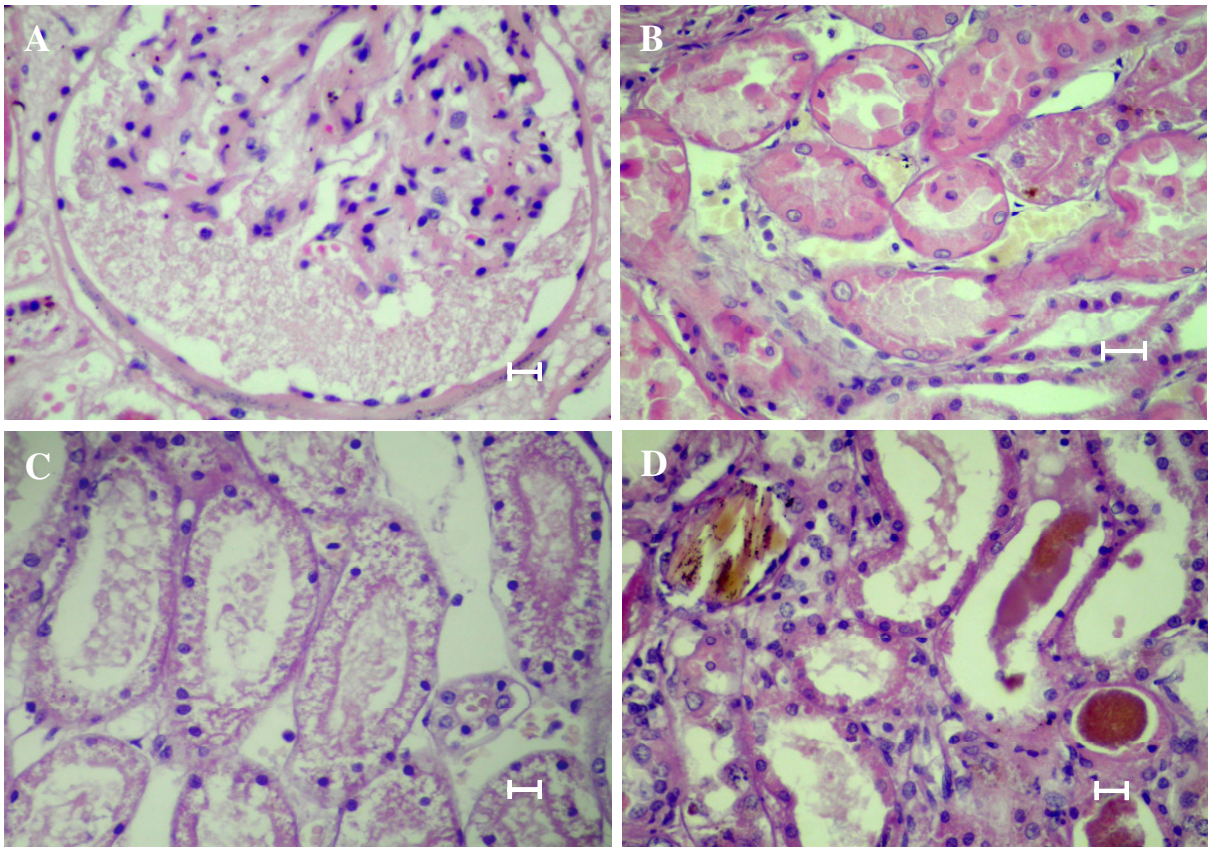

Figure 6.1. Renal sections of Babesia canis infected dogs treated with imidocarb.
(Haematoxylin and eosin stain, scale bar = 10 µm)
A. Cortical section showing absence of red blood cells in the glomerular capillaries. Protein rich ultrafiltrate is present in the lumen of the Bowman's capsule.
B. Various stages of degeneration are visible in renal tubular epithelial (RTE) cells ranging from vacuolar-hydropic degeneration to necrosis. Necrotizing RTE cells show karyopyknosis, karyolysis and acidophil cell necrosis. Detachment of RTE cells from the basement membrane can also be observed.
C. In some areas whole tubuli are necrotic. Debris of detached cells forms acidophil casts within the tubuli.
D. Haemoglobin casts can be observed in some tubuli, and there are haemoglobin panels in another one

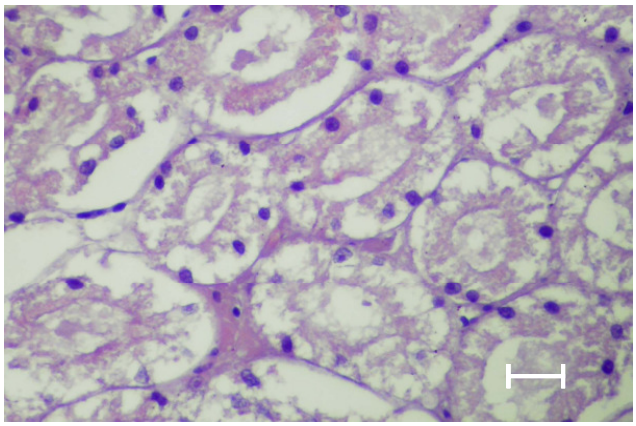

Figure 6.2. Renal section of the untreated *Babesia canis* infected dog demonstrating massive tubular necrosis
(Haematoxylin and eosin stain, scale bar = 10 µm)

Ultrastructural alterations of the kidneys

Degenerative nucleus alterations (nuclear membrane hyperchromatosis, pyknosis, and lysis) were seen in the RTE cells of almost all animals. Many times these cells collapsed, their electron-density was increased, and it was impossible to observe their structure (lesions compatible with acidophil cell necrosis).

The mitochondria of RTE cells showed turbid swelling in most of the animals: they were enlarged, their cristae were fragmented, and their electron-density was decreased. In other cases the mitochondria were collapsed: their structure degenerated and became more electron-dense (**Figure 6.3.**). Many times these organelles were visible in groups.

Vacuolar-hydropic degeneration manifested most often as water droplets in the folds of the endoplasmatic reticulum. However, sometimes hydropic degeneration also presented as nuclear oedema or water droplets in the microvilli (**Figure 6.4.**). The endoplasmatic reticulum was many times fragmented in the RTE cells. Disrupted cell membranes frequently appeared as whorled structures, resembling myelin sheaths (**Figure 6.5.**).

Both glomerular capillaries and the capillaries in the interstitium between renal tubuli were affected by degenerative endothelial cell damage: turbid swelling, vacuolar-hydropic degeneration and necrosis were observed. As a consequence, interstitial oedema between the tubuli was also present in some dogs (**Figure 6.6.**).

Haemoglobin casts or plates were very rarely seen in the renal tubuli. Absorbed haemoglobin droplets or bile granules were also infrequent findings.

Calcium salt deposits were detected in the glomerular capsule of one animal.

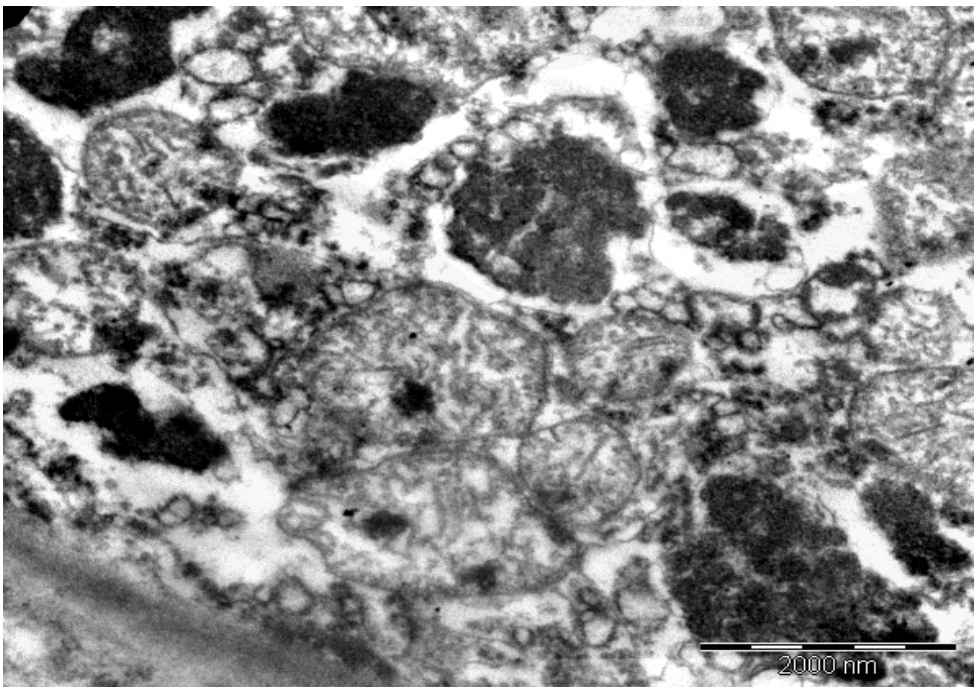

Figure 6.3. Electron microscopic image of the kidney in *B. canis* infection.
The mitochondria of renal tubular epithelial (RTE) cells are swollen, their cristae are fragmented, and their electron-density is decreased. Other mitochondria are collapsed: their structure degenerated and became more electron-dense

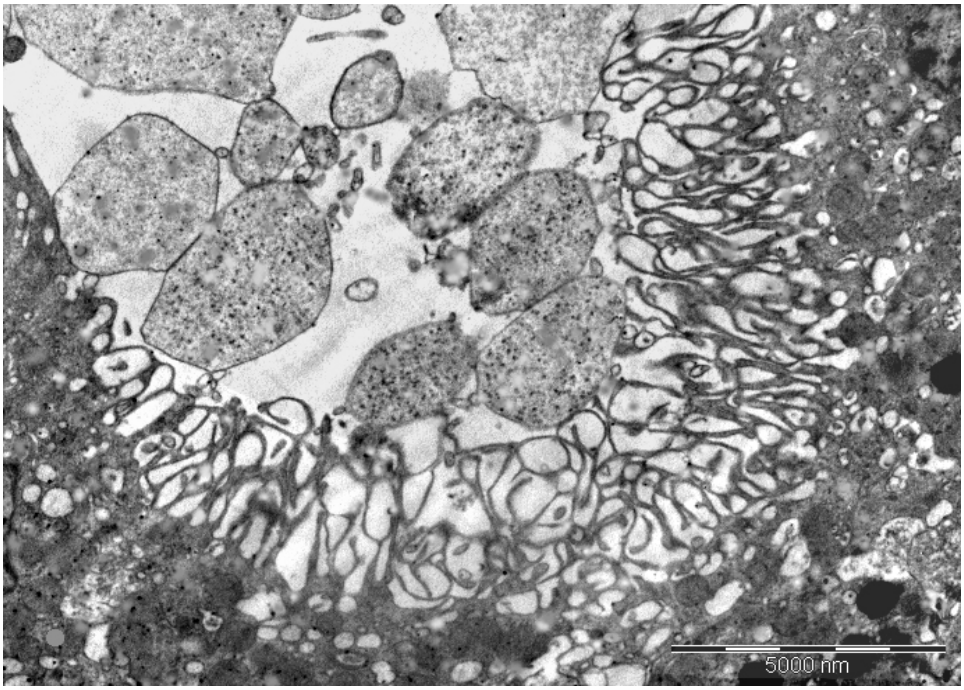

Figure 6.4. Electron microscopic image of the kidney in *B. canis* infection.
Vacuolar-hydropic degeneration causing swelling of the microvilli in RTE cells

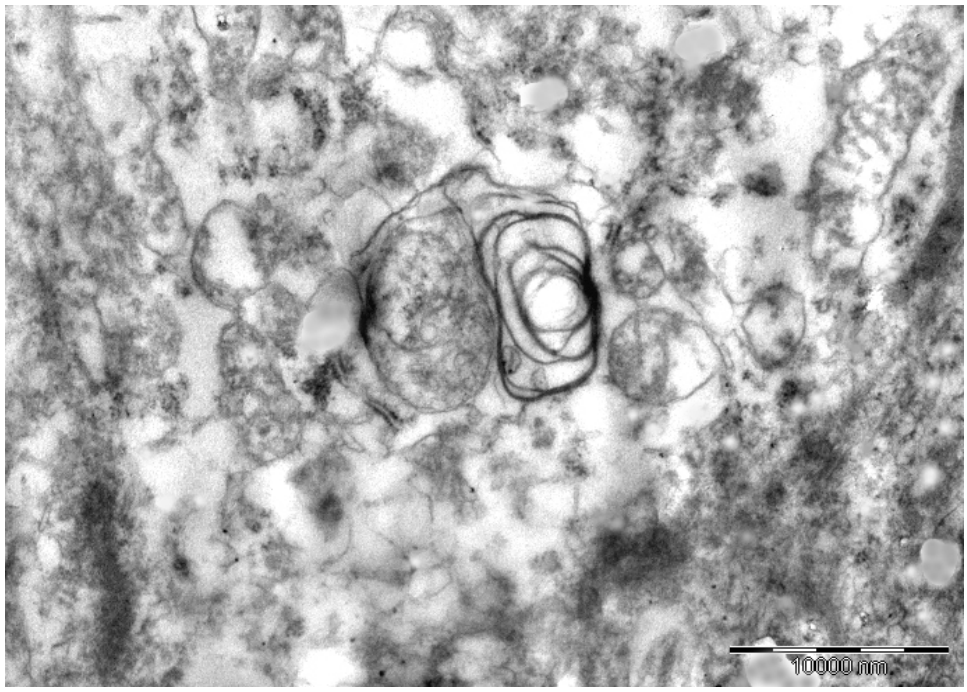

Figure 6.5. Electron microscopic image of the kidney in *B. canis* infection. Fragmented cell membrane appearing as a whorled structure

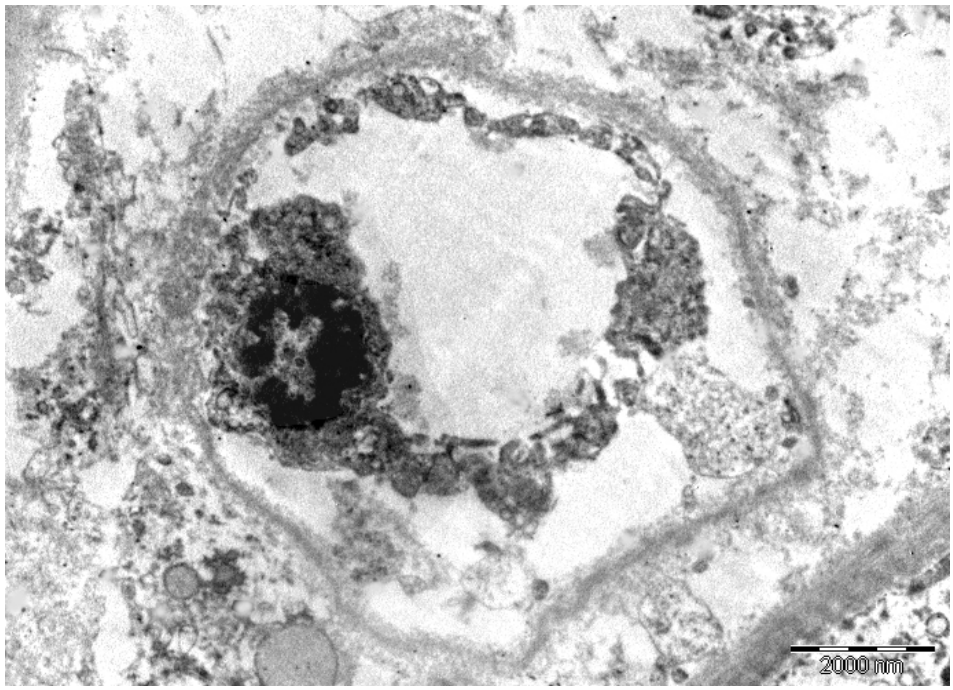

Figure 6.6. Electron microscopic image of the kidney in *B. canis* infection. Endothelial cell necrosis: karyopyknosis, increased electron-density with degeneration of organelles in the cytoplasm and detachment of the cell from the basement membrane are shown. Secondary interstitial oedema is also visible

6.3. Discussion

Gross pathological findings were very similar to those described by Irwin and Hutchinson (1991) in dogs naturally infected with *B. canis* in Australia.

Haemoglobinuric nephrosis was historically thought to cause nephropathy in babesiosis (Hildebrandt, 1981). It is characterised histologically by the presence of haemoglobin cast in the lumen and haemoglobin droplets in the RTE cells of proximal tubuli. This accumulation of proteinaceous material may lead to functional disability of RTE cells manifesting in ARF (Dobos-Kovács, 1988). Such alterations were only seen in the minority of our cases. Irwin and Hutchinson (1991) had similar findings; they detected haemoglobin resorption in RTE cells of 1/7 dogs having *B. canis* infection in Australia, while the dominating lesion was vacuolar degeneration of the proximal tubuli in their cases. The rarity of haemoglobin casts in our examination could be due to infusion and diuretic therapy of the dogs (i.e. haemoglobin might have been flushed off from the tubuli). The absence of haemoglobin droplets in RTE cells is perhaps due to an early functional deficit of the tubular epithelium preventing absorption of haemoglobin. Actually, severe haemoglobinaemia caused mild histological changes, but no significant nephropathy in dogs in an experimental model (Lobetti et al., 1996).

Histologically, degenerative changes of mainly the proximal renal tubuli were the most typical findings observed in this study. These included vacuolar-hydropic degeneration, karyopyknosis, -lysis and RTE cell necrosis, detachment of necrotic tubular cells from the basement membrane. Sometimes complete necrosis of the proximal tubulus was observed. The morphologic changes described are typical for hypoxic or toxic tubulonephrosis (Dobos-Kovács, 1988). However, the histological alterations were similar in dogs treated with imidocarb and the untreated animal, suggesting that renal pathology observed in our cases cannot be explained by the potential nephrotoxicity of imidocarb alone. Ultrastructural lesions were most prominent in the proximal tubuli, as well (degenerative nucleus and mitochondrion changes, cell necrosis, disruption of endoplasmatic reticulum and plasma membrane, hydropic swelling of microvilli), and suggested a similar aetiology. Hypoxia or toxicosis could also affect the vascular endothelium, consistent with our findings of degenerating glomerular and interstitial capillary endothelial cells.

There are many potential causes of tissue hypoxia in babesiosis, including severe anaemia, altered haemoglobin function, hypotension, pulmonary oedema, myocardial dysfunction, microvascular sequestration of parasitized erythrocytes, autoagglutination and disseminated intravascular coagulation (DIC) (Jacobson, 2006).

Severe anaemia was only present in 2/8 dogs examined in this study. Similar renal morphology was present in anaemic and non-anaemic individuals, suggesting that anaemic hypoxia is probably not the only cause of babesial nephropathy. Earlier Lobetti et al. (1996) described mild degenerative alterations of the renal tubular epithelium, without the development of serious ARF in dogs having artificially induced severe anaemia.

A qualitative deficit of haemoglobin can also occur with *B. canis* infections. The haemoglobin that remains in intact cells may function abnormally at tissue level, especially under acidic and hypercapnic conditions (Taylor et al., 1993). Other *Babesia* and *Plasmodium* species are known to contain enzymes that cleave haemoglobin. Preliminary electrophoretic studies with *B. canis* suggest that similar enzymes may be present in canine infections (Taylor and van Rensburg, 1995). It was not possible to investigate the altered function of haemoglobin in our cases.

Hypotension, a further possible cause for hypoxia, was present in half of the dogs suffering from complicated babesiosis in a South African study (Jacobson et al., 2000). It was suspected, that reduced vascular tone is the most common reason for decreased blood pressure. Hypotensive shock syndrome was described in a dog with *B. canis* infection earlier by Freeman et al. (1994). Recently, it has been demonstrated that acute myocardial damage also occurs during canine babesiosis, which could reduce cardiac output and cause hypotension (Lobetti, 2005). Acute tubular necrosis, typical of kidney shock syndrome was also observed in sheep experimentally infected with *B. ovis* (Habela et al., 1991). These observations make hypotension a likely cause of nephropathy in canine babesiosis. However, it is unknown whether animals involved in our investigation had lowered blood pressure, or not.

Various toxins could cause similar degenerative changes (i.e. tubulonephrosis) in the renal tubuli to those observed by us (Dobos-Kovács, 1988). Only mild tubular dilation and hydropic degeneration was present in kidney samples of dogs 5 days after experimental infusion of canine haemoglobin (Lobetti et al., 1996), so haemoglobin did not prove to be highly nephrotoxic.

Dosage dependent hepato- and nephrotoxicity of imidocarb was described earlier in cattle, horses and goats. Adams and Corrier (1980) reported on renal tubular necrosis and azotaemia developing in cattle repeatedly injected with 20 mg/kg imidocarb. Proteinaceous casts and regenerative epithelial hyperplasia were also visible 67 days post injection. In horses twice treated with 16 mg/kg imidocarb, azotaemia, diffuse acute cortical tubular necrosis and multifocal interstitial haemorrhage was described, mainly affecting the proximal convulated tubules 21 days post injection (Adams, 1981). Milder tubular lesions with proteinaceous casts

and regenerative epithelial hyperplasia were described at lower doses of imidocarb (4-8 mg/kg) in the same study. Healthy ponies were repeatedly injected with 4 mg/kg imidocarb in another trial (therapeutic dose), which resulted in mild azotaemia and increased urine γ-glutamyl transferase : creatinine ratios suggesting renal (tubular) dysfunction (Meyer et al., 2005). Corrier and Adams (1976, 1977) also described clinical and pathological changes in goats once treated with a lethal dose of 6.75 mg/kg imidocarb. The goats became azotaemic and died between postinjection day 4 and 8 due to acute necrosis of the proximal convulated tubuli. Proteinaceous casts and regeneration of the tubular epithelium were seen, as well. Ultrastructural renal lesions included plasma membrane disruption, swelling of mitochondria and fragmentation of their cristae in this study, resembling our observations in dogs suffering from babesiosis and treated with imidocarb.

However, there are few reports about the toxicity of imidocarb in the canine species. An accidental 10-fold overdose of imidocarb caused acute liver necrosis and death within a few hours due to hepatic failure in a dog (Kock and Kelly, 1991). Probably there was no time for the development of renal lesions in this case. One of 13 dogs used in a pharmacokinetic study died 8 days after receiving a single injection of 4 mg/kg imidocarb IV (Abdullah et al., 1984). The kidneys of the dog were grossly enlarged with extensive haemorrhage in the cortex and medulla. Marked tubulonephrosis with karyopyknosis and necrosis of RTE cells was exhibited microscopically. It is impossible to decide by morphologic evaluation, whether or not imidocarb therapy was detrimental to the kidneys of dogs having babesial nephropathy in our study. Histological and ultrastructural examinations from the kidneys and livers of dogs treated with different doses of imidocarb would be justified, in our opinion; especially that imidocarb is currently widely used for therapy and prevention of canine babesiosis world-wide. Until such results become available, it is probably wiser to use the lower dosage of imidocarb (i.e. 3 mg/kg) to control *Babesia* infection in patients suspected to have renal involvement. Furthermore, it is highly suggested to treat even mildly azotaemic dogs with proper intravenous fluid therapy simultaneously with imidocarb application, to decrease the risk of renal insufficiency.

Glomerular changes were mild and rare in the cases involved in this study. These included empty capillaries and sometimes the presence of proteinaceous ultrafiltrate within the Bowman's capsule. The latter observation is perhaps due to hypoxic damage of the capillary endothelium, resulting in dysfunction of the filtration barrier. Our observations regarding mild glomerular pathology are similar to those of Irwin and Hutchinson (1991) reporting on *B. canis* infection from Australia, but very different from those describing glomerulonephritis in infections with other *Babesia* species. Severe diffuse

membranoproliferative GN was found in one of 11 dogs having naturally acquired *B. gibsoni* infection in California, which was suspected as the result of chronic antigenic stimulation (Conrad et al., 1991). Membranoproliferative GN was also described in experimental *B. gibsoni* infections (Wozniak et al., 1997). Renal involvement also included tubular lesions in this latter study: the presence of haemoglobin droplets in RTE cells was noted in 4/6 dogs, without severe degenerative changes. Haemoglobin casts were rarely seen in their patients. *B. gibsoni* caused membranoproliferative GN in coyotes, as well, along with moderate tubulonephrosis (Roher et al., 1985). Endotheliomesangial GN was reported together with other renal lesions by Habela et al. (1991) in *B. ovis* infection of sheep. Both membranoproliferative GN and haemoglobinuric tubulonephrosis were described in Syrian hamsters experimentally infected with *B. microti* (Cullen and Levine, 1987). However, it is not known whether these lesions caused renal failure in the hamsters, or not. Although histological examinations were not performed, hypoalbuminaemia, hypercholesterolaemia, severe proteinuria and hyaline casts in the urine sediment suggested glomerular involvement in dogs infected with *Theileria annae*, a recently identified small piroplasma infecting dogs in Spain (García, 2006). These results suggest that *T. annae* might also cause GN in the dog; however, this should be further studied, in our opinion.

Moore and Williams (1979) reported on fibrin microthrombosis in the kidneys of 2 dogs suffering from babesiosis and having laboratory evidence of DIC. Urinalysis revealed renal involvement in these dogs (granular casts in the sediment and severe proteinuria), but these authors did not publish data about the presence or absence of azotaemia in their animals. In experimental *B. ovis* infection of sheep glomerular and interstitial haemorrhages were seen together with arterial thrombosis (Habela et al., 1991). DIC was suspected to be the end result of hypovolaemic shock in this experiment. Although macroscopic haemorrhages were occasionally visible in our cases, microthrombosis in the kidneys suggesting DIC was not observed.

In conclusion, histological and ultrastructural lesions compatible with haemoglobinuric nephrosis were rarely seen in our study, similarly to the findings of other researchers reporting on *B. canis* infections. Even when haemoglobinuric nephrosis occurs, the accumulation of haemoglobin droplets in the RTE cells and casts in the tubular lumen is unlikely to cause renal failure. The severe degeneration and necrosis of mainly proximal renal tubuli described in the present work are most probably the result of hypoxic renal injury. Systemic hypotension leading to vasoconstriction might be the most important cause of renal hypoxia in *B. canis* infections, but anaemia and alterations of haemoglobin may also contribute to inadequate oxygen supply. The possible presence and cause of hypotension

should be further studied in non-anaemic dogs developing ARF secondary to *B. canis* infection, and the role of haemoglobin dysfunction in babesial nephropathy should be investigated, as well. The potential nephrotoxicity of imidocarb resulting in acute tubulonephrosis has been reported in various species in different experimental settings. However, there are only sporadic reports on untoward effect of the drug in the canine species. Therefore, imidocarb should be applied with caution in dogs suspected to have nephropathy, until further data becomes available. Glomerular changes were insignificant in our current investigation, and very different from the inflammatory processes typical for the disease caused by *B. gibsoni* and sporadically reported in *B. ovis* or *B. microti* infections.

7. New scientific results

7.1. Clinical manifestations of canine babesiosis in Hungary

1. Similarly to French *Babesia canis* infections, most patients had babesiosis in the spring and autumn, 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 (ARF; 31%) and disseminated intravascular coagulation (DIC; 24%) were frequent complications, while immune-mediated haemolytic anaemia (10%), acute respiratory distress syndrome (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. Hepatopathy was common, while pancreatitis, severe ARF, ARDS and cerebral babesiosis were rare in *B. canis* infected South African dogs.
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 multiple organ dysfunction syndrome (MODS) more sensitively in this study than systemic inflammatory response syndrome (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.

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

9. 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.
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*.

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

13. 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.

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9. Author's publications in canine babesiosis

9.1. Full-text articles in referred scientific journals

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9.2. 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.

9.3. Academical reports

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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9.4. Award

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

10. Acknowledgements

I am grateful to all veterinarians and laboratory assistants who took part in the examinations and treatment of the patients involved in the retrospective study. The results reflect the work of approximately 40 people. Special thanks are due to Dóra Halmay DVM for the thorough examination of blood smears.

I also express my gratitude to Zoltán Diószegi DVM for taking the bone biopsy specimen and to Péter Vajdovich DVM, PhD for performing the cytological evaluations during the experimental infections. I would also like to thank the careful work of my colleagues, who were in charge of treating the experimental dogs. Many students helped with taking care of the dogs regularly, and providing a more comfortable environment for them. Special thanks are due to those students, who finally adopted the experimental animals.

The authors are also grateful to those colleagues who helped in the selection of appropriate cases for the study discussing renal pathology of babesiosis.

I owe a lot to my late teacher and colleague, László Papp DVM, who showed me all his little tricks in clinical examination. He inspired me a lot to become a clinician at the University. I will never forget his beaming character.

I wish to express my deep appreciation to my supervisor Prof. Károly Vörös, who taught me for the difficult profession of scientific writing and kept my belief alive when some projects collapsed.

And last but not least I am indebted to my loving family for their support over the years. The secure background they mean helped me a lot in completing this thesis.