Complications and risk factors regarding the outcomes of canine babesiosis in Central Europe – A retrospective analysis of 240 cases

ANJA STROBL[1], FRANK KÜNZEL[1], ALEXANDER TICHY[2] and MICHAEL LESCHNIK[1]*

[1] Department for Companion Animals, Small Animal Clinic, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210, Vienna, Austria

[2] Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Vienna, Austria

ORIGINAL ARTICLE

ABSTRACT

The purpose of this study was to investigate retrospectively the prevalence of the complicated and un-complicated forms of babesiosis and to evaluate various laboratory and clinical parameters of dogs infected with *Babesia canis* in order to assess their prognostic value regarding the outcomes of the disease. Medical records, complete blood count and serum biochemical analysis from the animal hospital information system of 240 dogs were reviewed and evaluated retrospectively. Binary logistic regression analysis was used to ascertain correlations between alterations in the obtained parameters and survival probability. The results showed that creatinine levels of more than 5 mg/dL and phosphate levels of more than 3 mmol/L have a highly significant link to death ($P \leq 0.001$). Albumin levels of <2.2 g/dL ($P = 0.003$) and a rectal body temperature below 38 °C ($P \leq 0.001$) may also serve as prognostic markers for the severity of the disease. If renal involvement was present, 33.9% of the dogs died, while 40.0% of the dogs died in the presence of pancreatitis. The parameters creatinine, phosphate, albumin and rectal temperature serve as reliable predictive markers of an increased risk of death in the case of an infection with *B. canis*.

INTRODUCTION

Canine babesiosis is a multisystemic, potentially life-threatening disease caused by the intra-erythrocytic protozoan *Babesia* spp. The piroplasm species detected in domestic dogs can be divided into large (*Babesia canis, Babesia vogeli, Babesia rossi, Babesia* spp.) and small (*Babesia gibsoni, Babesia vulpes* sp. nov., *Theileria equi, Theileria annulata*) organisms (Irwin, 2009). The dominant species found in Central Europe is *B. canis*, transmitted by the tick *Dermacentor reticulatus* (Máthé et al., 2006).

By invading red blood cells, the parasites activate various pathways which cause a combination of intra- and extravascular haemolysis. The destruction of erythrocytes is also supported by mechanisms such as the activation of the complement system, increased phagocytosis and oxidative stress. Haemolysis, hypotension, tissue hypoxia, vascular stasis and endogenously produced carbon monoxide lead to severe damage, especially in the kidneys or the central nervous system (Solano-Gallego and Baneth, 2011).

Typical clinical signs include fever, lethargy and abdominal pain, pale mucous membranes, jaundice and macroscopic haemoglobinuria (Máthé et al., 2006). However, clinical signs are often non-specific. The most common alterations found in blood analysis are anaemia, thrombocytopenia, leukopenia, and later in the course of infection, increased levels of reticulocytes (Kirtz et al., 2012).

*Corresponding author.
Tel.: +43 1250776862.
E-mail: michael.leschnik@vetmeduni.ac.at

Furthermore, infection can be classified into complicated and uncomplicated babesiosis. The uncomplicated form leads to anaemia and fever, whereas complicated babesiosis shows multi-organ involvement with an increased risk of death (Máthé et al., 2006). Typical organ involvements seen in complicated babesiosis are renal involvement, hepatopathy, pancreatitis, neurological signs, immune-mediated haemolytic anaemia (IMHA), acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), and disseminated intravascular coagulopathy (DIC) (Máthé et al., 2006).

The diagnosis of canine babesiosis in Central Europe is becoming increasingly important as the lethality range varies from 1.5 to 20%. The highest lethality rates, caused by infection with B. canis are reported in the central parts and in the northeast of Europe (12–20%). In the southwest of Europe, particular in Italy, France, Spain and Portugal, the lethality rate is less than 5%. These differences are hypothesised as being the result of the genetic diversity of B. canis strains, and the variable virulence of these strains (Carcy et al., 2015).

Concerning prognostic markers, Eichenberg et al. (2016) showed that in B. canis infections in Switzerland, dogs with severe thrombocytopenia, mild to moderate leukopenia, hyperlactataemia, hyperphosphataemia or hypoproteinaemia are more likely to die, compared to patients without these alterations. Nevertheless, the results should be interpreted with caution, due to the small sample size of 18 dogs in this study and the relatively high lethality rate.

The first aim of this retrospective study was to investigate the prevalence of the complicated and uncomplicated forms of babesiosis in dogs presented at the University of Veterinary Medicine, Vienna and to analyse the impact of each complication on the survival rate. The second aim was to evaluate the various laboratory and clinical parameters of dogs infected with B. canis to assess their predictive value regarding the outcome of the disease. Additionally, we calculated how the survival rate changed depending on an increase or decrease of the examined parameter.

MATERIALS AND METHODS

Two hundred and forty cases of babesiosis diagnosed at the University of Veterinary Medicine, Vienna were evaluated retrospectively. A total of 227 dogs of different breeds and of both sexes were presented between September 2001 and April 2017. In total, eleven dogs appeared twice, and one dog appeared three times as they showed assumed reinfections. The outcome is included in the results. The study population contained 177 (73.7%) purebreds and 63 (26.2%) mixed breeds. Among the 142 (59.2%) males, 114 were intact and 28 were castrated. Among the 98 (40.8%) females, 47 were intact and 51 were neutered. The median age was five years (range: 3 months to 15 years) and the median bodyweight was 25 kg (range: 2.8–60 kg). Concerning their travel history, 97 (40.4%) of the patients had travelled across Austrian borders, with the majority ($n = 71/97$, 73.2%) reported to have travelled to Western Hungary.

Inclusion criteria were a Babesia infection confirmed via blood smear and/or PCR. In dogs that were presented to the hospital several times, the median period between two infections was three years. In all 240 cases (100.0%) infection with B. canis was confirmed via blood smear, while in 52/240 cases (21.7%) the infection was further proven via PCR. PCR was done for B. canis and Anaplasma phagocytophilum and was carried out as described by Leschnik et al. (2012). Additionally, the dogs were divided into complicated and uncomplicated cases. The criteria for complicated babesiosis are presented in Table 1.

Specific treatment took the form of oxopirvedine (recommended dose: 15 mg/kg) or imidocarb dipropionate (recommended dose: 6.6 mg/kg). Oxopirvedine had been used until approximately 2001, as this was the medicine of choice, and the only medication available in treating canine babesiosis. After 2001, oxopirvedine was increasingly replaced by imidocarb dipropionate. Moreover, all dogs received intravenous fluid therapy (Ringer's solution or lactated Ringer's solution) and gastroprotective medication (omeprazol 1 mg/kg, intravenously or orally). Patients with abdominal pain (e.g. in cases of pancreatitis) received tramadol (2–4 mg/kg, intravenously or orally), buprenorphine (10–20 µg/kg, intravenously) or lidocaine via pump infusion. To control vomiting, patients were treated with maropitant (1 mg/kg, intravenously) or ondansetron (0.1–0.5 mg/kg, intravenously). Blood or packed cell volume (PCV) transfusions were administered if the haematocrit fell below 15%.

Table 1. Criteria used for classification (all of the classifications were used before in other surveys)

Complications	Criteria
Renal involvement	Persistently elevated creatinine, despite fluid therapy (Jacobson, 2006)
Pancreatitis	Confirmed sonographic alterations in combination with painful abdomen and/or vomitus (Máthé et al., 2006)
Hepatopathy	At least twofold alteration of ALT, without any other clinical signs (Máthé et al., 2006)
SIRS	At least three of the following alterations, one of which must be abnormal temperature or leukocyte count: Rectal temperature >39.2 °C or <37.8 °C, pulse rate >140 beats/min, respiratory rate >40 breaths/min, WBC count >12,000/µL or <4,000/µL or >10% band neutrophils (Matijatko et al., 2009; Wong and Wilkins, 2015)
IMHA	Positive Coombs test and autoagglutination (Jacobson, 2006) (tested via saline agglutination test)
Cerebral babesiosis	Neurological signs that cannot be attributed to any other cause (Goddard et al., 2013). Cerebral babesiosis was ruled out if dogs showed hypoglycaemia or were previously known as epileptic patients. Additionally, neurological signs were first observed during infection and did not return after successful therapy

ALT = alanine aminotransferase; WBC = white blood cell; SIRS = systemic inflammatory response syndrome; IMHA = immune-mediated haemolytic anaemia.

Blood samples

The following parameters were assessed: complete blood count (CBC), creatinine (CREA), total protein (TP), albumin, alkaline phosphatase (AP), alanine aminotransferase (ALT), urea, phosphate, sodium, calcium, potassium, chloride and glucose. Not all parameters were obtained for each dog.

Blood samples were taken at first admission and before any treatment. The CBC was performed using the haematology analyser Advia® 2120i, while biochemical analysis was performed using the mid-volume analyser Cobas® c501. A blood smear, stained with Diff-Quick, was done in all dogs to establish the diagnosis.

Statistical analysis

By binary logistic regression analysis, we evaluated how the probability of survival changed according to the concentrations of, or the alterations in, specific parameters. These parameters were selected because they were obtained for most of the dogs involved, and appeared to be most promising, according to our descriptive statistics and other surveys (Eichenberger et al., 2016; Máthé et al., 2006). The data of the logistic regression analysis are provided in figure and table format.

Chi-square tests were used to assess the significance of the regression analysis results. Furthermore, they were used to test the significance of a predisposition with regard to purebreds, to assess the significance of the relationship between the presence of complications and the survival rate, and to assess if specific anti-babesial treatment had a significant impact on survival.

Odds ratios and 95% confidence intervals (CI) were calculated for the survival probability in terms of the different complications, defined as the ratio of the odds of survival when the complication was present to those if it was absent. Bivariate Pearson correlation analysis was used to evaluate if sodium levels correlated inversely with creatinine levels.

All analyses were performed using IBM SPSS v24. For all analyses, a P value below 5% ($P < 0.05$) was defined as significant.

RESULTS

Study population and clinical signs

A total of 66,903 purebred and 21,292 mixed-breed dogs were presented to the hospital between 2001 and 2017. To evaluate if canine babesiosis was more common among purebreds than mixed breeds, both sets of data were compared, using Chi-square test [X^2 (df = 1) = 0.002; P = 0.969], but no significant predisposition could be found.

Clinical signs at admission were as follows: anorexia was found in 205/240 (85.4%) dogs, 139/240 (57.9%) dogs showed lethargy, 123/240 (51.2%) dogs showed discoloured urine and 68/240 (28.3%) dogs showed gastrointestinal signs. Moreover, 72/240 (30.0%) of the dogs had pale mucous membranes, and 37/240 (15.4%) dogs showed jaundice. None of the dogs showed ecchymosis, petechiae or spontaneous bleeding.

Ataxia was reported in 7/240 (2.9%) dogs, and 2/240 (0.8%) dogs showed at least one generalised seizure during hospitalisation. The median rectal temperature was 39.3 °C (range: 35.6 °C–41.1 °C). In total, 23 dogs were treated with oxopirvedine, and four of them died (17.4%, CI = 1.9–32.9%), 217 dogs received imidocarb, and 20 of them died (9.2%, CI = 5.4–13.1%). The Chi-square test showed no significant difference in survival between patients who received oxopirvedine or imidocarb ($P = 0.214$, odds ratio = 2.074).

Blood analysis

In all 240 dogs, the presence of the tear-shaped parasites within erythrocytes was confirmed via blood smear. PCR was performed in 52/240 dogs (21.7%) and in all those dogs infection with *B. canis* was confirmed. Only two dogs (0.8%) showed a positive PCR for *A. phagocytophilum*. Anaemia was observed in 167/228 dogs (73.2%). All 209/209 (100.0%) cases showed thrombocytopenia, 122/213 dogs (57.3%) showed leukopenia and 83/195 dogs (42.6%) showed neutropenia. All haematological alterations are displayed in Table 2.

The blood of 7/240 dogs (2.9%) was suspicious for macroscopic autoagglutination. Further diagnostics were done via saline test autoagglutination and Coombs test, and IMHA was confirmed in 5/7 dogs (71.4%).

Regarding biochemical analysis, 89/223 (39.9%) dogs showed increased levels of creatinine; elevated levels of urea were found in 86/124 (69.3%) dogs. Hypoproteinaemia was found in 108/205 (52.7%) patients, while the elevation of liver enzymes was only mild and unspecific [elevated values of ALT were found in 63/177 (35.6%) dogs, and elevated values of AP were found in 64/137 (46.7%) dogs]. Consequently, further evaluation of these enzymes was excluded from the regression analysis. The most common changes in electrolytes were as follows: hyponatraemia was found in 53/102 (52.0%) dogs, while 20/72 (27.8%) dogs showed hypochloraemia and 26/136 (19.1%) showed hypokalaemia. No significant inverse correlation was found between concentrations of sodium and concentrations of creatinine ($P = 0.101$; $P = 0.318$). All biochemical alterations are displayed in Table 3.

Complicated babesiosis

Complicated babesiosis was diagnosed in 116 dogs (48.3%), with 89 of these patients (76.7%) suffering from a single complication, whereas multiple complications were seen in 27 dogs (23.3%).

SIRS was the main complication ($n = 65$, 56.0%), followed by renal involvement ($n = 56$, 48.3%), pancreatitis ($n = 10$, 8.6%), cerebral babesiosis ($n = 9$, 7.8%), IMHA ($n = 5$, 4.3%) and hepatopathy ($n = 4$, 3.4 %). The Chi-square test showed significant differences in the frequency of complications [X^2 (df = 3) = 59.3; $P \le 0.01$]. Renal involvement and pancreatitis had the poorest prognosis: 33.9% of the patients died when renal involvement was present ($P \le 0.001$) and 40% of the dogs died when pancreatitis was diagnosed ($P = 0.001$). Lethality was not significantly increased with regard to the other complications, but due to the small sample sizes, the results should be interpreted with caution (Table 4).

Table 2. Haematological parameters

Haematological parameters	↑	↓	~	Total number of dogs	Mean ± SD	Minimum–maximum range	Reference values
Erythrocytes (μL)	0	149	47	196	4.71 ± 1.21	1.39–7.25	5.5–8
Haematocrit (%)	0	167	61	228	31.72 ± 8.06	9.0–50.0	37–55
Haemoglobin (g/dL)	1	83	68	152	11.31 ± 3.33	3.1–32.2	12–18
MCV (fL)	2	10	138	150	65.93 ± 4.13	51–80.9	60–77
MCH (pg)	62	2	83	147	23.69 ± 1.67	11.3–28.7	19–24
MCHC (g/dL)	131	1	19	151	35.99 ± 2.27	19.4–43.2	31–34
Reticulocytes/μL	25	1	9	35	55,488.6 ± 88,864.22	5,200–425,952	6,000–15,000
Leukocytes/μL	16	122	75	213	6,990.94 ± 4,906.60	740–33,000	6,000–15,000
Lymphocytes/μL	14	64	118	196	1,711.70 ± 1,705.46	97.6–14,731.4	780–4,500
Band cells/μL	10	0	121	131	133.32 ± 469.31	0–4,200	0–500
Neutrophils/μL	10	83	102	195	4,734.58 ± 4,432.25	362.6–44,110.6	3,300–11,250
Mononuclear leukocytes/μL	63	0	99	162	617.81 ± 782.64	0–6,893.1	0–500
Eosinophils/μL	0	0	150	150	46.93 ± 88.42	0–700	0–800
Basophils/μL	5	0	132	137	20.92 ± 39.37	0–201.24	0–150
Thrombocytes 10^3/μL	0	209	0	209	35.36 ± 23.23	0–116	150–500

↑: Number of dogs with parameters above reference values; ↓: Number of dogs with parameters below reference values; ~ Number of dogs within reference values; Mean ± SD and minimum–maximum range of all dogs.

Table 3. Biochemical parameters

Biochemical parameters	↑	↓	~	Total number of dogs	Mean ± SD	Minimum–maximum range	Reference values
Creatinine (mg/dL)	89	2	132	223	1.66 ± 1.75	0.2–10.9	0.4–1.2
Total protein (g/dL)	4	108	93	205	5.83 ± 0.89	3.3–8	6–7.5
Albumin (g/dL)	0	50	56	106	2.56 ± 0.54	0.37–3.9	2.58–4.73
AP (U/L)	64	0	73	137	148.52 ± 92.98	20–650	0–130
ALT (U/L)	63	0	114	177	88.61 ± 87.63	12–563	0–80
Total bilirubin (mg/dL)	38	0	30	68	4.03 ± 8,03	0.15–38.2	0–0.8
Urea (mg/dL)	86	15	23	124	105.075 ± 109.37	9–546.8	20–40
Phosphate (mmol/L)	38	1	39	78	1.91 ± 1.05	0.5–5.73	0.8–1.6
Calcium (mmol/L)	0	1	67	68	2.345 ± 0.21	1.99–2.9	2–3
Sodium (mmol/L)	0	53	49	102	140.63 ± 7.15	119–160	142–164
Chloride (mmol/L)	20	3	72	95	108.08 ± 7.53	72–122	95–113
Potassium (mmol/L)	6	26	104	136	3.90 ± 0.60	2.4–5.5	3.5–5
Glucose (mg/dL)	61	0	78	139	101.21 ± 22.40	59–228	55–100

↑: Number of dogs with parameters above reference values; ↓: Number of dogs with parameters below reference values; ~ Number of dogs within reference values; Mean ± SD and minimum–maximum range of all dogs.

Table 4. Impact of the different complications on lethality, and significance and odds ratio with a confidence interval of 95%

Complication	Odds ratio	Significance	X^2	df	95% CI
SIRS	0.583	0.226	1.47	1	0.24–1.41
Renal involvement	0.054	0.000	46.47	1	0.02–0.15
Pancreatitis	0.143	0.001	10.43	1	0.04–0.55
Cerebral babesiosis	0.200	0.174	5.66	1	0.05–0.86
IMHA	0.434	0.451	0.57	1	0.05–4.05
Hepatopathy	–	0.501	0.501	1	–

df = degree of freedom.

Table 5. Outcome if complications were absent or present

		Presence of complications		Total
		No	Yes	
Outcome complications	Number of dogs that died	0	24	24
	Number of recovered dogs	124	92	216
Total		124	116	240

Contingency tables, analysed by the use of the Chi-square test, showed that lethality was significantly associated with the presence of complications ($P < 0.001$) (Table 5).

Binary logistic regression analysis

An increase of creatinine from 3.0 mg/dL to 5.0 mg/dL let the survival rate drop from 80 to 40%. Creatinine levels above 6.0 mg/dL led to a survival rate below 20% (Fig. 1A).

Although concentrations of five times elevated urea (200 mg/dL) still come along with a survival probability of 80%, high levels of urea are significantly associated with lethality ($P \leq 0.001$) (Fig. 1B).

Serum phosphate behaves in a manner similar to serum creatinine, and therefore seems to be a very good prognostic marker ($P \leq 0.001$). Levels above 3 mmol/L correlate with poor outcomes (Fig. 1C).

Due to the narrow range of survival, sodium is not thought to be a significant prognostic marker ($P = 0.769$). The lowest measured level of 118 mmol/L still suggests a survival rate of more than 74% (Fig. 1D).

No significant correlation between survival and levels of serum total protein could be observed ($P = 0.387$). Survival rate varies between 85 and 95%; moreover, severe hypoproteinaemia (3.3 g/dL) is still linked to an estimated survival probability of 85% (Fig. 2A).

In contrast to total protein, serum albumin seems to be a better indicator of disease severity. A decrease in albumin is associated with an increased probability of death ($P = 0.003$). If concentrations are below 2.2 g/dL, the survival probability drops from 83 to 10% (Fig. 2B).

The severity of anaemia is not linked to death ($P = 0.196$). Even if haematocrit falls below 20%, the survival probability still varies between 86 and 82% (Fig. 3A).

Leukopenia does not seem to be prognostic ($P = 0.185$) as a number of 500 leukocytes/μL is still related to a survival probability of more than 90%. Only high numbers of leukocytes seem to be linked to a drop in survival probability (Fig. 3B).

Our findings suggest severe thrombocytopenia to be more favourable for survival than mild to moderate thrombocytopenia ($P = 0.002$). Counts over 20,000 platelets/μL are associated with a drop in the survival probability from 92 to 52% (Fig. 3C).
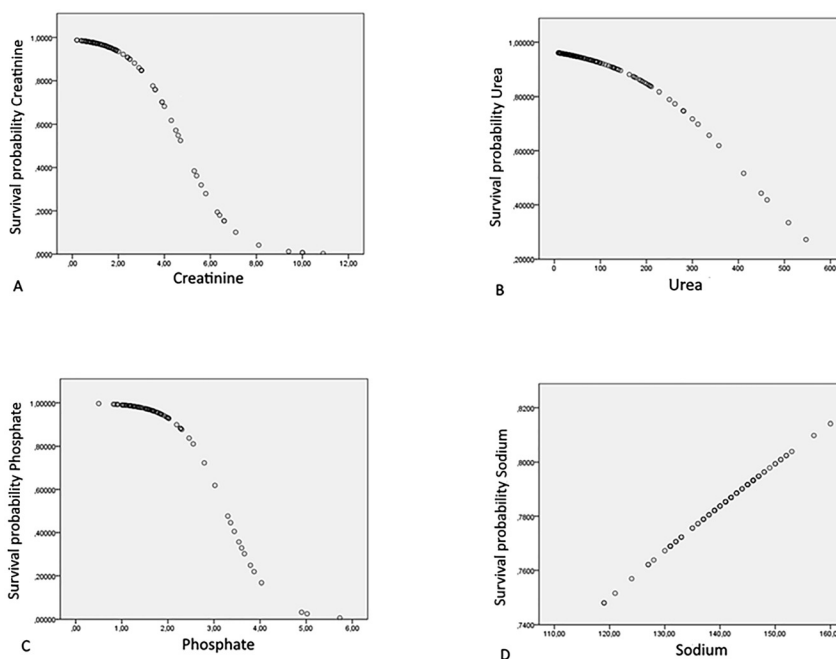

Fig. 1. Renal function. Expected survival rate depending on different concentrations of serum creatinine (A), urea (B), serum phosphate (C) and serum sodium (D)
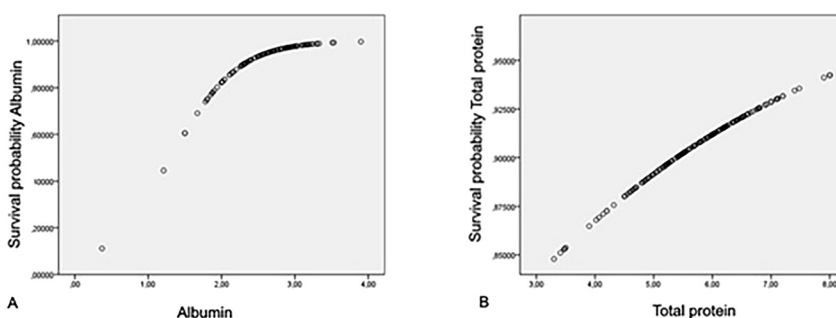

Fig. 2. Biochemical alterations. Expected survival rate depending on different concentrations of serum total protein (A) and serum albumin (B)
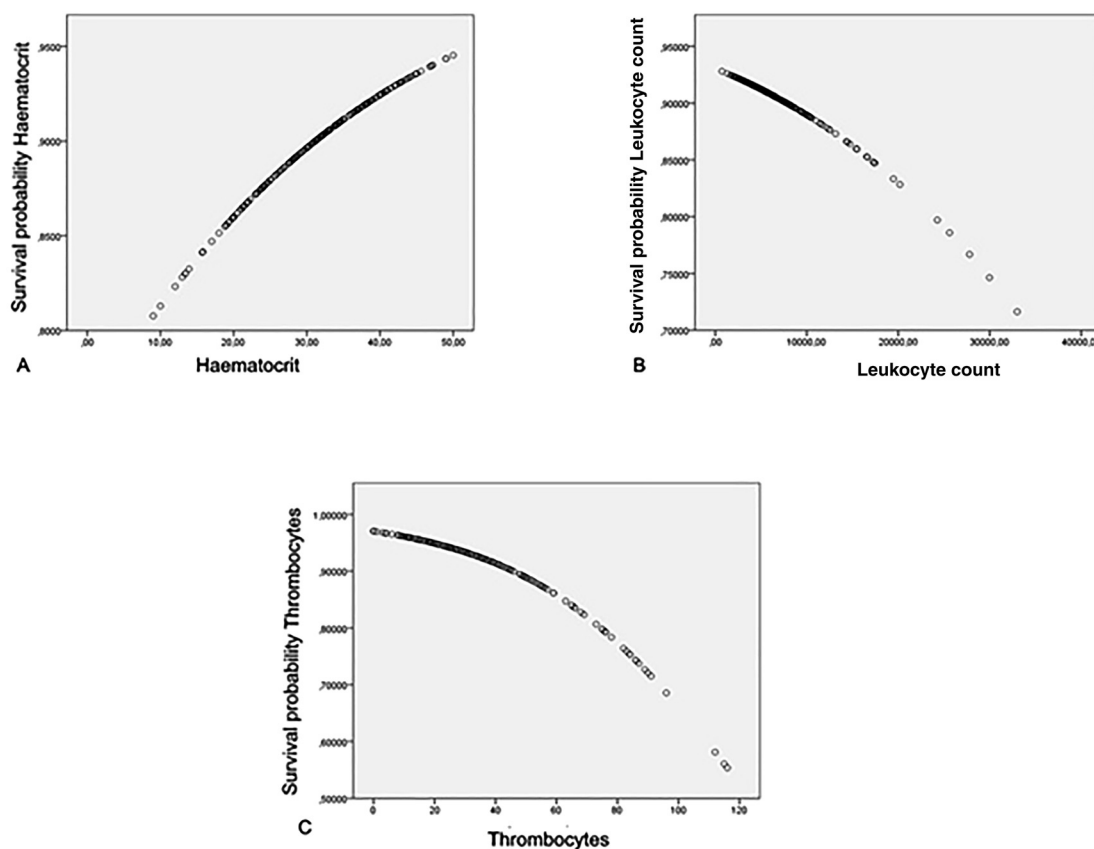
Fig. 3. Haematological abnormalities. Expected survival rate depending on different concentrations of haematocrit (A), leukocyte count (B) and thrombocyte count (C)
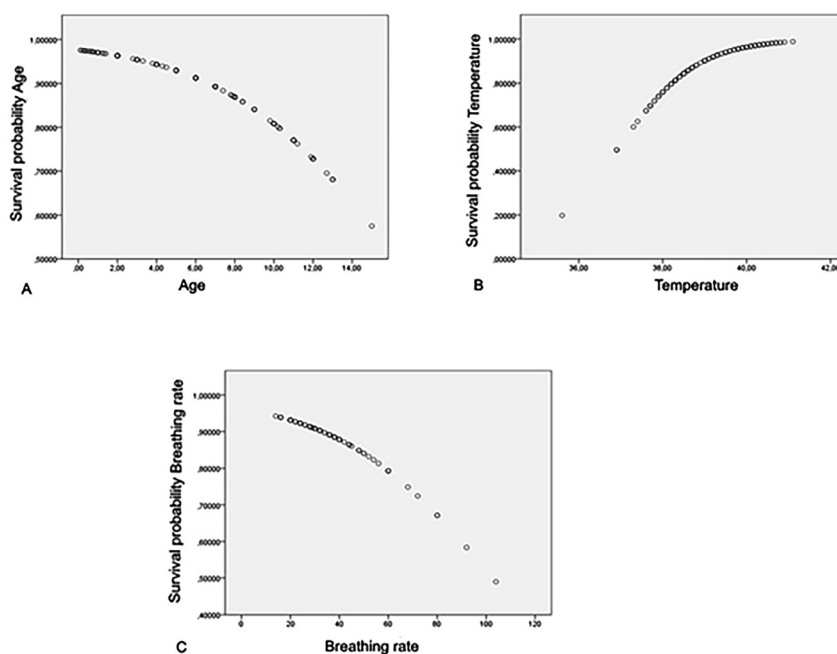

Fig. 4. Clinical signs. Expected survival rate depending on different ages (A), different values of inner body temperature (B) and different breathing rates (C)

Older dogs were at a greater risk of death due to babesiosis than younger dogs ($P = 0.001$). Between six and 14 years, the survival rate varied from 88 to 52% (Fig. 4A). Dogs of more than eight years old had a survival probability between 80 and 55%.

Hypothermia correlates significantly with poor outcome ($P \leq 0.001$). An inner body temperature of 41 °C still comes along with nearly 100% survival probability. If the body temperature fell below 38 °C,

Table 6. Expected survival probability according to the different levels of the specific parameters

Parameter	Survival probability					
	5%	25%	50%	75%	90%	95%
Creatinine (mg/dL)	7.9	6.0	4.8	3.6	2.5	1.7
Urea (mg/dL)	775	545	410	270	135	40
Phosphate (mmol/L)	4.7	3.8	3.25	2.72	2.2	1.8
Haematocrit (%)					31	54
Thrombocytes/µL	230,000	164,000	125,000	85,000	47,000	20,000
Age					6.6	3.3
Temperature (°C)	34.15	35.85	36.9	37.05	39.0	39.65
Breathing rate/min	200	140	103	68	35	10

Table 7. Odds ratio and significance for the data of the regression analysis

Parameter	Odds ratio	Significance
Creatinine	0.386	0.000
Urea	0.992	0.000
Phosphate	0.127	0.000
Sodium	1.010	0.769
Haematocrit	1.035	0.196
Leukocytes	1.000	0.185
Thrombocytes	0.972	0.002
Albumin	9.213	0.003
Total protein	1.258	0.387
Age	0.797	0.001
Temperature	2.894	0.000
Breathing rate	0.969	0.042

the survival probability dropped from 70 to 20% (Fig. 4B).

If the breathing rate exceeded 30 breaths/min, the survival rate dropped from 90 to 45%, and therefore the breathing rate is associated with lethality (P = 0.042) (Fig. 4C).

The data assessed in the logistic regression analysis are additionally provided in table format. Not all parameters are provided, as not all survival rate figures range from 0 to 100%, and some calculated probabilities are not in the possible biological range for dogs (Table 6).

The odds ratios and significance of the parameters mentioned above are displayed in Table 7 – creatinine, urea, phosphate and body temperature had the best prognostic significance regarding the survival rate, followed by age, thrombocytes and albumin.

DISCUSSION

In this study, significant differences in clinical and laboratory findings were observed between survivors and non-survivors in the event of an infection with *B. canis*. The parameters involved are therefore considered to have significant prognostic relevance. Furthermore, the survival rate changed significantly depending on the presence and the type of the complications involved.

Concerning the biochemical analysis, increased levels of serum creatinine, serum phosphate and urea indicate an increased risk of death, and serve as reliable negative prognostic markers. Those parameters are highly indicative of reduced glomerular filtration rate (GFR). The term 'renal dysfunction' seems to be more fitting in the case of canine babesiosis than acute renal failure (Kuleš et al., 2018). Tissue hypoxia, systemic hypotension and haemoglobinuria seem to be favourable reasons for transitory renal involvement, leading to hyperphosphataemia and elevated concentrations of creatinine and urea. The dysfunction seems to be a result of presumed glomerular damage and proximal tubular damage (Kuleš et al., 2018). Interestingly, in experimentally infected dogs, some showed decreased levels of creatinine at the very beginning of the infection, resulting from presumed dilution due to water retention, but in our study only two dogs showed decreased serum creatinine concentrations (Schetters, 2019). Perhaps the moment when dogs show clinical signs and are taken to hospital is not the early beginning of the infection, and this is why we see low creatinine values relatively infrequently in the course of babesiosis.

Concerning hyperphosphataemia, other mechanisms leading to increased levels of serum phosphate are lactic acidosis and high glucocorticoid levels (Stockham and Scott, 2008). Matijatko et al. (2014) demonstrated that lethality was significantly elevated in infected dogs with high cortisol concentrations. High levels of glucocorticoids may lead to hyperphosphataemia, bearing in mind that both alterations are risk factors for death. A further explanation for hyperphosphataemia could be lactic acidosis, although the mechanisms are not fully understood yet (Stockham and Scott, 2008). Hyperlactataemia correlates with a poor outcome, as does hyperphosphataemia (Eichenberger et al., 2016).

Other factors assessed for their prognostic value were sodium and total protein, but they do not seem to be of prognostic value at all. Hyponatraemia does not result from loss via the kidneys, thus Zygner et al. (2012) suggest that decreased sodium levels are caused by dilution. Hypotension and reduced renal perfusion stimulate the renin–angiotensin–aldosterone system, which triggers the retention of sodium and water, and decreased water excretion leads to the dilution of sodium. The same would be appropriate for hypoproteinaemia, which may also be a result of hypotension and dilution (Schetters et al., 2009).

Albumin is demonstrated to be a better prognostic indicator than total protein. One possible explanation could be

glomerular dysfunction, as microalbuminuria is often reported in dogs with early glomerular dysfunction and urine albumin is supposed to be a reliable marker for this damage (Kuleš et al., 2018). In our study, renal involvement was a marker for poor prognosis, which supports the assumption. Additionally, albumin is known as a negative acute phase protein, which is decreased in concentration in the course of an infection, and seems to be indicative of an acute phase response, suggesting that the level of hypoalbuminaemia correlates with disease severity (Eckersall and Bell, 2010).

In dogs with babesiosis, haematocrit levels are often low. Due to the acute onset of the infection, anaemia is normocytic and normochromic, suggesting that it is non-regenerative, but later in the course of the disease it becomes regenerative; these results are in accordance with the findings of another Austrian study concerning the diagnosis of canine babesiosis in Central Europe (Kirtz et al., 2012). Haematocrit does not serve as a prognostic marker, which could be explained by the fact that the level of anaemia and the level of parasitaemia do not correlate (Nel et al., 2004).

Regarding the white blood cell (WBC) count, leukopenia does not serve as a reliable indicator of increased risk of death. Leukopenia is associated with SIRS, a common complication in canine babesiosis. The decreased leukocyte counts may also result from possible sequestration in the spleen (Máthé et al., 2006). Leukocytosis linked to a higher risk of lethality could be the response to a relatively longer duration of the infection, or to co-infections or co-morbidities which may weaken the dog's organism.

The most unexpected observation was made regarding the platelet count: as expected, all patients suffered from thrombocytopenia, and no dog showed signs of haemorrhage. Interestingly, patients with more severe thrombocytopenia were more likely to survive than those with milder alterations. The explanation for our findings could be a statistical artefact due to the current way in which statistical analysis is applied. All thrombocyte counts were below the reference values. Some of the dogs survived, although they showed very low thrombocyte counts, and some of the non-survivors had more thrombocytes than the survivors, but these counts were still below the reference values. This creates the impression that non-survivors had a milder thrombocytopenia than did the survivors.

After successful therapy, the thrombocyte counts of the patients included in our study began to increase within three or four days (data not shown). Therefore, the most likely reasons for thrombocytopenia might be destruction or sequestration. DIC, another reason for thrombocytopenia, is not a frequent complication in canine babesiosis in Europe (Furlanello et al., 2005).

Various studies show that spontaneous bleeding is not a common complication in canine babesiosis in Europe. This is consistent with our findings, presuming that alterations in clotting times, if present, and thrombocytopenia are not clinically relevant in the event of an infection with B. canis (Furlanello et al., 2005; Solano-Gallego and Baneth, 2011).

Another factor included in the analysis was age. Although it is known that younger dogs are more likely to show excessive immune reactions and to develop a severe form of babesiosis, they seem to recover more easily than older dogs. Our results suggest that older dogs are at a higher risk of death than younger dogs. They may additionally suffer from other systemic diseases which weaken the immune system, or generally show some age-related reduced immune response which may influence the outcome (Máthé et al., 2006; Solano-Gallego et al., 2016).

The study showed a negative correlation of survival rate with low rectal temperature and high breathing rate. Although these are no marked alterations, they could be suggestive of SIRS or shock. In our study, dogs with hypothermia were at a greater risk of death than were dogs with hyperthermia. The reason could be that hypothermia is more indicative of shock, followed by multi-organ dysfunction (Wong and Wilkins, 2015).

Dogs without complications were more likely to recover than those which developed complicated babesiosis. Only a few dogs suffered from pancreatitis, but according to our statistical analysis these dogs had the lowest probability of survival. Pancreatitis may be the result of hypotensive shock, leading to septic shock and death (Matijatko et al., 2009). Concerning the survival probability, the same is true for renal involvement. These findings are comparable to those from other authors (Máthé et al., 2006).

Although not in all dogs, the blood smear findings of large Babesia species were additionally confirmed with PCR. Consequently, it is quite likely that all dogs suffered from B. canis infection. The majority of the dogs in our study population had a history of staying in Austria or travelling to Hungary, both of which are endemic areas for B. canis and its vector D. reticulatus (Solano-Gallego et al., 2016). Furthermore, Rhipicephalus sanguineus is mostly active from spring to early autumn, whereas D. reticulatus shows its main activity in March and in October (Cacció et al., 2002; Duscher et al., 2013). Therefore, it is unlikely that one of the dogs, for which PCR is missing, suffered from B. vogeli infection.

Two dogs with clinical babesiosis were also PCR positive for A. phagocytophilum. Both dogs survived. However, co-infections of B. canis with A. phagocytophilum are rare, as the vector of the latter, Ixodes ricinus, shows different peaks of activity (April/May and September) compared to D. reticulatus (Duscher et al., 2013).

Eleven dogs were presented twice, and one dog three times – these dogs were supposed to have had reinfections because, in all cases, successful therapy was confirmed via negative Babesia spp. PCR in a follow-up examination about one week after hospitalisation.

Several studies indicate that purebreds are more likely to suffer from canine babesiosis than mixed breeds (Martinod et al., 1986; Hornok et al., 2006). Also, in the present study, purebreds were detected more often than mixed breeds, but compared to the hospital population in the same period, there was no difference in the number of purebreds, hence it was not possible to say whether or not purebreds are more likely to suffer from canine babesiosis.

This study has some limitations. First, as this is a retrospective study, not all parameters were assessed for each

patient. As a result, the numbers of complications in the form of IMHA, hepatopathy or pancreatitis in particular might be too low, and more dogs might have suffered from these complications. Second, the results of urine and venous blood gas analysis were excluded due to the small sample size. Third, we did not have data about parameters such as lactate or cortisol, given that they could have some prognostic relevance. Consequently, the addition of these missing data could help expand and specify the spectrum of prognostic factors regarding infections with *B. canis*.

In conclusion, canine babesiosis caused by *B. canis* can be a multisystemic disease if complications are present. Systemic inflammatory response syndrome, renal involvement and pancreatitis were the most common complications in our study. Increased levels of creatinine, hyperphosphataemia, hypoalbuminaemia, and hypothermia seem to be the best predictors of a poor outcome; these parameters can be obtained quickly. Dogs suffering from complications are at a greater risk of death compared with dogs having uncomplicated babesiosis. Taken together, the results of this study demonstrate that several laboratory and clinical parameters serve as reliable prognostic markers of canine babesiosis.

REFERENCES

Cacció, S. M., Antunovic, B., Moretti, A., Mangili, V., Marinculic, A., Rafaj Baric, R., Slemenda, S. B. and Pieniazek, N. J. (2002): Molecular characterisation of *Babesia canis canis* and *Babesia canis vogeli* from naturally infected European dogs. Vet. Parasitol. **106**, 285–292.

Carcy, B., Randazzo, S., Depoix, D., Adaszek, L., Cardoso, L. and Baneth, G. (2015): Classification of *Babesia canis* strains in Europe based on polymorphism of the Bc28.1-gene from the *Babesia canis* Bc28 multigene family. Vet. Parasitol. **211**, 111–123.

Duscher, G., Feiler, A., Leschnik, M. and Joachim, A. (2013): Seasonal and spatial distribution of ixodid tick species feeding on naturally infested dogs from Eastern Austria and the influence of acaricides/repellents on these parameters. Parasit. Vectors **6**, 76.

Eckersall, P. D. and Bell, R. (2010): Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Vet. J. **185**, 23–27.

Eichenberger, R. M., Riond, B., Willi, B., Hofmann-Lehmann, R. and Deplazes, P. (2016): Prognostic markers in acute *Babesia canis* infections. J. Vet. Intern. Med. **30**, 174–182.

Furlanello, T., Fiorio, F., Caldin, M., Lubas, G. and Solano-Gallego, L. (2005): Clinicopathological findings in naturally occurring cases of babesiosis caused by large form *Babesia* from dogs of northeastern Italy. Vet. Parasitol. **134**, 77–85.

Goddard, A., Wiinberg, B., Schoeman, J. P., Kristensen, A. T. and Kjelgaard-Hansen, M. (2013): Mortality in virulent canine babesiosis is associated with a consumptive coagulopathy. Vet. J. **196**, 213–217.

Hornok, S., Edelhofer, R. and Farkas, R. (2006): Seroprevalence of canine babesiosis in Hungary suggesting breed predisposition. Parasitol. Res. **99**, 638–642.

Irwin, P. J. (2009): Canine babesiosis: from molecular taxonomy to control. Parasit. Vectors **2** (Suppl. 1)**,** S4.

Jacobson, L. S. (2006): The South African form of severe and complicated canine babesiosis: clinical advances 1994–2004. Vet. Parasitol. **138**, 126–139.

Kirtz, G., Leschnik, M., Hooijberg, E. H., Tichy, A. and Leidinger, E. (2012): In-clinic laboratory diagnosis of canine babesiosis (*Babesia canis canis*) for veterinary practitioners in Central Europe. Tierärztl. Prax. **40**, 87–94.

Kuleš, J., Bilić, P., Beer Ljubić, B., Gotić, J., Crnogaj, M., Brkljačić, M. and Mrljak, V. (2018): Glomerular and tubular kidney damage markers in canine babesiosis caused by *Babesia canis*. Ticks Tick Borne Dis. **9**, 1508–1517.

Leschnik, M. W., Khanakah, G., Duscher, G., Wille-Piazzai, W., Hörweg, C., Joachim, A. and Stanek, G. (2012): Species, developmental stage and infection with microbial pathogens of engorged ticks removed from dogs and questing ticks. Med. Vet. Entomol. **26**, 440–446.

Martinod, S., Laurent, N. and Moreau, Y. (1986): Resistance and immunity of dogs against *Babesia canis* in an endemic area. Vet. Parasitol. **19**, 245–254.

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

Matijatko, V., Kis, I., Torti, M., Brkljacić, M., Kucer, N. and Baric Rafaj, R. (2009): Septic shock in canine babesiosis. Vet. Parasitol. **162**, 263–270.

Matijatko, V., Torti, M., Kiš, I., Šmit, I., Štoković, I., Vranješ-Đurić, T., Milanović, S., Mrljak, V. and Brkljačić, M. (2014): Serum cortisol and insulin concentrations in dogs naturally infected with *Babesia canis*. Vet. Arh. **84**, 551–562.

Nel, M., Lobetti, R. G., Keller, N. and Thompson, P. N. (2004): Prognostic value of blood lactate, blood glucose, and hematocrit in canine babesiosis. J. Vet. Int. Med. **18**, 471–476.

Schetters, T. (2019): Mechanisms involved in the persistence of *Babesia canis* infection in dogs. Pathogens **8**, 94.

Schetters, T. P., Kleuskens, J. A., Van De Crommert, J., De Leeuw, P. W., Finizio, A. L. and Gorenflot, A. (2009): Systemic inflammatory responses in dogs experimentally infected with *Babesia canis*; a haematological study. Vet. Parasitol. **162**, 7–15.

Solano-Gallego, L. and Baneth, G. (2011): Babesiosis in dogs and cats – expanding parasitological and clinical spectra. Vet. Parasitol. **181**, 48–60.

Solano-Gallego, L., Sainz, Á., Roura, X., Estrada-Peña, A. and Miró, G. (2016): A review of canine babesiosis: the European perspective. Parasit. Vectors **9**, 336.

Stockham, S. L. and Scott, M. A. (2008): Calcium, phosphorus, magnesium, and their regulatory hormones. In: Stockham, S. L. and Scott, M. A. (eds) Fundamentals of Veterinary Clinical Pathology. 2nd edition. Wiley-Blackwell, Oxford. pp. 539–638.

Wong, D. M. and Wilkins, P. A. (2015): Defining the systemic inflammatory response syndrome in equine neonates. Vet. Clin. North Am. Equine Pract. **31**, 463–481.

Zygner, W., Gójska-Zygner, O. and Wędrychowicz, H. (2012): Strong monovalent electrolyte imbalances in serum of dogs infected with *Babesia canis*. Ticks Tick Borne Dis. **3**, 107–113.