Thrombocytopenia in canine babesiosis and its clinical usefulness

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ABSTRACT
Canine babesiosis is a common cause of thrombocytopenia but there are few formal studies that have investigated this haematological finding in dogs. Thrombocyte counts from full blood counts were retrospectively analysed for the years 1996–2002. Thrombocyte counts and mean platelet volumes of dogs with babesiosis were compared with those of dogs, seen over the same period of time, that did not have babesiosis. There were 1162 cases in the Babesiosis group and 10 808 in the Non-babesiosis group. A frequency distribution of the thrombocyte counts showed a trimodal distribution in the Non-babesiosis group compared to a bimodal distribution in the Babesiosis group, with a strong positive skewness. The modes for the frequency distributions were 10, 40, 500 and 10, 35 × 10⁹ thrombocytes, respectively. The median thrombocyte count in the Babesiosis group was 14 × 10⁹ and 282 × 10⁹ in the Non-babesiosis group. There was a statistically significant difference in the median thrombocyte count between the Babesiosis group and the Non-babesiosis group. In the Babesiosis group, 99% of the thrombocyte counts were below the lower reference range value (250 × 10⁹) and 62% of thrombocyte counts were below 25 × 10⁹. The mean platelet volume (11.1 fl) for the Babesiosis group was greater than the reference range (6–10 fl) and significantly larger than in the Non-babesiosis group (median 9.7 fl). Thrombocyte counts greater than 110 and 250 × 10⁹ had a predictive value that the dog was not suffering from babesiosis of 99.3% and 99.8%, respectively. There was a statistically significant difference between the thrombocyte counts of dogs with babesiosis when grouped by parasitaemia scores. The mechanisms of the thrombocytopenia are not fully understood, and multiple mechanisms, including concomitant thrombocytopenia-inducing diseases such as ehrlichiosis, probably result in this haematological finding. Babesiosis in the South African canine population is associated with thrombocytopenia in nearly all patients and is severe in the majority of them. In the absence of thrombocytopenia, babesiosis is an unlikely diagnosis.

Key words: babesiosis, Babesia canis, mean platelet volume, negative predictive value, parasitaemia, thrombocytopenia.

INTRODUCTION
Babesiosis of dogs is a tick-transmitted intracellular haemoprotozoal disease that is commonly diagnosed at the Onderstepoort Veterinary Academic Hospital (OVAH) and accounts for approximately 11% of hospital admissions each year. Canine babesiosis is caused by Babesia gibsoni and Babesia canis. In South Africa it is the subtype Babesia canis rossi that causes disease. Thrombocytopenia is well described in animal embryos, calves, lambs, ewes, cattle, and specifically canine (and specifically Babesia canis spp) Babesiosis. Thrombocytopenia is also associated with other haemoprotozoal diseases, such as malaria and trypanosomosis. It is unknown whether thrombocytopenia is associated with the degree of red blood cell parasitaemia or severity of disease in babesiosis. While it appears that the degree of anaemia, and severity of disease, is not determined by the level of parasitaemia in natural expression of B. c. rossi, others have found an association between the parasitaemia and B. c. rossi disease severity, although the same authors could not find such an association in B. c. canis. There is a small experimental study that showed an association between infection with Babesia parasitaemia and thrombocytopenia in dogs co-infected with Ehrlichia canis. There are also limited data that suggest that the severity of disease influences the degree of thrombocytopenia. Thrombocytopenia is commonly recognised at the OVAH in dogs with babesiosis and is often severe, with thrombocyte counts lower than 50 × 10⁹/µl being common. This does not appear to be the case for Babesia infections in cats.

Babesiosis is easily diagnosed by identifying the Babesia canis parasites on thin blood film stained with a Romanowsky-type stain and should not provide a diagnostic dilemma. Red blood cell parasitaemia varies from minimal to grossly obvious. However, on occasions, patients present with clinical signs typical of babesiosis (fever with pale mucus membranes, anaemia, splenomegaly, listlessness) and are suspected to be infected with B. canis, but where the diagnosis cannot be confirmed on multiple thin blood smear evaluation. In these patients, it is possible that the parasitaemia is very low or the patient is presented before the presence of significant parasitaemia. Such patients must then be closely monitored and repeat blood film evaluations periodically performed. While polymerase chain reaction (PCR) assay is the gold standard for confirming a diagnosis of subclinical canine babesiosis in the absence of visible parasitaemia, this diagnostic test may not be available and is potentially unhelpful by virtue of the time delay while awaiting results. It would be beneficial to have a test that could help predict whether these borderline cases have B. canis infections or not, thereby providing ease of mind to both owner and clinician that this potentially acutely fatal disease is not present. It has been the subjective assessment of the clinicians at the OVAH that in the absence of thrombocytopenia, babesiosis is unlikely (i.e. the assumption of a high predictive value of a negative result has been used intuitively).

The predictive value of a negative test result is the probability of the disease being absent with a negative test. This value is dependent on the sensitivity and prevalence of the disease and is calculated as the number of true negative test results as a proportion of all negative test results. Receiver operator characteristic (ROC) curves graphically display the full spectra of true positive ratios (sensitivity), relative to the false positive ratio (1-specificity), for a particular test and are usually used to compare different tests employed to diagnose a condition.
Dogs for which a blood film evaluation was positive for babesiosis 2 weeks or more after the initial diagnosis and treatment, were assumed to be reinfected or relapsed, and their data were included in the study.

Mean platelet volume data/response patterns were also evaluated. Only dogs with platelet counts below the laboratory’s reference range (250–500 × 10⁹/L) were included in the MPV analysis, as the release of larger platelets into circulation is expected to occur during thrombocytopenia.

The Kolmogorov-Smirnov test was used to test for normality. Non-parametric data were compared using the Mann-Whitney rank sum test (P < 0.001) for pairwise comparisons and the Kruskal-Wallis 1-way ANOVA on ranks for multiple group comparisons. The significance level, P, was set at 0.001. Statistical analyses were performed using SigmaStat (Jandel Corporation, Chicago). The ROC was plotted in Excel (Microsoft Corporation, Redmond, USA).

Four statistical analyses were conducted: 1) descriptive and comparative statistics were performed on the 2 groups of dogs (Babesiosis and Non-babesiosis) for both thrombocyte counts and 2) MPV; 3) the sensitivity and specificity of using thrombocyte counts for diagnostic purposes by ROC analysis; 4) evaluation of the association between parasitaemia and the thrombocyte count.

RESULTS

There were 11 970 canine FBC records with thrombocyte counts: 1162 in the Babesiosis group and 10 808 in the Non-babesiosis group. Of the records in the Babesiosis group, 600 (52 %) had parasitaemia scores. There were 130 MPV results for the Babesiosis group, and 1422 for the Non-babesiosis group (for which the platelet counts were <250 × 10⁹/L). The low number of MPV results is due to the Cell-Dyn analyser not providing MPV data in cases where the platelet histogram did not meet the expected non-log normal distribution (Cell-Dyn 3700 Manual, November 2000, Abbott Laboratories, Santa Clara, USA).

Thrombocyte counts

A frequency distribution of the thrombocyte counts showed a trimodal distribution in the Non-babesiosis group compared to a bimodal distribution in the Babesiosis group, but with a strong positive skewness (Fig. 1). The distribution patterns for both groups in the thrombocytopenic range were remarkably similar. The modes for the 2 groups were: 10, 40, 300 and 10, 35 × 10⁹/L thrombocytes, respectively, there being essentially no cases in the Babesiosis group to produce a recognisable distribution pattern for counts >150. The thrombocyte count distribution for both groups failed the test for normality.

Babesiosis group: the platelet count ranged from 0 to 721 × 10⁹/L. The median was 14 × 10⁹/L (mean was 34.6 × 10⁹/L). Ninety nine percent (1148/1162) of the babesiosis dogs had a thrombocyte count below the lower reference range value (250 × 10⁹/L). Sixty two percent of thrombocyte counts were lower than 25 × 10⁹/L.

Non-babesiosis group: the thrombocyte count range was 0 to 1429 × 10⁹/L, with a median of 282 × 10⁹/L (mean of 288 × 10⁹/L). Forty percent of the patients had a thrombocyte count below the reference range and 7 % of thrombocyte counts were lower than 25 × 10⁹/L.

There was a statistically significant difference in the median thrombocyte count between the 2 groups.

Mean platelet volume

Babesiosis group: the MPV data were normally distributed. The MPV ranged from 6.7–16.4 fl, with both a mean and median of 11.1 fl. The standard deviation was 1.7 fl.

Non-babesiosis group: the MPV data failed the test for normality. The MPV ranged from 4.2–22.7 fl and had a median of 9.7 fl (mean 10.2 fl).

There was a statistically significant difference in the median MPV between the 2 groups. Linear regression analysis with MPV on platelet counts was performed (Fig. 2). The Babesiosis group had a weak correlation with a positive regression line (R = 0.170), although there was insufficient power (Power = 0.49, alpha = 0.05) to draw any conclusions. The Non-babesiosis group had a weak (although significant) correlation, with a negative slope of the MPV on platelet count regression line (R = 0.219, Power = 1).

Sensitivity and specificity

A ROC was plotted for the range of thrombocyte counts that would be diagnostic for babesiosis (Fig. 3). For a sensitivity of 95 %, the thrombocyte count was 110 × 10⁹/L. At the lower end of the reference range (250 × 10⁹/L), sensitivity was 99 %. The negative predictive values for
babesiosis were 99.3 % and 99.8 % at a thrombocyte counts of 110 and 250 × 10^9/, respectively, while the positive predictive values were 35.6 % and 20.1 %, respectively.

**Parasitaemia**

The parasitaemia was subjectively quantified from 1 to 5 (least to most) in 600 cases (N_1=196; N_2=230; N_3=96; N_4=51; N_5=27). None of the 5 groups were distributed normally. The median thrombocyte counts were 16.4, 10.8, 13.5, 9.0 and 8.6 × 10^9/, respectively (Fig. 4). The ANOVA demonstrated a statistically significant difference in the median values among the 5 groups. No assessment of the parasitaemia was made in the remaining 562 patients. A comparison between the pooled group of 600 patients and the non-scored group, showed a significant difference in the median value: 12.3 and 17.0 × 10^9/, respectively.

**DISCUSSION**

The Babesiosis group accounted for 9.5 % of all the FBC counts performed, which is similar to the previously reported 11 % incidence^29^ of canine babesiosis at the OVAH. The reference range for thrombocytes in dogs at the Clinical Pathology laboratory is 250–500 × 10^9/. Ninety-nine percent (1148/1162) of patients in the Babesiosis group had a platelet count lower than 250 × 10^9/ and this is in agreement with the literature in that dogs with babesiosis are thrombocytopenic^17,20,32^. These results also support the subjective assessment that the majority of canine babesiosis cases at the OVAH are thrombocytopenic and provides objective prevalence data. Dogs with babesiosis had significantly fewer thrombocytes than the Non-babesiosis group. Sixty two percent of thrombocyte counts in the Babesiosis group were lower than 25 × 10^9/.
10/\text{\(L\)}, supporting the 2nd observation that severe thrombocytopenia is common in canine babesiosis diagnosed at the OVAH. There is some evidence in the literature that the severity of disease may influence the degree of thrombocytopenia. In 1 study, dogs with mild B. canis infections were less thrombocytopenic than those with severe clinical disease. \textsuperscript{21} Babesia gibsoni infections cause less severe disease than B. canis. In a B. gibsoni study\textsuperscript{17}, thrombocytopenia was present, with a mean value that was far higher than the study's results (mean: 155 × 10\textsuperscript{9}/\(L\), range: 35–375 × 10\textsuperscript{9}/\(L\)). Only 1 of the 18 dogs in that study\textsuperscript{17} was clinically ill at the time of diagnosis. Thrombocytopenia in the absence of visible parasitaemia may occur. Eight of the 18 polymerase chain reaction assay (PCR) B. gibsoni-positive dogs were microscopically negative on blood smear evaluation.\textsuperscript{17} The incidence and severity of thrombocytopenia may be species dependent. In cats infected with Babesia felis, thrombocytopenia appears to be an inconsistent finding and was confirmed to be present in only 25 % of cats, unlike the 99 % prevalence found in this population.

The median (and mean) MPV in the Babesiosis group was greater than our laboratory’s reference range (6–10 fl) as well as greater than the median of the Non-babesiosis group. Similar findings have been reported elsewhere in babesiosis\textsuperscript{15} and malaria.\textsuperscript{4,13} It suggests that the body is responding to the thrombocytopenia with release of larger platelets. It is surprising that the MPV of the Non-babesiosis group was within reference range, as thrombocytopenia should stimulate the release of platelets with larger volumes.\textsuperscript{14} The negative MPV-thrombocyte regression line of the Non-babesiosis group does, however, reflect this as one would predict. The apparent contradiction is probably due to the large spread of platelet sizes (Fig. 2), and that these data represent a multitude of disease processes. Similarly, one would expect a negative regression line in the Babesiosis group, which was not the case (Fig. 2). However, there was insufficient statistical power to be certain of these results.

The thrombocyte counts proved to be remarkably sensitive for babesiosis. The ROC was employed in this report to graphically display the sensitivity and specificity of using thrombocytopenia as a diagnostic test for babesiosis. The sensitivity was 95 % at a thrombocyte threshold value of 110 × 10\textsuperscript{9}/\(L\). At the lower end of the reference range (250 × 10\textsuperscript{9}/\(L\)), the sensitivity was 99 %. At thrombocyte threshold values of 110 × 10\textsuperscript{9}/\(L\) and 250 × 10\textsuperscript{9}/\(L\), the negative predictive value was 99.3 % and 99.8 %, respectively. Therefore, at platelet counts of above 250 × 10\textsuperscript{9}/\(L\), one is 99.8 % certain (at the prevalence of babesiosis at the OVAH) that babesiosis is not present, and another cause for illness should be sought. While the disease is common at the OVAH, in other parts of the world it is rare. Thrombocytopenia is not specific for babesiosis and therefore the observation does not assist in making a diagnosis of babesiosis, nor helps differentiate it from dogs with thrombocytopenia caused by other diseases such as ehrlichiosis or immune-mediated conditions. This is reflected by the low positive predictive values (35.6 % and 20.1 % at thrombocyte counts of 110 and 250 × 10\textsuperscript{9}/\(L\), respectively) and is graphically illustrated in the histograms for both groups (Fig. 1). At the thrombocytopenic tail of the histogram, the Babesiosis and Non-babesiosis groups are remarkably similar. Thrombocytopenia will need to precede diagnostically significant parasitaemia (i.e. B. canis seen on blood smear), if the negative predictive value is to be diagnostically helpful. This does appear to be the case,\textsuperscript{17} but may not always be so. In a study of bovine
babesiosis, thrombocytopenia was seen initially after experimental infection with *Babesia bovis*, before thrombocytopenia was noted\(^3\). However, these patients were splenectomised and the trial was performed soon (2 weeks) after the splenectomy. Both surgical blood loss and splenectomy can cause a reactive thrombocytosis\(^1\). An initial thrombocytosis was also seen in 1 *B. caballi* study\(^1\). This may explain the thrombocytosis outliers seen in the Babesiosis group. Despite this, the negative predictive value is unlikely to be challenged by these other findings. The platelet count decreased within 4 days after infection in both cited studies\(^1,3\), well within the 10–21 day incubation period seen in *Babesia canis* infections\(^4\).

The mechanism of the thrombocytopenia seen in babesiosis is not clear. Falciparum malaria and babesiosis appear, to a degree, to share a common pathogenesis\(^5\). Some of the mechanisms described for *Plasmodium*-induced thrombocytopenia may therefore apply to *Babesia* infection. Thrombocytopenia may result from platelet destruction (both immunological and non-immunological, and increased utilisation), increased sequestration, or decreased production\(^6\). All 3 processes have been described in either babesiosis or malaria. The mechanism of inducing thrombocytopenia in babesiosis may not be specific to *Babesia* spp., but rather to the inflammatory response that accompanies the infection\(^1\). It is possible, in fact even probable, that a number of the cases on which these data are based could have suffered from concurrent diseases that would also have induced thrombocytopenia. One of the more likely of these is canine ehrlichiosis and has been reported\(^2\). It is, however, unlikely that all 1162 canine babesiosis cases suffered from concomitant canine ehrlichiosis or other diseases.

At platelet counts below \(10-20 \times 10^3/\mu l\) spontaneous bleeding may occur\(^1,12\). Forty-one percent of patients with babesiosis in this study had a thrombocytopenia of \(<10 \times 10^3/\mu l\). Clinical bleeding would be seen in most disorders that cause platelet destruction (immunemediated or consumption) to the levels of thrombocytopenia seen in these patients. It is therefore conceivable that part of the thrombocytopenia seen is relative, and not an absolute decrease in total body thromocyte numbers. Studies of malaria have also demonstrated that platelets adhere to red cell membranes, resulting in apparent auto-agglutination of erythrocytes\(^2\) and that automated blood count machines are unable to count this complex as 2 cells, resulting in a pseudo-thrombocytopenia\(^3\). Clumping of thromocytes\(^2\) may similarly cause false low thromocyte counts. All the cases in this study also had a blood film examined and the low platelet count was confirmed for each case. A large proportion (up to one third) of total body thromocytes are normally stored in the spleen and are in equilibrium with circulating thromocytes\(^3\). In patients with enlarged spleens, this sequestration may be excessive and cause concurrent thrombocytopenia\(^4\). In such cases, however, decreased thromocyte counts are usually only moderate and it is unlikely to be clinically significant unless other haemostatic disease processes occur concurrently\(^7\). Splenomegaly is usually marked in canine babesiosis and although the thrombocytopenia seen in these patients is severe, rather than moderate, it is likely that splenic sequestration plays some role in the observed thrombocytopenia. However, thrombocytopenia was seen in 1 study in which splenectomised calves were experimentally infected with *B. bovis*\(^8\), suggesting that splenomegaly and sequestration may not always play a major role. Splenic sequestration, and decreased platelet half-lives, have been described in both malaria\(^6\) and trypanosomosis\(^5\). Platelet-mediated bleeding, seen clinically as cutaneous petechia and ecchymosis, is rarely seen in canine babesiosis patients presented to the OVAH, despite the low platelet counts described above, and suggests that primary haemostatic function is probably still largely normal. This also seems to be the case in malaria\(^6\). However, small animal clinicians at the OVAH often report prolonged ‘needle-prick’ bleeding in dogs with babesiosis. In 1 experimental study, babesia-infected dogs only showed excessive bleeding from ears pin-pricked for blood smear preparations\(^9\), with no other clinical evidence of primary haemostatic dysfunction. Another possible reason that babesia patients do not bleed spontaneously in the face of thrombocytopenia is that although platelet numbers are low, they are still functional, which may not be the case in conditions of increased platelet destruction, such as immune-mediated thrombocytopenia\(^9\). Alternatively, patients with disseminated intravascular coagulation (DIC) have other non-platelet associated coagulatory disturbances that coexist, which may synergistically result in bleeding. Disseminated intravascular coagulation manifests itself as a continuum from subclinical to fulminant, with clinical bleeding. Mild cases of babesiosis may experience some or only early changes of DIC, without the associated bleeding. Studies of malaria have demonstrated activated, but controlled coagulation activity\(^1\). Intravascular micro-thrombi are described in babesiosis, unassociated with clinical bleeding (i.e. severe DIC). While fulminant DIC is described in canine babesiosis\(^10,11,22\), it is likely to be present only in severe cases. Disseminated intravascular coagulation certainly may add to the cause of thrombocytopenia, but this is unlikely to be the sole or primary mechanism.

The finding of a significant difference in the thrombocyte count in dogs with various degrees of parasitaemia was unexpected, although Van Heerden et al\(^12\) noted, in dogs experimentally infected with *Babesia canis*, that thrombocyte counts often decreased at periods of peak parasitaemia. Strains of falciparum malaria, which cause more severe disease in humans, result in proportionally more platelet-mediated erythrocyte clumping\(^6\) and may therefore be expected to cause greater thrombocytopenia. Similarly, human patients in intensive care units (for a variety of causes) with thrombocytopenia have been associated with a poorer prognosis\(^2\). In malaria, thrombocytopenia has also been correlated with the parasitaemia\(^13\). Severity of disease has been associated with the level of parasitaemia in *B. c. rossi*, but not *B. c. canis*\(^13\). Different laboratory staff subjectively assess the parasitaemia and scoring is likely to suffer from bias to some degree. In support of the latter: those groups with parasitaemia grading originated from the same population as that subgroup of patients that did not have parasitaemia scores and yet a Mann-Whitney Rank sum tests showed a significant difference between the babesiosis patients with no parasitaemia scores and a pooled group of the parasitaemia-scored animals. Further prospective studies of the affect of parasitaemia on thrombocyte counts are needed, with emphasis on eliminating bias while scoring parasitaemia.

**CONCLUSIONS**

Thrombocytopenia in *Babesia canis*-infected dogs at the OVAH is very common, and in the majority of cases is severe, although without apparent clinical effect (bleeding diatheses). This high incidence of thrombocytopenia can be helpful in ruling out babesiosis in those patients where the clinical signs are consistent with babesiosis, but for which a blood film evaluation is negative. Although a significant association was found between thrombocytopenia and level of parasitaemia, significant bias may have resulted in this finding and further prospective analysis is needed to substantiate this observation. Thrombocytopenia is...
probably due to a combination of factors, including concurrent disease such as canine monocytic ehrlichiosis, and explanations using only 1 mechanism may result in an incomplete understanding. Based on the differences in severity of thrombocytopenia caused by different Babesia species in the same host species, it appears that the degree of thrombocytopenia is largely determined by the pathogenicity of the parasite species and type. The 99.8% predictive value of excluding canine babesiosis, in the absence of thrombocytopenia, is fortuitously independent of the putative causes of thrombocytopenia — canine babesiosis and subsequent concurrent disease (such as canine ehrlichiosis) do not alter this statistic.

REFERENCES