Received: 13 September 2022 | Revised: 16 November 2022 | Accepted: 7 December 2022

DOI: 10.1111/vcp.13228

Veterinary Clinical Pathology
An International Journal of Laboratory Medicine
WILEY

ORIGINAL ARTICLE

Regenerative response in dogs naturally and experimentally infected with *Babesia rossi*

Chandini Seejarim | Yolandi Rautenbach | Emma H. Hooijberg | Andrew L. Leisewitz | Johan P. Schoeman | Amelia Goddard

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

Correspondence
Amelia Goddard, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Old Soutpan Road, Onderstepoort, Pretoria, 0110, South Africa.
Email: amelia.goddard@up.ac.za

Funding information
South African Agency for Science and Technology Advancement, Grant/Award Number: NRF CPRR160425163064

Abstract

Background: The regenerative response following *Babesia rossi* infection in dogs is mild, despite severe hemolytic anemia.

Objective: We aimed to compare the admission absolute reticulocyte count (ARC) and reticulocyte indices in 103 dogs naturally infected with *B. rossi* with 10 dogs suffering from immune-mediated hemolytic anemia (IMHA) and 14 healthy control dogs. The regenerative response was also evaluated in five dogs experimentally infected with *B. rossi*.

Methods: This is a retrospective observational study of records generated on the ADVIA 2120 hematology analyzer.

Results: The median hematocrits (HCT) of the *B. rossi* and IMHA groups were significantly lower than the control group ($p < .001$ for both); however, no differences were seen between the *B. rossi* and IMHA groups. Compared with the control group, the median ARC was significantly higher in the *B. rossi* ($p = .006$) and IMHA ($p = .019$) groups but significantly lower in the *B. rossi* group than the IMHA group ($p = .041$). In the experimentally infected dogs, there was a sudden decrease in the ARC approximately 48 h after the detection of peripheral parasitemia, which was followed by an increase after treatment. Reticulocytes of naturally infected *B. rossi* dogs were larger, with more variation in cellular volume. The reticulocytes of the experimentally infected dogs decreased in size with decreasing hemoglobin concentrations as the study progressed.

Conclusions: The regenerative response in dogs naturally infected with *B. rossi* is inadequate, given the severity of the anemia observed, and it might be a result of direct suppressive action by the parasite or host response on the bone marrow.

KEYWORDS
absolute reticulocyte count, anemia, babesiosis, canine

1 | INTRODUCTION

Babesiosis is caused by the virulent *Babesia rossi* species and is a common intra-erythrocytic tick-borne disease that affects dogs in southern Africa. The main consequence of babesiosis in dogs is acute hemolytic anemia, which may be intravascular as well as extravascular.[1] The *Babesia* parasite causes direct damage to erythrocytes during replication, resulting in intravascular hemolysis, while extravascular hemolysis results from increased phagocytosis of parasitized and non-parasitized erythrocytes in the spleen and liver.[2] The anemia caused by *B. rossi* is multifactorial, with a number of mechanisms resulting in erythrocyte destruction, including the binding of antibodies to the erythrocyte surface and complement activation, the production of serum hemolytic factors, erythrocyte oxidative damage, the creation of spherocytes, and an increase in the osmotic fragility of erythrocytes.[2]

Hemolytic anemias are considered regenerative anemias, in which the bone marrow responds appropriately to erythrocyte destruction by increasing production of erythrocyte precursors. These precursors can later be recognized as reticulocytes in the circulation.[3] Hemolytic anemias are also usually more regenerative than hemorrhagic anemias because the liberated iron from lysed erythrocytes is recycled in the production of new cells.[3] In contrast, non-regenerative anemias result from decreased erythrocyte production or defective erythropoiesis, which indicates a poorly or non-responsive bone marrow. In these cases, the reticulocyte response would be normal or only minimally increased for the degree of anemia.[4] As a result, non-regenerative anemias commonly present as normocytic and normochromic and may be a consequence of extramedullary diseases, such as is seen with anemia of inflammatory disease (AID).[4,5]

To date, three studies have recorded poor regenerative responses in dogs naturally infected with *B. rossi*, showing moderate to severe anemia.[6–8] The first study noted that the mean reticulocyte percentage (RET%) of the admission blood samples was non-regenerative (RET% = 0.74%) in moderately anemic dogs (hematocrit; HCT = 0.15–0.30 L/L) and mildly regenerative (RET% = 3.43%) in severely anemic dogs (HCT < 0.15 L/L).[6] However, the absolute reticulocyte count (ARC) was not determined in these cases. The second study described the regenerative response as mild (ARC = 60–150 × 10^9/L) to moderate (ARC = 150–300 × 10^9/L) in dogs requiring a blood transfusion. The transfused group had a median HCT of 0.09 L/L at presentation. Similarly, a mild to moderate regenerative response was seen in dogs that did not receive a blood transfusion. Nonetheless, the overall regenerative response, recorded over a period of 6 days, was never marked, despite the severity of anemia observed.[7] The third, more recent study supported these findings and showed that at presentation, 61.9% of infected dogs had an ARC <100 × 10^9/L, with a median HCT of 0.24 L/L.[8] None of these three studies investigated this apparent inadequate regenerative response any further.

Recently, more information has become available on the use of reticulocyte indices in an attempt to describe and explain the underlying pathogenesis of various anemias.[9] The ADVIA 2120 (Siemens Healthcare), an automated hematology analyzer, is able to provide information regarding the ARC and reticulocyte indices using a nucleic acid-binding dye (oxazine 750) and the light scatter properties of reticulocytes.[9,10] Notably, several reticulocyte indices are better indicators of iron-restricted anemia in dogs than conventional hematologic or biochemical indices because they reflect the real-time function of the bone marrow.[9,11]

The objectives of this study were to (1) describe the admission ARC and selected reticulocyte indices in dogs naturally infected with *B. rossi* and compare the results with those of healthy control dogs as well as those with acute erythrocyte destruction (unrelated to babesiosis); and (2) describe the ARC and selected reticulocyte indices throughout the disease course in dogs experimentally infected with *B. rossi* in an attempt to better understand the regenerative response in these dogs. We hypothesized that the admission ARC in dogs naturally infected with *B. rossi* would be significantly lower compared with dogs with acute non-*Babesia*-associated erythrocyte destruction, that the reticulocyte indices would be different than those seen in healthy control dogs and dogs with acute non-*Babesia*-associated erythrocyte destruction, and that the ARC and reticulocyte indices in dogs experimentally infected with *B. rossi* would change over the course of the disease and may be associated with parasitemia.

2 | MATERIALS AND METHODS

2.1 | Study design

This was an observational study that retrospectively evaluated the ARC and a number of erythrocyte and reticulocyte indices in dogs naturally and experimentally infected with *B. rossi*, as well as dogs with immune-mediated hemolytic anemia (IMHA) and healthy control dogs. Research ethics approval was granted by the Research Ethics Committee of the Faculty of Veterinary Science (REC202-19) and the Animal Ethics Committee of the University of Pretoria for the three study cohorts (V055-11, V091-13, and V003-18). The records generated on the ADVIA 2120 (Siemens Healthcare) were retrospectively analyzed.

2.1.1 | Study design for naturally infected dogs

The first study cohort included 85 *Babesia*-infected dogs and 14 healthy control dogs, collected from October 2013 to July 2015, whereas the second study cohort included 18 *Babesia*-infected dogs collected from January to December 2014. These dogs were compared with 10 dogs with IMHA, unrelated to babesiosis, that presented as clinical cases to the Veterinary Academic Hospital between 2014 and 2020. For both *Babesia* study cohorts, dogs were included if they were >12 weeks of age, weighed >3 kg, and had demonstrable parasitemia. Infection with *B. rossi* was confirmed by PCR and

reverse line blot (RLB).[12] Dogs were excluded if they were infected with *B. vogeli*, *Ehrlichia canis*, *Theileria*, or *Anaplasma* spp., based on the results of PCR and RLB. Dogs were also excluded if they had any comorbidities, such as cardiac, neoplastic, infectious, or traumatic conditions or diseases, or any treatment with anti-inflammatory drugs at presentation or within 4 weeks prior to presentation. The standard treatment for the dogs with babesiosis included diminazene aceturate (Berenil RTU 0.07 g/mL, Intervet), an antibabesial drug, at 3.5 mg/kg. Additional supportive treatments of packed red cell transfusions and intravenous fluids were administered as needed. Any complications were treated accordingly at the discretion of the attending clinician. Fourteen healthy dogs formed part of the control group. They were client-owned and were presented to the Veterinary Academic Hospital for routine procedures, such as ovariohysterectomy, castration, or blood donation. The control dogs were deemed healthy based on history, a full clinical examination, peripheral blood smear evaluation, CBC, full biochemistry profile, as well as PCR and RLB to rule out infection with *B. rossi*, *B. vogeli*, *E. canis*, *Theileria*, and *Anaplasma* spp. Owner consent was obtained for the enrollment of infected cases and healthy control dogs. At presentation and before any treatments were administered, blood was collected in EDTA and serum vacutainer tubes from *Babesia*-infected and control dogs. The EDTA blood was used to perform a CBC on the ADVIA 2120 within 1 h, which included the ARC and reticulocyte indices. A small aliquot of this sample was used for DNA extraction by PCR and RLB.

The database of the Clinical Pathology laboratory was retrospectively searched for dogs with IMHA, unrelated to babesiosis, that were presented to the Veterinary Academic Hospital between 2014 and 2020. Although PCR was not performed on these cases to confirm the absence of *Babesia* parasites, the blood smears were evaluated for the presence of *Babesia* parasites, and the hematology results were evaluated for typical changes seen with babesiosis. Furthermore, it has been reported that a platelet count of $>110\times10^9$/L has a 99.3% predictive value that the dog is not suffering from babesiosis.[13] Therefore, all dogs with IMHA and a platelet count less than 110×10^9/L were excluded. This cohort of cases was included to compare their reticulocyte response with that seen in dogs with babesiosis. The inclusion criteria for this cohort of cases were in line with those set by the American College of Veterinary Internal Medicine (ACVIM) consensus statement on the diagnosis of IMHA,[14] which include: (1) anemia (below the laboratory-generated reference interval); (2) signs of immune-mediated erythrocyte destruction such as spherocytosis (≥5 spherocytes per ×100 oil immersion field on blood smear evaluation), a positive saline agglutination test, and/or demonstration of anti-erythrocyte antibodies; and (3) evidence of hemolysis, such as spherocytosis, hyperbilirubinemia (in the absence of functional hepatic disease, cholestasis or sepsis), hemoglobinemia, hemoglobinuria, or erythrocyte ghosts. Owner consent for the use of the data forms part of the standard disclaimer that owners have to sign when their pets are admitted to the Veterinary Academic Hospital.

2.1.2 | Study design for the experimentally infected dogs

Data collected during a longitudinal experimental study from February 28 to March 8, 2019, that included six 6-month-old purpose-bred sterilized male Beagle dogs, were retrospectively included in our study. This study was approved by the University of Pretoria's Animal Ethics Committee (V003-18). The Beagles were housed at the university's academic research unit from 8 weeks of age until the end of the experimental period, after which the dogs were rehomed. One dog was randomly selected, splenectomized, and used to raise a viable parasite inoculum from cryopreserved, PCR-confirmed wild-type *B. rossi*. The parasitemia of the splenectomized dog was determined manually twice daily on central venous blood collected every 12 h from 24-h post-infection. The remaining five dogs were experimentally infected (day one) with an intravenous injection of fresh whole blood, diluted to achieve the two parasite doses required. Two of the five dogs received a low-dose (LD) *B. rossi* parasite inoculum (10^4 parasitized RBC/mL), and three dogs received a high-dose (HD) *B. rossi* parasite inoculum (10^8 parasitized RBC/mL). The five dogs finally infected with the *B. rossi* inoculum acted as their own baseline controls. The splenectomized dog was cured with diminazine aceturate. Blood was collected on a daily basis in EDTA vacutainer tubes from all five dogs for four consecutive days. Unfortunately, one dog in the HD group died; the remaining four dogs were cured with diminazene aceturate when their clinical status reached a priori criteria of severity. Blood was collected for an additional 4 days post-treatment (8 days in total). The EDTA blood was used to perform a CBC on the ADVIA 2120 within 1 h of collection.

2.2 | Methodologies

CBC reports, measured by the ADVIA 2120, were retrospectively analyzed. Specific variables produced by the ADVIA 2120 from all study data sets that were evaluated included: HCT, mean cell volume (MCV), hemoglobin concentration in whole blood (HGB), mean cell hemoglobin concentration (MCHC), cell hemoglobin concentration mean (CHCM), ARC, reticulocyte mean cell volume (MCVr), distribution width (variability) of reticulocyte cell size (RDWr), reticulocyte cell hemoglobin concentration mean (CHCMr), reticulocyte hemoglobin content (CHr), and distribution width (variability) of CHr (CHDWr).

2.3 | Statistical analysis

A commercial software package (SPSS Statistics version 26 IBM) was used. For the naturally infected and IMHA dogs, the normality assumption was evaluated using the Shapiro–Wilk test, and the data distribution was determined to be non-parametric. The Kruskal–Wallis test was used to determine the significance across

groups for each variable. If significance was present, the Mann–Whitney *U* test was used as a post-hoc analysis. Sex proportions between groups were assessed using the Chi-square test. The data are presented as the median and interquartile range (IQR). The significance level was set at 5%. Due to the small sample size of the experimentally infected dogs, statistical analysis could not be performed; instead, the trend over time was described for each variable. The data for these dogs are presented as median and range.

3 | RESULTS

3.1 | Study population characteristics

For the naturally infected study, data from 103 client-owned dogs infected with *B. rossi*, 10 dogs with IMHA, and 14 healthy control dogs were included. The age of the *Babesia* group (18 months; 9–36) was significantly lower than that of the IMHA group (66 months; 54–93; $p < .001$) and the control group (56 months; 18–84; $p = .034$). In contrast, there was no significant age difference between the IMHA and control groups. The weight of the *Babesia* group (18 kg; 9–26.8) was significantly lower than that of the control group (29 kg; 12–34.5; $p = .042$). Yet, there was no significant weight difference between the *Babesia* and IMHA (15 kg; 9.8–29.6) groups, nor between the IMHA and control groups. The ratio of male:female was as follows: control group (5:9), *Babesia* (69:34), and IMHA (4:6), with significantly more male dogs in the *Babesia* group ($p = .023$). The five experimentally infected dogs were all from the same litter of beagle dogs and were all males; there were no differences in age or weight between them.

3.2 | Hematologic variables, including ARC, for naturally infected dogs with babesiosis compared with dogs with IMHA and healthy control dogs

Table 1 contains a summary of all the variables for the various groups at presentation. For the *Babesia* and IMHA groups, the HCT ($p < .001$ for both), HGB ($p < .001$ for both), and CHCM ($p < .001$ for both) were significantly lower than in the control group. In contrast, the HGB ($p = .027$) and CHCM ($p < .001$) were significantly higher in the *Babesia* group compared with the IMHA group. The HCT did not differ significantly between the *Babesia* and IMHA groups. The MCHC was significantly lower in the *Babesia* group ($p < .001$) compared with the control group, but there was no difference between the *Babesia* and IMHA groups or the IMHA and control groups. The MCV was significantly higher in the IMHA group compared with the *Babesia* and control groups ($p < .001$ for both); however, there was no significant difference between the *Babesia* and control groups. Compared with the control group, the ARC was significantly higher in the *Babesia* ($p = .006$) and IMHA ($p = .019$) groups (Figure 1). However, the ARC was significantly lower in the *Babesia* group compared with the IMHA group ($p = .041$), despite there being no significant difference in the HCT between groups.

3.3 | Reticulocyte indices for naturally infected babesiosis dogs, compared with dogs with IMHA and healthy control dogs

Table 1 contains a summary of all the variables for the various groups at presentation. The MCVr ($p = .005$; Figure 2) and RDWr ($p = .003$) were

TABLE 1 Hematologic and reticulocyte variables at presentation for naturally infected *B. rossi* dogs, dogs with IMHA, and healthy control dogs.

Variable	Unit	Control group median (IQR)	*Babesia* group median (IQR)	IMHA group median (IQR)
Hemoglobin concentration (HGB)[a,b,c]	g/L	179.5 (154.0–190.8)	50.0 (39.0–86.0)	40.0 (31.0–45.8)
Hematocrit (HCT)[a,b]	L/L	0.52 (0.45–0.57)	0.16 (0.12–0.27)	0.15 (0.12–0.17)
Mean cell volume (MCV)[c]	fL	68.0 (66.6–69.5)	70.5 (66.7–76.3)	97.7 (79.1–108.9)
Mean cell hemoglobin concentration (MCHC)[a]	g/L	340 (336–341)	322 (300–333)	312 (294–395)
Cell hemoglobin concentration mean (CHCM)[a,b,c]	g/L	346 (339–353)	325 (299–340)	278 (267–290)
Reticulocyte percentage (RET%)[a,b,c]	%	0.61 (0.44–0.75)	3.55 (1.46–7.64)	28.2 (3.3–35.6)
Absolute reticulocyte count (ARC)[a,b,c]	$\times 10^9$/L	42.1 (33.8–62.6)	82.1 (48.6–174.9)	256.7 (50.1–559.9)
Reticulocyte mean cell volume (MCVr)[a]	fL	88.6 (86.3–89.3)	95.9 (88.0–103.5)	98.8 (83.5–123.2)
Distribution width (variability) of reticulocyte cell size (RDWr)[a,b]	%	13.1 (12.1–14.3)	14.6 (13.4–15.8)	15.4 (13.2–20.9)
Reticulocyte cell hemoglobin concentration mean (CHCMr)[a,b,c]	g/L	286 (278–303)	273 (262–289)	254 (234–261)
Reticulocyte hemoglobin content (CHr)	pg/cell	25.6 (23.6–26.8)	25.9 (24.5–27.4)	25.4 (20.9–29.1)
Distribution width (variability) of CHr (CHDWr)[a,b]	pg	3.04 (2.91–3.21)	3.37 (3.10–3.82)	3.79 (3.7–5.9)

[a]Significance between control and naturally infected *B. rossi* dogs ($p < .05$).

[b]Significance between control and IMHA dogs ($p < .05$).

[c]Significance between naturally infected *B. rossi* and IMHA dogs ($p < .05$).

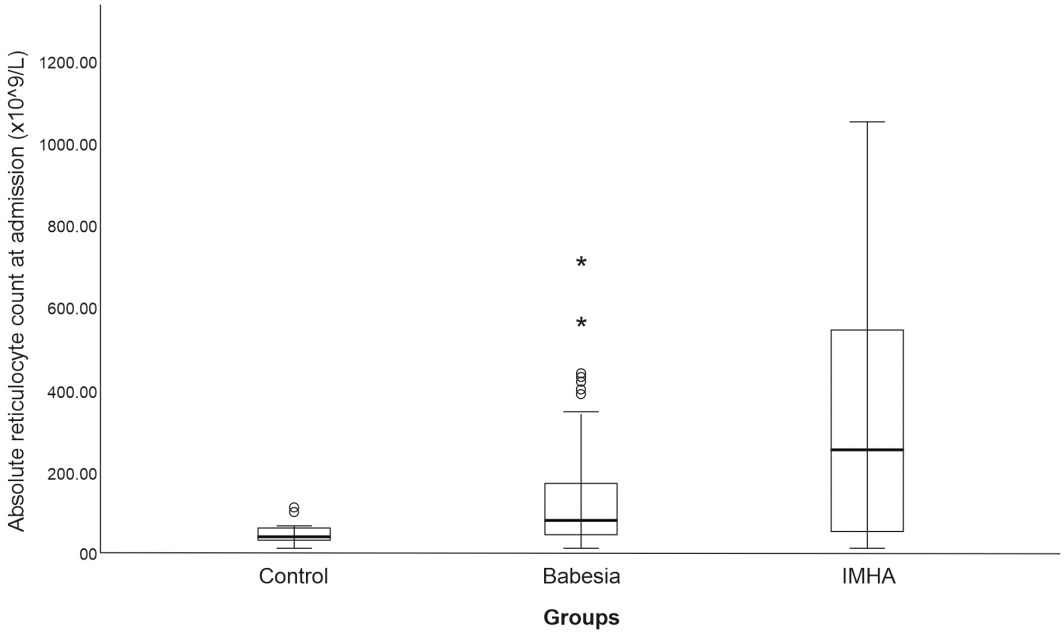

FIGURE 1 Boxplot demonstrating the admission ARC results for the control, *Babesia*, and IMHA groups. The median is represented by the horizontal line that runs through the box. The lower and upper edges of the box represent the first and third quartiles, respectively. The lower and upper edges of the whiskers represent the minimum and maximum values, respectively. The outliers are indicated by circles, and the extreme outliers by asterisks.

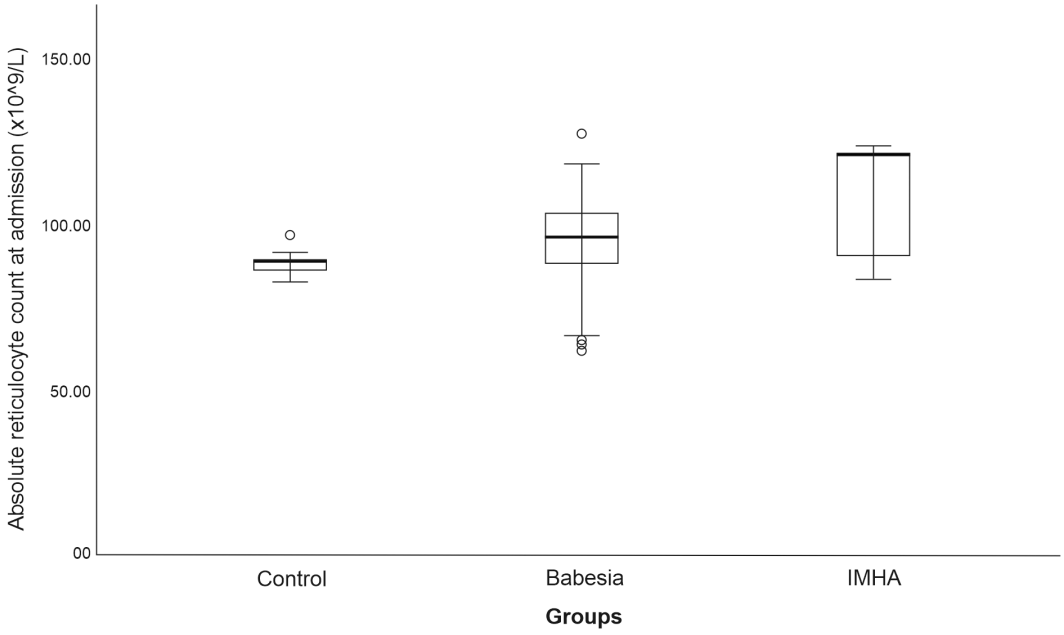

FIGURE 2 Boxplot demonstrating the admission MCVr results for the control, *Babesia*, and IMHA groups. The median is represented by the horizontal line that runs through the box. The lower and upper edges of the box represent the first and third quartiles, respectively. The lower and upper edges of the whiskers represent the minimum and maximum values, respectively. The outliers are indicated by circles.

significantly higher in the *Babesia* group compared with the control group, but there were no significant differences between the *Babesia* and IMHA groups. The RDWr was significantly higher ($p = .031$) in the IMHA group compared with the controls, but the MCVr was not significantly different between the two groups. Although there were no significant differences between groups for CHr, the CHCMr was significantly lower in the *Babesia* ($p = .007$) and IMHA ($p < .001$) groups compared with the control group and significantly higher in the *Babesia* group compared with the IMHA group ($p = .003$). Compared with the control group, the CHDWr was significantly higher in the *Babesia* ($p = .002$) and IMHA ($p = .001$) groups, with no significant difference between the *Babesia* and IMHA groups.

3.4 | Temporal changes of the hematologic and reticulocyte variables in experimentally infected *B. rossi* dogs

Blood was collected for a total of 8 days, 4 days before and 4 days after treatment. Results for each variable on each day are displayed in Table 2. Parasitemia was first detected on peripheral blood smears on days 2 (HD) and 3 (LD).

The HGB concentration and HCT (Figure 3) began to decrease by day 3, reached their lowest values by days 5 (HD) and 6 (LD), and thereafter increased again. The MCV remained unchanged, except for an increase in the HD group on day 4, with a return to baseline on day 5. Similarly, the CHCM remained unchanged, except for a decrease in the HD group on day 4 and a return to baseline on day 5. The MCHC demonstrated a gradual decrease for both groups over the period, with a return to baseline on day 8. The ARC (Figure 4) increased from day 2, reaching a peak on days 3 (HD) and 4 (LD). Thereafter, it suddenly decreased to return to baseline values by days 4 (HD) and 6 (LD). All dogs were drug-treated and cured of the parasitemia on day 4. An increase in the ARC was seen again on day 8. The MCVr and CHr increased on days 2 and 3 for the LD group, after which they showed a steady decline for both groups, reaching their lowest values on days 4 (HD) and 6 (LD) before peaking again on day 8. The RDWr showed a gradual increase for both groups, reaching its highest values on days 6 (HD) and 7 (LD), thereafter decreasing to baseline values. The CHCMr decreased throughout the study for both groups. The CHDWr demonstrated a steady increase in the LD group, reaching its highest value on day 7, after which there was a marked decline to baseline values. For the HD group, the CHDWr peaked on day 4, after which there was a gradual decline to baseline values.

4 | DISCUSSION

This study demonstrated that the erythrocyte regenerative response in dogs naturally and experimentally infected with *B. rossi* was poor, despite the observed severity of the anemia. However, the regenerative response subsequently increased shortly after treatment, suggesting a potential direct effect of the *Babesia* parasite or host response on erythrocyte production.

As expected for a hemolytic disease, the admission HCT for the naturally infected *Babesia* and IMHA groups was significantly lower, whereas the ARC was significantly higher compared with the control group. However, the admission ARC was significantly lower in the *Babesia* group compared with the IMHA group, despite no significant difference in the HCT between the two groups. Assuming that the rate of erythrocyte loss in both conditions is similar, an equally low HCT in the *Babesia* group should have a similar ARC to the IMHA group because it is expected for severe hemolytic anemia to evoke a greater stimulus for increased erythrocyte production.[15] Therefore, the regenerative response in the naturally infected *Babesia* group was inadequate, in agreement with previous studies.[6–8]

An interesting phenomenon was observed in the experimentally infected dogs, which may serve as an explanation for the inadequate response in naturally infected dogs. The ARC gradually increased from day one and reached its highest on days 3 (HD) and 4 (LD) post-infection. Yet, clinically significant anemia (HCT < 30 L/L) was only observed a day later for both groups. A substantial reticulocyte response is usually observed 3 to 5 days after an anemic insult.[3] Instead, in some instances, proinflammatory cytokines from an inflammatory or infectious insult can inhibit the constant rate of erythrocyte production from the bone marrow and result in the release of immature reticulocytes, known as stress reticulocytes, from extra-medullary organs such as the spleen and liver.[16] While erythropoiesis in the bone marrow is suppressed during stress erythropoiesis, the spleen and liver contain specialized cells that respond to stimulatory signals, which allow for the differentiation of immature progenitor cells into early stress-erythroid progenitors.[17,18] The marked host proinflammatory response reported in dogs infected with *B. rossi*[19,20] may be responsible for the initial reticulocyte changes noted in the experimental group, namely an early release of stress reticulocytes, recorded as an increased ARC before significant anemia was observed.[16,17] The MCVr was also at its highest on days 2 and 3 compared with the subsequent days of experimental infection in the LD group, which indicates a greater average volume of the reticulocyte population and supports the above explanation. On days 4 (HD) and 6 (LD), there was a sudden decrease in the ARC to baseline values, only to show an increase again after treatment on day 8. This rapid reduction in reticulocytes occurred in the presence of anemia and was, therefore, unexpected. Previous studies conducted in different species found that stress reticulocytes have a longer maturation time in circulation compared with normal reticulocytes. These reticulocytes are reported to decrease in size within 4 h and disappear within 3 days after their production.[21] In our study, clinically significant anemia was present within 3 days after the suspected stress-erythropoiesis response, during which a return to steady-state erythropoiesis is expected to maintain a circulating erythrocyte concentration. This lack of a reticulocyte response in dogs 4–6 days after an anemic insult indicates that the anemia may be exacerbated by insufficient erythrocyte production in the bone marrow.[4] Furthermore, the sudden decrease in the ARC occurred 48 hours after parasitemia was detected on a peripheral blood smear for both groups. This finding may indicate a possible suppressive action of the *Babesia* parasite on the bone marrow during the time of parasitemia, resulting in insufficient erythropoiesis. Moreover, the presence of inflammatory cytokines modifies the response of erythroid precursor cells to erythropoietin, as described in malaria infections in humans.[22] For our study, it is therefore postulated that after treatment that killed the *B. rossi* parasite, a consequential decrease in proinflammatory cytokines and possibly parasite products ensued, thus alleviating the suppression of erythropoiesis and allowing the ARC to increase. It must, however, be noted that dogs with IMHA are reported to have a greater concentration of proinflammatory cytokines

TABLE 2 Daily changes for hematologic and reticulocyte variables in dogs experimentally infected with *B. rossi*.

Variable	Inoculum dose	Experimental group median (range) values							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Hemoglobin concentration (HGB; g/L)	Low	135.5 (134.0–137.0)	134.5 (134.0–135.0)	129.5 (128.0–131.0)	135.5 (133.0–138.0)	93.0 (89.0–97.0)	79.5 (77.0–82.0)	78.0 (77.0–79.0)	91.5 (88.0–95.0)
	High	133.0 (133.0–141.0)	135.0 (128.0–141.0)	120.0 (112.0–121.0)	89.0 (70.0–95.0)	58.0 (49.0–67.0)	65.0 (54.0–76.0)	83.5 (71.0–96.0)	83.0 (77.0–89.0)
Hematocrit (HCT; L/L)	Low	0.38 (0.36–0.39)	0.41 (0.40–0.41)	0.38 (0.36–0.40)	0.39 (0.37–0.40)	0.28 (0.26–0.29)	0.25 (0.24–0.25)	0.25 (0.24–0.26)	0.28 (0.27–0.29)
	High	0.40 (0.33–0.42)	0.40 (0.38–0.42)	0.34 (0.31–0.36)	0.27 (0.12–0.28)	0.17 (0.14–.19)	0.20 (0.17–0.23)	0.26 (0.22–0.29)	0.25 (0.23–0.26)
Mean cell volume (MCV; fL)	Low	67.8 (67.8–67.8)	67.9 (67.7–68.1)	67.9 (67.6–68.1)	68.3 (67.8–68.7)	68.6 (68.0–69.1)	67.6 (67.5–67.7)	70.0 (69.9–67.7)	68.1 (67.6–68.6)
	High	67.4 (65.6–67.8)	67.2 (66.1–67.3)	68.3 (66.7–69.0)	71.4 (70.8–78.7)	69.6 (68.0–71.2)	68.6 (67.6–69.5)	69.7 (68.2–71.1)	68.6 (67.2–70.0)
Mean cell hemoglobin concentration (MCHC; g/L)	Low	358 (341–376)	334 (332–337)	342 (329–356)	351 (332–370)	335 (334–337)	325 (323–328)	315 (309–321)	328 (328–328)
	High	336 (331–399)	338 (337–341)	333 (333–387)	337 (328–595)	351 (348–355)	323 (320–327)	323 (320–327)	339 (339–339)
Cell hemoglobin concentration mean (CHCM; g/L)	Low	337 (334–341)	336 (335–338)	338 (338–339)	335 (334. – 336)	327 (327–328)	331 (326–337)	313 (310–317)	328 (327–329)
	High	337 (336–338)	338 (331–340)	326 (326–330)	298 (277–299)	315 (315–316)	324 (323–326)	324 (322–327)	340 (339–342)
Reticulocyte percentage (RET%)	Low	0.94 (0.90–0.98)	1.01 (0.92–1.10)	1.25 (1.17–1.33)	3.81 (3.70–3.92)	2.06 (1.70–2.42)	1.16 (1.09–1.22)	1.37 (1.19–1.54)	1.88 (1.39–2.36)
	High	1.23 (1.22–1.28)	2.28 (1.89–2.81)	3.50 (2.87–3.81)	1.44 (0.75–1.89)	1.57 (0.96–2.18)	1.16 (1.01–1.31)	1.20 (1.17–1.22)	3.72 (3.24–4.19)
Absolute reticulocyte count (ARC; $\times 10^9$/L)	Low	52.1 (51.8–52.3)	59.6 (55.1–64.1)	69.8 (69.3–70.3)	215.4 (124.6–216.1)	83.8 (65.8–101.7)	41.8 (38.2–45.4)	48.0 (43.4–52.5)	76.0 (59.5–92.5)
	High	76.8 (60.1–78.1)	135.5 (107.6–174.5)	172.2 (134.1–199.3)	31.6 (25.8–57.10)	39.3 (19.6–58.9)	33.1 (31.5–34.7)	43.8 (39.4–48.2)	132.7 (109.0–156.3)
Reticulocyte mean cell volume (MCVr; fL)	Low	89.0 (87.9–90.0)	90.5 (89.4–91.6)	91.4 (91.2–91.6)	86.6 (86.5–86.7)	79.4 (79.0–79.7)	71.3 (70.1–72.4)	79.4 (78.0–80.8)	92.5 (91.5–93.5)
	High	88.3 (86.9–89.0)	86.0 (85.0–87.9)	78.8 (76.5–83.7)	81.1 (80.0–83.7)	81.7 (81.6–81.8)	83.0 (79.1–86.8)	94.1 (93.6–94.5)	109.7 (107.6–111.7)
Distribution width (variability) of reticulocyte cell size (RDWr; %)	Low	11.9 (11.8–12.0)	12.2 (11.9–12.5)	10.9 (10.7–11.0)	12.4 (12.2–12.5)	14.1 (13.5–14.7)	15.7 (15.5–15.9)	19.4 (18.8–19.9)	17.0 (15.9–18.0)
	High	11.9 (11.8–12.9)	12.1 (11.9–12.5)	13.7 (12.4–13.9)	(19.9–19.9 – 21.2)	19.3 (17.9–20.7)	21.6 (19.0–24.1)	17.5 (16.9–18.0)	13.2 (12.7–13.7)
Reticulocyte cell hemoglobin concentration mean (CHCMr; g/L)	Low	287 (286–289)	283 (283–284)	286 (285–287)	281 (283–283)	276 (275–277)	270 (268–272)	265 (264.– 266)	267 (265–270)
	High	286 (284–287)	283 (281–287)	285 (283–293)	270 (264–270)	272 (263–282)	279 (269–290)	277 (274–281)	260 (258–262)
Reticulocyte hemoglobin content (CHr; pg/cell)	Low	25.4 (25.0–25.8)	25.6 (25.2–25.9)	26.1 (25.9–26.2)	24.2 (24.1–24.3)	21.9 (21.8–21.9)	19.3 (19.1–19.4)	21.0 (20.7–21.2)	24.6 (24.5–24.6)
	High	25.1 (24.8–25.1)	24.2 (24.0–24.7)	23.3 (22.3–23.8)	21.5 (21.4–22.6)	22.15 (21.4–22.9)	22.9 (22.7–23.0)	25.7 (25.3–26.1)	28.2 (27.9–28.4)
Distribution width (variability) of CHr (CHDWr; pg)	Low	2.84 (2.81–2.87)	2.79 (2.75–2.83)	2.69 (2.68–2.70)	3.18 (3.06–3.30)	3.90 (3.84–3.95)	3.75 (3.67–3.82)	4.24 (4.04–4.43)	3.44 (3.27–3.60)
	High	2.65 (2.58–2.95)	2.86 (2.62–2.99)	4.06 (3.67–4.11)	5.29 (4.85–5.71)	4.20 (3.91–4.49)	4.19 (3.94–4.43)	3.42 (3.35–3.49)	3.18 (2.93–3.42)

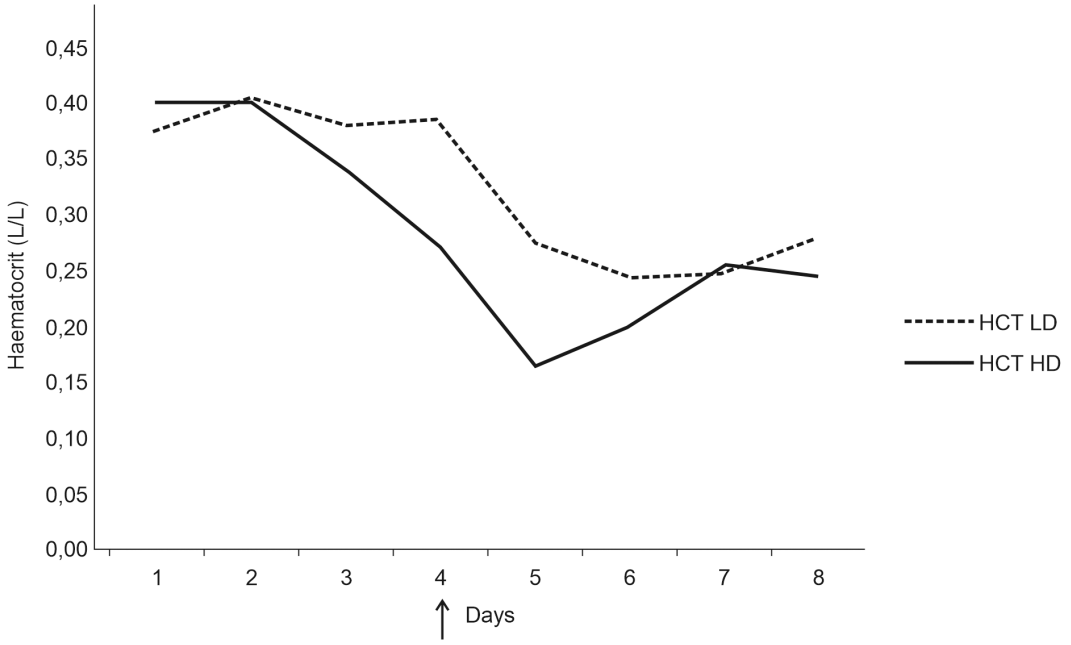

FIGURE 3 Line graph displaying the trend of the median HCT over 8 days of high-dose (10^8 parasitized RBC/mL) (solid line) and low-dose (10^4 parasitized RBC/mL) (broken line) experimentally *Babesia*-infected dogs. The arrow denotes the day of treatment.

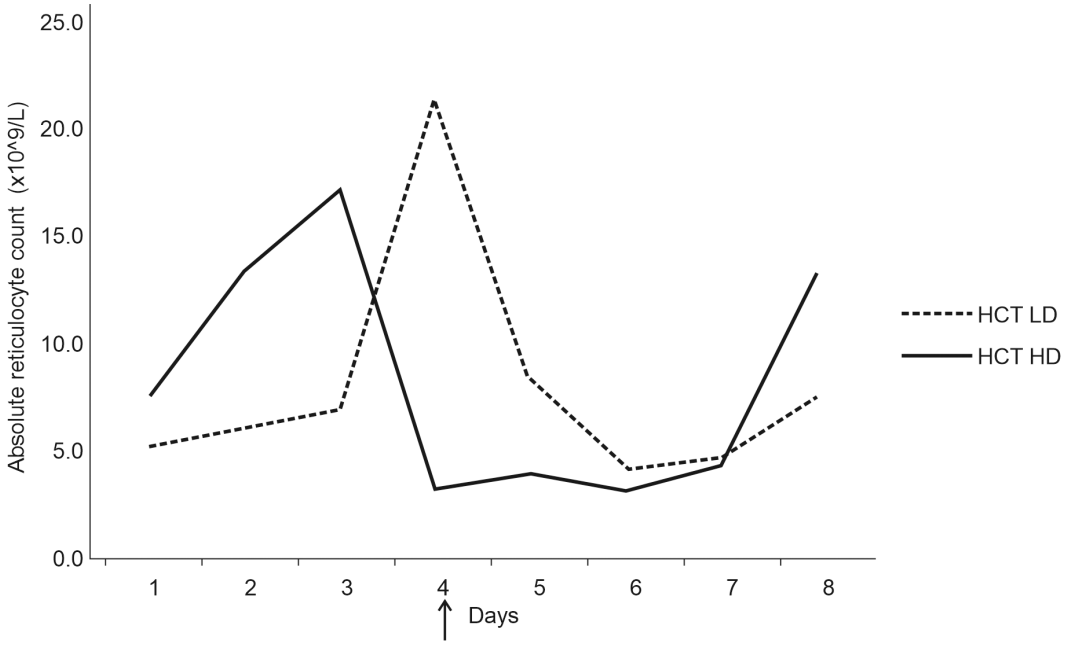

FIGURE 4 Line graph displaying the trend of the median ARC over 8 days of high-dose (10^8 parasitized RBC/mL) (solid line) and low-dose (10^4 parasitized RBC/mL) (broken line) experimentally *Babesia*-infected dogs. The arrow denotes the day of treatment.

compared with dogs that have other non-immune-mediated inflammatory conditions.[23] This, along with the small sample size, may indicate inaccuracies in our findings. Another possible reason for this finding is the direct effect of treatment on possible *B. rossi* parasites within the bone marrow itself. The presence of parasites within the bone marrow and subsequent downregulation of erythroid maturation genes have been reported in malaria infections.[24] A recent study that evaluated the cytologic, histologic, and immunohistochemical staining of bone marrow pathology in six dogs naturally infected with *B. rossi* demonstrated intraerythrocytic parasites in the bone marrow microvasculature.[25]

Human malaria and canine babesiosis have been described as related intra-erythrocytic protozoal diseases that share a similar pathogenesis and clinical course.[6,26–28] Previous studies on *Plasmodium falciparum* infection have reported suboptimal reticulocyte counts in untreated cases of malaria in the face of ongoing hemolysis coupled

with high serum erythropoietin levels.[22,29,30] The anemia caused by malaria is, therefore, considered to be multifactorial and not only attributed to the hemolysis of infected and uninfected RBCs, but also to an inability to replenish the lost erythrocytes due to an inadequate erythroid response.[22,30,31] The causes of impaired erythropoiesis in *P. falciparum* malaria may be categorized into direct and indirect effects. Direct effects suggest that the parasite produces specific factors that have a direct toxic effect on erythroid progenitor cells, erythroid precursor cells, or both.[22] A more substantiated explanation may be the indirect effects, which postulate that erythropoiesis is indirectly disturbed by parasite products, specifically hemozoin and hemozoin derivatives, which may be directed at T-lymphocytes or hemopoietic microenvironmental cells.[22] Malaria is also associated with the release of cytokines such as interferon-ɣ, tumor necrosis factor-α, interleukin (IL)-2, IL-12, and IL-10, which impair or suppress erythropoiesis by inhibiting erythroid progenitor cells or blunting their response to erythropoietin.[22] Although the *B. rossi* organism has not been shown to produce a hemozoin-type product, a similar excessive proinflammatory cytokine response has been described in affected dogs, which may inhibit the regenerative response.[19,20] Further research is required to determine whether *Babesia* organisms have any specific direct or indirect toxic effects.

A study that investigated genes and pathways that were differentially expressed over time, using the same cohort of experimentally infected dogs as our study, showed that genes with functions related to erythropoiesis and heme biosynthesis increased in expression as the infection progressed and returned to normal during recovery. The rise and fall in erythrocyte transcripts paralleled the ARC, peaking on days 3 (HD) and 4 (LD), and began to rise again after treatment. The authors also attributed the anemia to a combination of hemolysis and inadequate erythropoiesis due to a possible suppressive action of the *B. rossi* parasite on the bone marrow.[32] Similarly, based on the findings of our study, the anemia caused by *B. rossi* may be attributed to two mechanisms. The first is largely due to processes that result in the destruction of parasitized and non-parasitized erythrocytes, as previously mentioned.[2] The second mechanism, based on the inadequate production of reticulocytes, could indicate a disturbance along any of the erythrocyte production lines prior to reticulocyte release, which include erythropoietin production, growth factor and cytokine function (IL-3, IL-9, and IL-11), colony-stimulating factors (GM-CSF or G-CSF), progenitor cell division and maturation (BFU-E and CFU-E), or erythroid precursor development and their maturation into reticulocytes.[33] The study that evaluated the bone marrow pathology of six *Babesia*-infected dogs demonstrated a hypercellular bone marrow, mainly because of erythrocyte precursor proliferation, notably rubriblasts. The number of metarubricytes decreased in the *Babesia*-infected dogs compared with the five healthy control dogs included, and dyserythropoietic changes were evident within the metarubricyte population.[25]

Reticulocyte indices may be better than erythrocyte indices at providing information regarding current or real-time bone marrow function because reticulocytes are recently produced and released into the blood.[34] Admission MCVr and RDWr were significantly increased in the naturally infected *B. rossi* group compared with the control group. This finding was similar to what was observed on days 2 and 3 of the experimentally infected (LD) group and indicated the likely presence of larger reticulocytes within the bloodstream that may have been released earlier than normal, consistent with a finding of stress reticulocytes in circulation. Although the CHr was not significantly different between groups, there was more variation in the CHr and the presence of hypochromic reticulocytes in the *B. rossi* and IMHA cases based on the CHDWr and CHCMr, respectively. This finding was compounded by the fact that the erythrocyte MCHC and CHCM were significantly lower in the *B. rossi* group and the CHCM in the IMHA group compared with the healthy control dogs, indicating hypochromic anemia. A common cause of this finding is iron-restricted anemia, which in this case, may be attributed to AID.[5] In addition to hypochromasia, AID is usually associated with smaller erythrocytes, represented by a decreased MCV.[35] However, macroreticulocytosis, together with reticulocyte hypochromasia, has been reported in previous studies in dogs with iron-restricted anemia.[9,36] Reticulocyte size has also been evaluated in three anemia-induced states in humans, namely phlebotomy-induced, folate or vitamin B12 deficiency and drug-induced bone marrow aplasia. In all subjects, the reticulocytes were initially macrocytic before returning to their normal size or becoming microcytic. The macroreticulocytes were ultimately immature stress reticulocytes that were released in response to acute, severe anemia.[37] The macroreticulocytosis in the naturally infected babesiosis dogs of our study was accompanied by severe anemia and a significantly lower CHCMr, which therefore supports the above explanation. The significantly increased MCV observed in the IMHA group was most likely a result of erythrocyte agglutination.[38]

For the experimentally infected dogs, the MCVr of both the HD and LD groups decreased on days 2 and 4, respectively, after an initial increase was observed in the LD group on days 2 and 3. This supposed microcytosis was coupled with an increasing RDWr for both groups on those days, thus indicating a greater variation in reticulocyte volume. The MCVr started to increase again a few days after treatment, with a value higher than baseline seen on day 8 (both groups). The initial decreasing CHr and CHCMr in both the HD and LD groups were consistent with the hypochromasia seen in the naturally infected group. Based on previous studies, decreased MCVr, CHr, and CHCMr have been reported as more sensitive indicators of decreased hemoglobin concentration, as seen with iron-restricted anemia,[10,34,35] associated with AID.[11,39,40] During the inflammatory host response reported in dogs with *B. rossi* infection, both IL-6 and IL-10 are significantly raised in the serum.[19,20] Increased serum IL-6 and IL-10, along with other inflammatory cytokines, stimulate hepcidin production, which in turn stimulates the mechanisms resulting in functional and transport pool-related iron-restricted anemia.[39] These mechanisms include iron sequestration, decreased production of erythrocyte progenitor cells, and a shortened life span of erythrocytes.[40] The study of the bone marrow of dogs naturally infected with *B. rossi* also showed, using Perl's Prussian Blue stain for iron,

that there was an abundance of iron within the bone marrow. As a result, iron deficiency is not the cause of the muted reticulocyte response. However, these findings support the fact that iron may be sequestered and unavailable for erythropoiesis in the marrow – a finding consistent with the effect of the host inflammatory cytokine response.[25]

Our study had some limitations. The naturally infected babesiosis dogs presented at different stages during the disease process, which likely resulted in significant variation in results based on the duration of illness prior to presentation. Despite this, an obvious and consistent effect of infection is seen. It is likely that many naturally infected dogs had been infected for longer compared with the experimentally infected dogs. In fact, the HD group followed an unnaturally acute disease course that is not usually seen in natural infections. This may have resulted in altered reticulocyte production kinetics, affected by the balance of inhibitory and stimulatory cytokines. The severity of the infection may also have influenced the intensity of the reticulocyte response in both groups. Furthermore, any subclinical comorbidities, ecto- or endoparasites, or nutritional deficiencies that were not excluded during the initial screening process of the naturally infected dogs may have influenced the results. In the experimental study, the results may have been influenced by the low number of cases. Similarly, the IMHA group had a relatively small sample size. Admittedly, the presence of other comorbidities (which could have potentially influenced the results) was not excluded in the IMHA group because they consisted of clinical cases that had previously presented to the Veterinary Academic Hospital.

5 | CONCLUSIONS

This study has shown that the regenerative response of dogs with babesiosis is inadequate for the severity of anemia and that the anemia may be attributed to multiple factors, such as hemolysis, dyserythropoiesis, and inflammation. Furthermore, the changes observed in the reticulocyte indices in the naturally and experimentally infected dogs were consistent with iron-restricted anemia, probably related to AID. The return of reticulocytosis after treatment in the experimental group suggests the involvement of parasite-associated toxins, the sudden downregulation of a host-derived factor, or possibly a sequestered mass of *Babesia* parasites within the bone marrow of infected animals, all of which would require further research.

FUNDING INFORMATION

This observational arm of the research did not require any specific grants from funding agencies in the public, commercial, or not-for-profit sectors. The experimental arm of the research was funded by a National Research Foundation grant held by Prof. A. Leisewitz (NRF CPRR160425163064).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

ORCID

Yolandi Rautenbach https://orcid.org/0000-0002-9798-1874
Emma H. Hooijberg https://orcid.org/0000-0002-4367-799X
Amelia Goddard https://orcid.org/0000-0002-8415-4802

REFERENCES

1. Schoeman JP. Canine babesiosis: tick-borne diseases. *Onderstepoort J Vet Res*. 2009;76(1):59-66.
2. Solano-Gallego L, Baneth G. Babesiosis in dogs and cats—expanding parasitological and clinical spectra. *Vet Parasitol*. 2011;181(1):48-60.
3. Cowgill ES, Neel JA, Grindem CB. Clinical application of reticulocyte counts in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2003;33(6):1223-1244.
4. Grimes CN, Fry MM. Nonregenerative anemia: mechanisms of decreased or ineffective erythropoiesis. *Vet Pathol*. 2015;52(2):298-311.
5. Chikazawa S, Dunning MD. A review of anaemia of inflammatory disease in dogs and cats. *J Small Anim Pract*. 2016;57(7):348-353.
6. Reyers F, Leisewitz AL, Lobetti RG, Milner RJ, Jacobson LS, van Zyl M. Canine babesiosis in South Africa: more than one disease. Does this serve as a model for falciparum malaria? *Ann Trop Med Parasitol*. 1998;92(4):503-511.
7. Scheepers E, Leisewitz AL, Thompson PN, Christopher MM. Serial haematology results in transfused and non-transfused dogs naturally infected with *Babesia rossi*. *J S Afr Vet Assoc*. 2011;82(3):136-143.
8. Leisewitz AL, Goddard A, Clift S, et al. A clinical and pathological description of 320 cases of naturally acquired *Babesia rossi* infection in dogs. *Vet Parasitol*. 2019;271:22-30.
9. Fry MM, Kirk CA. Reticulocyte indices in a canine model of nutritional iron deficiency. *Vet Clin Pathol*. 2006;35(2):172-181.
10. Schaefer DMW, Stokol T. The utility of reticulocyte indices in distinguishing iron deficiency anemia from anemia of inflammatory disease, portosystemic shunting, and breed-associated microcytosis in dogs. *Vet Clin Pathol*. 2015;44(1):109-119.
11. Fuchs J, Moritz A, Grussendorf E, et al. Canine reticulocyte hemoglobin content (RET-He) in different types of iron-deficient erythropoiesis. *Vet Clin Pathol*. 2017;46(3):422-429.
12. Matjila PT, Leisewitz AL, Jongejan F, Penzhorn BL. Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. *Vet Parasitol*. 2008;155(1–2):152-157.
13. Kettner F, Reyers F, Miller D. Thrombocytopaenia in canine babesiosis and its clinical usefulness. *J S Afr Vet Assoc*. 2003;74(3):63-68.
14. Garden OA, Kidd L, Mexas AM, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *J Vet Intern Med*. 2019;33(2):313-334.
15. Oishi A, Sakamoto H, Shimizu R, Ohashi F, Takeuchi A. Evaluation of phlebotomy-induced erythropoietin production in the dog. *J Vet Med Sci*. 1993;55(1):51-58.
16. Bennett LF, Liao C, Quickel MD, et al. Inflammation induces stress erythropoiesis through heme-dependent activation of SPI-C. *Sci Signal*. 2019;12:598.
17. Lenox LE, Perry JM, Paulson RF. BMP4 and Madh5 regulate the erythroid response to acute anemia. *Blood*. 2005;105(7):2741-2748.
18. Liao C, Prabhu KS, Paulson RF. Monocyte-derived macrophages expand the murine stress erythropoietic niche during the recovery from anemia. *Blood*. 2018;132(24):2580-2593.
19. Goddard A, Leisewitz AL, Kjelgaard-Hansen M, Kristensen AT, Schoeman JP. Excessive pro-inflammatory serum cytokine concentrations in virulent canine babesiosis. *PloS One*. 2016;11:0150113.
20. Leisewitz A, Goddard A, De Gier J, et al. Disease severity and blood cytokine concentrations in dogs with natural *Babesia rossi* infection. *Parasite Immunol*. 2019;41(7):e12630.

21. Shimada A. The maturation of reticulocytes. II. Life-span of red cells originating from stress reticulocytes. *Acta Med Okayama*. 1975;29(4):283-289.
22. Wickramasinghe SN, Abdalla SH. Blood and bone marrow changes in malaria. *Best Pract Res CL HA*. 2000;13(2):277-299.
23. Swann JW, Woods K, Wu Y, Glanemann B, Garden OA. Characterisation of the immunophenotype of dogs with primary immune-mediated Haemolytic Anaemia. *PloS One*. 2016;11(12):e0168296.
24. Brito MAM, Baro B, Raiol TC, et al. Morphological and transcriptional changes in human bone marrow during natural *Plasmodium vivax* malaria infections. *J Infect Dis*. 2022;225(7):1274-1283.
25. Bumby MM. *Cytological and Histopathological Bone Marrow Findings in Dogs with Natural Babesia Rossi Infection [Thesis]: Veterinary Pathology*. University of Pretoria; 2022.
26. Jacobson LS. The South African form of severe and complicated canine babesiosis: clinical advances 1994–2004. *Vet Parasitol*. 2006;138(1):126-139.
27. Krause PJ, Daily J, Telford SR, Vannier E, Lantos P, Spielman A. Shared features in the pathobiology of babesiosis and malaria. *Trends Parasitol*. 2007;23(12):605-610.
28. Djokic V, Rocha SC, Parveen N. Lessons learned for pathogenesis, immunology, and disease of erythrocytic parasites: *Plasmodium* and *Babesia*. *Front Cell Infect Mi*. 2021;11:685239.
29. Joice R, Nilsson SK, Montgomery J, et al. Plasmodium falciparum transmission stages accumulate in the human bone marrow. *Sci Transl Med*. 2014;6(244):244re5.
30. Casals-Pascual C, Roberts DJ. Severe malarial anaemia. *Curr Mol Med*. 2006;6(2):155-168.
31. Pathak VA, Ghosh K. Erythropoiesis in malaria infections and factors modifying the erythropoietic response. *Anemia*. 2016;2016:1-8.
32. Smith RL, Goddard A, Boddapati A, et al. Experimental *Babesia rossi* infection induces hemolytic, metabolic, and viral response pathways in the canine host. *BMC Genomics*. 2021;22(1):619.
33. Weiss DJ, Wardrop KJ. *Schalm's Veterinary Hematology*. 6th ed. John Wiley & Sons; 2011.
34. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. *Crit Rev Clin Lab Sci*. 2000;37(2):93-130.
35. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. *Clin Chem*. 2003;49(10):1573-1578.
36. Kotisaari S, Romppanen J, Penttilä I, Punnonen K. The Advia 120 red blood cells and reticulocyte indices are useful in diagnosis of iron-deficiency anemia. *Eur J Haematol*. 2002;68(3):150-156.
37. Clarkson DR, Moore EM. Reticulocyte size in nutritional anemias. *Blood*. 1976;48(5):669-677.
38. Porter RE Jr, Weiser MG. Effect of immune-mediated erythrocyte agglutination on analysis of canine blood using a multichannel blood cell counting system. *Vet Clin Pathol*. 1990;19(2):45-50.
39. Meléndez-Lazo A, Tvarijonaviciute A, Cerón JJ, Planellas M, Pastor J. Evaluation of the relationship between selected reticulocyte parameters and inflammation determined by plasma C-reactive protein in dogs. *J Comp Pathol*. 2015;152(4):304-312.
40. Radakovich LB, Santangelo KS, Olver CS. Reticulocyte hemoglobin content does not differentiate true from functional iron deficiency in dogs. *Vet Clin Pathol*. 2015;44(4):511-518.

How to cite this article: Seejarim C, Rautenbach Y, Hooijberg EH, Leisewitz AL, Schoeman JP, Goddard A. Regenerative response in dogs naturally and experimentally infected with *Babesia rossi*. *Vet Clin Pathol*. 2023;52:422-432. doi:10.1111/vcp.13228