Research Article

Seroprevalence of Canine Ehrlichiosis and Microscopic Screening for Canine Babesiosis in Dogs in Harare, Zimbabwe, 2016-2017

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A cross-sectional study was done to determine ehrlichiosis seroprevalence and babesiosis prevalence in dogs that were presented to selected veterinary clinics in Harare. Sera from randomly selected dogs were tested for antibodies to Ehrlichia spp. using an enzyme-linked immunosorbent assay while microscopy of peripheral blood smears was used to confirm babesiosis. Overall, 75.2% (88/117, 95% CI: 66.2–82.5) of sera samples tested were positive to Ehrlichia spp. antibodies while the prevalence of canine babesiosis was 47.9% (56/117, 95% CI: 38.6–57.3). Age, breed, and sex were found not to be associated with the two disease conditions (p > 0.05).

Most of the dogs with babesiosis (82.1%, 46/56) were also positive to Ehrlichia spp. antibodies. Hypoalbuminaemia (53.8%, 63/117), anaemia (53.0%, 62/117) and thrombocytopaenia (40.2%, 47/117) were the most common laboratory findings. Thrombocytopaenia and hypoalbuminaemia was more pronounced in dogs with babesiosis only while anaemia was more marked in dogs with ehrlichiosis and positive to Ehrlichia spp. antibodies.

1. Introduction

Canine ehrlichiosis, a potentially fatal disease of dogs is caused by Ehrlichia species. The disease has acute, subclinical and chronic stages [1] and clinical findings in dogs vary with the stage of the infection [2]. Clinical signs observed in the acute phase of the disease include fever, anorexia, oculo-nasal discharges, vomiting, weight loss, hepatosplenomegaly, lymphadenopathy and, rarely epistaxis and haemorrhage [1, 3]. The chronic phase is marked by epistaxis, haematuria, petechiae, ecchymosis distributed over skin surface, respiratory distress, ocular abnormalities, and CNS signs [1]. Previous canine ehrlichiosis studies in Zimbabwe showed an overall seroprevalence of 42% [4]. Dogs are naturally infected with several species; E. canis, E. chaffeensis, and E. ewingii [5] with E. canis being the most common and causing the most severe clinical disease in Africa and Asia [6]. Although several Ehrlichia spp. are able to cause natural disease in dogs, only E. canis and E. ruminantium are known to occur in southern Africa [6]. However, serological evidence of antibodies against E. chaffeensis, E. ewingii, and E. ruminantium from dog sera in South Africa and Zimbabwe has been documented [7–10]. Some studies from Venezuela and Costa Rica have suggested that E. canis might be zoonotic [11, 12].

Canine babesiosis is a disease of worldwide significance that causes fever, haemolytic anaemia, haemoglobinuria and death [13]. It is an important disease of domestic and wild canidae [14]. The most common clinical signs associated with babesiosis are anorexia, fever, depression/lethargy, pale mucosae, splenomegaly, and weight loss [13]. Canine babesiosis studies in Zimbabwe are limited, with two studies reporting a prevalence of 6.9% and 26% [4, 15]. The disease is caused by three strains of the large Babesia canis namely, B. canis, B. rossi, and B. vogeli; the small B. gibsoni and the microbabesiae; B. microti, B. vulpes and B. conraeda [16]. In Africa, the small-sized Babesia has been reported in East and North Africa [13, 17, 18] with the rest of Africa reporting the large-sized Babesia [13, 19, 20] and there is no report of the microbabesiae [21, 22]. However, currently there is no literature on Babesia spp. infecting dogs in Zimbabwe.
Dogs can have concurrent infections with various *Babesia*, *Bartonella*, *Ehrlichia* and *Rickettsia* species [23] and those with a heavy tick exposure can be infected at a higher rate with multiple and potentially zoonotic tick-borne pathogens [23]. Worldwide, tick-borne diseases are an important cause of morbidity and mortality in dogs with the brown dog tick, *Rhipicephalus sanguineus* being implicated as a vector of *E. canis*, *B. vogeli*, and *B. gibsoni* [1, 22]. Hence, the transmission occurs when *R. sanguineus* takes a blood meal from the dog [1, 2]. Concurrent infections of *E. canis* with *Babesia* spp. have been reported [4, 24, 25] leading to more severe case outcomes [26]. The epidemiology of canine tick-borne diseases may change due to the effects of climate change and the ease of international travel [27]. Studies about the prevalence of *Babesia* and *Ehrlichia* spp. co-infections in dogs in Zimbabwe are limited [4]. The first objective of this study was to determine the seroprevalence of ehrlichiosis and the prevalence of babesiosis. The second objective was to determine the prevalence of *Ehrlichia* spp. seropositivity in dogs with babesiosis and the common clinicopathological findings.

2. Materials and Methods

2.1. Study Location, Design and Collection of Blood Samples. This study was conducted in urban Harare, Zimbabwe where a cross-sectional study was employed to collect blood samples from dogs between October 2016 and March 2017. The blood samples were collected from dogs presented for routine elective surgery or ill-health at randomly selected private veterinary practices. A systematic random sampling technique was used to select dogs presented at the selected private veterinary practices; the first dog being selected using simple random sampling and every tenth dog thereafter. The selected dogs were restrained manually and whole blood was collected from the cephalic vein into 5 ml plain and ethylene diamine tetra-acetic acid (EDTA) tubes. Serum obtained through centrifugation at 2500 rpm for 10 minutes using a Sigma 3E-1 centrifuge (Sigma Harz, Germany) was stored at −20°C prior to use for *Ehrlichia* spp. serological testing. The EDTA blood was used for complete blood counts and microscopy of peripheral blood from an ear vein was used for detection of *Babesia* piroplasms from Giemsa-stained thin blood smears. Data recorded during blood samples collection included age, sex, and breed of the dog.

2.2. Testing for *Ehrlichia* spp. Antibodies and *Babesia* Piroplasms. The ImmunoComb* Canine *Ehrlichia* Antibody Test Kit (Biogal-Galed Laboratories, Israel) was used to detect IgG antibodies against *Ehrlichia* spp. from the collected dog sera. The test was carried out according to the manufacturer’s instructions (http://www.biogal.co.il) and as previously described [28, 29]. The results were read with a calibrated colour Comb Scale (graded 50–56), which was provided with the test kit. A scale of 53, which is equivalent to a positive immune response at a titre of 1:80 by an indirect fluorescent antibody (IFA) test, was considered as the “cut-off” level of IgG antibodies (http://www.biogal.co.il). Hence, in this study serum samples with a Comb Scale score of ≥53 (i.e. ≥1:80 titre) were considered to be positive for *Ehrlichia* spp. antibodies. Microscopy was used for the detection of *Babesia* piroplasms. Giemsa-stained, thin peripheral blood smears were prepared from peripheral blood from an ear vein. Two peripheral blood smears were made and from each, a minimum of 100 fields was microscopically examined for the presence of *Babesia* piroplasms. Careful examination of the Giemsa-stained thin peripheral blood smears was done by well-trained and experienced technologists.

2.3. Haematology and Clinical Chemistry. EDTA blood of dogs testing positive for *Babesia* and *Ehrlichia* spp. antibodies was subjected to a complete blood count using an automated haematology analyzer (BC-2008 Vet-Shenzhen Mindray Bio-medical Electronics, China). The blood samples were analyzed to measure haematocrit (HCT), total number of erythrocytes (RBC), haemoglobin concentration (HB), mean erythrocyte volume (MCV), mean corpuscular haemoglobin content (MCH), mean haemoglobin concentration in erythrocytes (MCHC), erythrocyte distribution width (RDW), and total number of platelets (PLT). The leukogram measurement included total number of leukocytes (WBC), absolute number of neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), and eosinophils (EOS). An automated chemistry analyzer (Humastar 180™—Human GmbH, Wiesbaden, Germany) was used to measure albumin and globulin. Reference values for all the parameters were adopted from Diagnopath Veterinary laboratory (Pvt) Ltd, Harare.

2.4. Data Analysis. The recording and editing of the data was performed using Microsoft Excel®. A statistical package called Epicalc (2000) version 2, was used to analyze all the raw data. The total number of positive/seropositive animals was calculated from the total number of samples tested over the study period and expressed as a percentage. Positive/seropositive animals were examined in relation to sex, breed, and age. The Chi-square test was used to measure differences in proportions between generated categories of babesiosis and ehrlichiosis status and values of p < 0.05 were considered as significant. Seropositivity/positivity was also analyzed according to HCT, RBCs, MCV, hemoglobin, MCHC, platelets, WBCs, total protein, and albumin. Association between the above blood parameters was evaluated by calculating the Chi-square test, relative risk and the 95% confidence interval using Epicalc version 2.

3. Ethical Considerations

Ethical approval for use of dogs and for all protocols in this study was obtained from the Ethical and the Higher Degree committees of the Faculty of Veterinary Science reference number VEHDC 2016/05. The purpose of this study was well explained to the veterinary practitioners stationed at all the veterinary clinics in Harare, who all expressed consent to participate in the study. Verbal and written consent was obtained from owners whose dogs were selected for the study. Standard operating procedures were followed for collection of blood samples.
4. Results

4.1. Canine Ehrlichiosis Seroprevalence and Babesiosis Prevalence. Tables 1 and 2 show the distribution of sampled dogs, ehrlichiosis seroprevalence and babesiosis prevalence according to different categories. A total of 117 serum samples were collected and the overall ehrlichiosis seroprevalence was 75.2% (88/117; 95% CI: 66.2–82.5) whilst babesiosis prevalence was 47.9% (56/117; 95% CI: 38.6–57.3). Seropositivity/positivity to the two diseases was found not to be associated with the age, breed, sex and health status of the dogs. Of the ehrlichiosis seropositive dogs, 65.9% (58/88) had a titer of 1:80 and 34.1% (30/88) a titer of >1:80. Five and seven dogs that were ehrlichiosis seropositive had a titer of 1:160 and 1:320, respectively. Of the total serum samples tested, 39.3% (46/117; 95% CI: 30.5–48.8) were Babesia spp. positive and also seropositive to Ehrlichia spp. Most of the dogs with babesiosis (82.1%, 46/56) were positive to Ehrlichia spp. antibodies.

4.2. Haematological Findings. There were no significant differences (p > 0.05) noted on the erythrogram and leukogram of apparently healthy and ill dogs. The changes of the erythrogram and leukogram are presented in Table 3. Hypoalbuminaemia (53.8%, 63/117), anaemia (53.0%, 62/117), and thrombocytopenia (40.2%, 47/117) were the most common laboratory findings. Anaemia, evidenced by decreased HCT, absolute RBC and HB values was the main erythrogram change noted. A significantly (p < 0.01) higher percentage of dogs positive to both Babesia and Ehrlichia spp. recorded decreased values of these parameters compared to those seropositive to Ehrlichia spp. only. The anaemia, as measured by the decreased mean HCT was more pronounced in those dogs positive to both Babesia and Ehrlichia spp. (24.1 ± 0.6) compared to those positive to Babesia spp. only (25.3 ± 1.4) and Ehrlichia spp. only (27.6 ± 2.8). On the leukogram, eosinopenia and monocytopenia, accompanied with a neutrophilic and lymphocytic leukocytosis were the main changes noted. Lymphocytosis and neutrophilia was more pronounced in dogs positive to both Babesia and Ehrlichia spp. However, no significant differences (p > 0.05) in the values of these parameters were noted among the different groups of dogs.

Thrombocytopenia, as measured by decreased platelets count was recorded in 52% of dogs positive to both Babesia and Ehrlichia spp., 30% and 26% of those positive to Babesia spp. only and Ehrlichia spp. antibodies only, respectively. The difference in percentages was significant (p < 0.05) between dogs positive to both Babesia and Ehrlichia spp. and those positive to Ehrlichia spp. antibodies only. The decreased mean
Table 3: Percent of dogs with below and above the normal range measured haematological parameters according to infection status.

| Parameter  | Normal range | % dogs with values below [above] normal range |
|------------|--------------|-----------------------------------------------|
| HCT (%)    | 37–55        | Babesiosis only (n = 42) 70.0 [0.0] 82.6 [2.2] |
| RBC (10^12/L) | 5.5–8.5 | Babesiosis only (n = 10) 50.0 [0.0] 71.7 [0.0] |
| HB (g/L)   | 12–18        | Babesiosis only (n = 10) 70.0 [0.0] 67.4 [2.2] |
| MCV (fL)   | 60–77        | Babesiosis only (n = 10) 0.0 [30.0] 4.3 [13.0] |
| MCH (pg)   | 19–23        | Babesiosis only (n = 10) 10.0 [30.0] 4.3 [58.7] |
| MCHC (g/L) | 32–36        | Babesiosis only (n = 10) 40.0 [0.0] 15.2 [19.6] |
| RDW (%)    | 11.6–14.8    | Babesiosis only (n = 10) 14.3 [35.7] 40.0 [20.0] |
| PLT (10^9/L) | 200–500 | Babesiosis only (n = 10) 26.2 [2.4] 30.0 [0.0] |
| WBC (10^9/L) | 6–17 | Babesiosis only (n = 10) 2.4 [26.2] 20.0 [10.0] |
| NEU (10^9/L) | 3–12.5 | Babesiosis only (n = 10) 2.4 [31.0] 10.0 [20.0] |
| LYM (10^9/L) | 1–4 | Babesiosis only (n = 10) 4.8 [21.4] 10.0 [40.0] |
| MONO (10^9/L) | 0.1–1.35 | Babesiosis only (n = 10) 33.3 [11.9] 50.0 [20.0] |
| EOS (10^9/L) | 0.1–1.25 | Babesiosis only (n = 10) 95.3 [0.0] 90.0 [10.0] |
| ALB (g/L)  | 28–40        | Babesiosis only (n = 10) 45.2 [0.0] 80.0 [0.0] |

HCT: Haematocrit; RBC: red blood cells; HB: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin content; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width; PLT: platelets; WBC: white blood cells; NEU: neutrophils; LYM: Lymphocytes; MONO: monocytes; EOS: eosinophils; ALB: albumin.

platelets count was lowest for those positive for Babesia spp. only (116.7 ± 21.5), followed by dogs positive to both Babesia and Ehrlichia spp. (134 ± 18.7) and lastly for those positive to Ehrlichia spp. antibodies only (139 ± 22.3) but the differences were not significant (p > 0.05).

Eighty percent of dogs with babesiosis only, 69.6% positive to both Babesia and Ehrlichia spp. and 45.2% with ehrlichiosis only had hypoalbuminemia as measured by a decreased value of albumin. The difference in percentages was significant (p < 0.05) between the dogs positive to both Babesia and Ehrlichia spp. and those positive for Ehrlichia spp. antibodies only. The hypoalbuminemia was more pronounced in those positive to Babesia only (21.9 ± 1.5) compared to dogs positive to both Babesia and Ehrlichia spp. (23.8 ± 0.7) and Ehrlichia spp. antibodies only (24.2 ± 0.8).

5. Discussion

The lack of significant differences noted on the erythrogram and leukogram of apparently healthy and ill dogs was an unexpected finding. This was probably because the ill dogs had a low grade of babesiosis and/or ehrlichiosis infection. Due to lack of clinical details and follow-up of ill dogs, the intensity of babesiosis and/or ehrlichiosis infection could not be determined. The erythrograms of healthy dogs were not determined. In addition, ehrlichiosis seroprevalence could probably have been underestimated as dogs with Ehrlichia infection will self-cure and remain seropositive for variable periods thereafter [6]. Cross-reactivity between antibodies against Ehrlichia spp. has been reported [7–9] and in areas where they co-exist their serological differentiation may therefore not be possible and this is a limitation to our study. The use of molecular techniques (PCR and sequencing) using parasite-specific primers would have provided a better diagnostic tool in terms of both sensitivity and specificity and along with the identification of the infecting species.

Blood smear examination is a useful diagnostic tool for clinical babesiosis in dogs and microscopy evaluation continues to be the easiest and most accessible diagnostic test for most laboratories [32]. Although microscopy is highly specific and can be used to diagnose the large forms of Babesia, the
small piroplasms are hard to observe by light microscopy which has poor to moderate sensitivity and expertise is needed [22, 33]. In addition, the limit of parasites detection in a thin blood smear is reported to be parasitemias of 0.5% [34]. For these reasons, our study might have underestimated the prevalence of babesiosis in the studied dogs and more sensitive molecular PCR-based methods should be used in future studies.

Our study found a higher ehrlichiosis seroprevalence (75.2%) than that previously (52%) recorded [4]. Similarly, the current study reported a higher canine Babesia prevalence (47.9%) than an earlier report of 26% [4]. A recent PCR-based study on Babesia in apparently healthy rural dogs in the country also found a low prevalence (6.9%) of Babesia spp. antigen [15]. The prevalence was also much higher than that reported by Rautenbach et al. [35]. Differences in study areas and in testing regimes (e.g. for Ehrlichia spp.) could probably account for the variations. Babesia spp. identification was a limitation of the present study and there is no literature on the species occurring in the country. However, B. gibsoni and B. rossi have been confirmed in neighbouring Zambia [14] while B. rossi and B. vogeli are known to occur in South Africa [13]. Further studies are required to determine the tick vectors and, the Babesia and Ehrlichia spp. present in dogs in the country and also to assess their roles in the clinical and pathological picture of the diseases. In support of earlier observations in the country [4], co-presence was shown with over 80% of Babesia spp. positive dogs also being seropositive to Ehrlichia spp. antibodies. The ehrlichiosis/babesiosis concurrent prevalence found in this study (39.3%) was more than twice that was previously reported (17%) in the country [4] and this phenomenon has been reported elsewhere [25, 36–38]. Rhipicephalus sanguineus, the brown dog tick transmits both parasites [37] and this is likely to account for the observed findings.

Anaemia and thrombocytopenia have been demonstrated as consistent laboratory findings in canine babesiosis and ehrlichiosis [13, 25, 36, 39, 40]. In an earlier study in the country, the two were the most common laboratory abnormalities observed in dogs with Babesia and Ehrlichia infection [4]. Despite having no signs of the disease, Babesia and Ehrlichia positive dogs in the current study had anaemia and thrombocytopenia. However, thrombocytopenia was observed in less than a third of dogs with either babesiosis or ehrlichiosis but in more than half of those with both. In Zambia, thrombocytopenia was also observed in less than one-fifth of Babesia infected dogs [14]. The low number of dogs with thrombocytopenia might be probably due to the fact that serology was performed for ehrlichiosis which indicates past exposure rather than active infection.

In this study, leukocyte abnormalities were nonspecific and most dogs had eosinopaenia and monocytopaenia while neutrophilia, lymphocytosis, and leukocytosis were observed in some of the dogs. Niwetpathomwat et al. [36, 40] also observed nonspecific leukocyte abnormalities in Babesia and Ehrlichia infected dogs. The observed abnormalities of eosinopaenia, monocytopaenia and neutrophilia are in agreement with other studies for ehrlichiosis [37, 39]. One of the predominant biochemical abnormalities found in dogs infected with E. canis is hypoalbuminaemia [38, 41]. In this study, hypoalbuminaemia was constantly observed in dogs either positive to one or both pathogens.

Thrombocytopenia and hypoalbuminaemia was more pronounced in dogs positive to Babesia spp. only while anaemia was more marked in those positive to both Babesia and Ehrlichia spp. The severity of anaemia and thrombocytopenia in Babesia–Ehrlichia concurrent infections was intermediate to that of individual infections [4]. In Babesia–Ehrlichia mixed infection the disease was reported to be more severe [42]. Manzillo et al. [43] reported that anaemia and thrombocytopenia are more common in dogs with babesiosis than those with ehrlichiosis. Single infections with B. gibsoni or B. canis gave more severe haematological results than mixed infections [14]. The observed differences in severity of the laboratory abnormalities are likely dependent on Babesia/Ehrlichia spp. in individual and concurrent infections. Hence, it is difficult to ascribe haematological abnormalities to any of the Babesia/Ehrlichia spp. Tsachev et al. [37] indicated that variations in haematological profiles in Ehrlichia infected dogs may be related to differences in the virulence of Ehrlichia spp. strains, antigen heterogeneity of this bacterial agent and the clinical form of the disease. In this study, no particular stage of ehrlichiosis was selected for evaluation.

The laboratory abnormalities observed, particularly anaemia and thrombocytopenia can affect the outcome of routine surgeries [30]. Hence, before such procedures are done, testing for Ehrlichia/Babesia in dogs from the study area should be considered. According to Kelly [6], although apparently healthy, dogs subclinically infected with E. canis usually have laboratory abnormalities, especially thrombocytopenia, anaemia, hypoalbuminaemia and leukocytosis singly or in combination. In our study, the dogs positive to Ehrlichia spp. antibodies were apparently healthy and had the above mentioned laboratory abnormalities further supporting that many natural E. canis infections are subclinical [26].

In conclusion, this study showed a high prevalence of Babesia/Ehrlichia presence in the study dogs. The haematology and biochemical profiles are similar to the results observed in other reports with the most significant abnormalities being anaemia, thrombocytopenia and hypoalbuminaemia. Thrombocytopenia and hypoalbuminaemia was more pronounced in dogs with Babesia only while anaemia was more marked in those positive to both Babesia and Ehrlichia spp. However, there were no laboratory abnormalities which could be utilized to differentiate between individual and concurrent presence. Further studies are required to determine the Babesia and Ehrlichia spp. present in dogs in the country.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no financial or personal relationships(s) that may have inappropriately influenced them in writing this article.
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