



Short communication

The epidemiology of canine *Babesia* infections in ZambiaKing S. Nalubamba*, Careen Hankanga, Ntombi B. Mudenda¹, Maxwell Masuku

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ABSTRACT

This study of 1196 dogs over a period of 18 months determined the seasonal infection patterns of canine babesiosis in Lusaka, the capital city of Zambia. The work also describes a retrospective study of the prevalence of canine babesiosis in laboratory clinical blood samples submitted to the University of Zambia, School of Veterinary Medicine for routine haematological examination from the year 1994 to 2009. A cross-sectional study was also performed to determine the levels of *Babesia* in a low-income society (during the dry season and the wet season of the year), where 361 samples were collected from dogs presented for mass rabies vaccination campaigns. Morphology of the *Babesia* indicated that all were of the large-sized *Babesia canis* infection. *Babesia*-positive dogs had significantly higher rectal temperatures than negative ones, and dogs younger than 1 year were more likely to be *Babesia* positive followed by those between 2 and 5 years old. Seasonal trends indicate two peaks, one in the rainy season (November–March) and another in the cold dry season (June/July). Monthly prevalence rates of *Babesia* ranged from 0% to 2.4% in natural populations and from 0% to 28.6% in laboratory specimens. This study shows that Zambia has lower *Babesia* prevalence than reported in other African countries.

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Canine babesiosis is a vector-borne disease caused by intra-erythrocytic protozoa that induces anaemia, fever, jaundice, splenomegaly, thrombocytopaenia and, occasionally, haemoglobinuria. It is caused by protozoa of the genus *Babesia* and diagnosis of canine babesiosis is made by the microscopic detection of parasites in peripheral blood smears or by serological tests, flow cytometry and polymerase chain reaction (PCR). There are many strains of canine *Babesia* species (Kjemtrup et al., 2000), with *Babesia canis* and *B. gibsoni* being two organisms that are commonly the cause of the disease. The two organisms cause clinically indifferent disease manifestations but can be differentiated by microscopy (*B. canis* is larger ~4–5 µm; *B. gibsoni* is smaller ~1–2.5 µm (Ayoob et al., 2010; García,

2006)), serology and PCR. Packed cell volumes (PCVs) in *Babesia* infections are reported to be between 8 and 28% in hyperacute cases and between 35 and 41% in chronic cases (Abdullahi et al., 1990), with other haematological changes such as thrombocytopaenia, anisocytosis and neutropenia reported (Ayoob et al., 2010; Mathe et al., 2006; Zygnier et al., 2007).

Infections may be asymptomatic but the disease sometimes presents or degenerates into hyper-acute septic shock-like syndromes, which are highly fatal in dogs (Matijatko et al., 2009) with mortality rates of 10–15% compared with rates of less than 2% in uncomplicated infections. In Africa, cases of *Babesia*-induced septic shock syndromes have been reported in South Africa (Jacobson, 2006) and Nigeria (Abdullahi et al., 1990).

Canine *Babesia* is transmitted by tick vectors of the genera *Dermacentor* (*B. canis canis* in Europe), *Rhipicephalus* (*B. canis vogeli* in tropics and subtropical countries) and *Haemaphysalis* (*B. canis rossi* in South Africa). Some authors suggest direct transmission of *Babesia* via dog fights (Bashir et al., 2009).

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Various authors describe seasonal peaks in *Babesia* prevalence related to vector and other transmission dynamics (Leschnik et al., 2008; Maia et al., 2007; Martinod and Gilot, 1991; Oduye and Dipeolu, 1976) in Austria, Brazil, France and Nigeria, respectively. The seasonal trends of *Babesia* infection in dogs in Zambia has not been previously studied or reported, though it is commonly thought to increase during the rainy season when the vector burdens also increase. There is no published literature on the species of the parasite that exists in this country despite there being numerous cases of the infection that are diagnosed microscopically. This study was thus designed to address the lack of information on the prevalence and epidemiology of canine babesiosis in Zambia.

1. Materials and methods

1.1. Study area

The study was carried out in Lusaka, Zambia, Central Africa. Zambia has a tropical climate, with three seasons described. It has a distinct warm, wet rainy season between November and April the following year, followed by a cooler dry season (May–July) and, finally, a hot dry season that precedes the rainy season. The retrospective study was carried out using records from the University of Zambia School of Veterinary Medicine, service laboratory. The University Laboratory receives samples from veterinarians and clients in Lusaka. Monthly meteorological data were obtained from the meteorological department of Zambia for the duration 1994–2009.

1.2. Sampling and laboratory analysis

1.2.1. Private practice samples

Blood samples ($n = 1196$, (683 = male and 513 = female)) from dogs from within and around Lusaka that were seeking routine attention at a private veterinary clinic were collected from March 2008 to August 2009. This pseudo-longitudinal study involved sampling each subject at presentation to the practice for routine procedures such as vaccination, deworming and grooming, and the data collated monthly. Samples were conveniently obtained from pets whose owners agreed to the sampling and data captured on a form. Informed consent was sought from owners to participate in the survey and collect blood from their dogs. Patient data recorded at the time of blood collection included age, sex, colour of mucous membranes, presence of ectoparasites, rectal temperature, body condition score and presence/absence of lymphadenopathy.

Ethylene diamine tetraacetic acid (EDTA)-anticoagulated blood was collected from the cephalic vein of the dogs for Giemsa-stained thin blood smear examination to detect *Babesia* and complete blood counts (Jain, 1995). Two blood smears were made from each sample and a minimum of 100 fields of each stained blood smear were examined under oil immersion to determine parasitaemia.

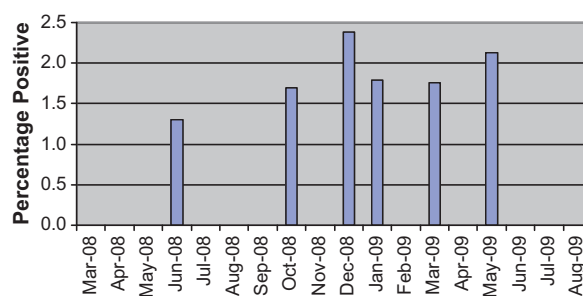


Fig. 1. Mean monthly percentage positive of *Babesia* by microscopic examination of blood smears in dogs presenting for routine prophylactic procedures at a Private practice in Lusaka March 2008 to August 2009 ($n = 1196$).

1.2.2. Mass rabies vaccination campaign samples

Blood samples ($n = 361$, 202 males and 159 females) were also collected for a cross-sectional survey from dogs presented for a mass rabies vaccination campaign in two low-income residential areas of Lusaka during the dry season (October 2008) and also during the wet season (December 2008). Owner, patient and blood sample processing were as described in the private practice sampling above.

1.2.3. Laboratory records

Retrospective analysis of laboratory records ($n = 7197$) from the University of Zambia School of Veterinary Medicine small animal clinic service laboratory was also carried out to diagnose *Babesia* infections in routine patient blood samples submitted to the laboratory. Records that were examined were from October 1994 to December 2009 (excluding the period from September 2003 to August 2004, due to missing laboratory files).

1.3. Statistical analysis

Statistical analysis and data graphing was done using MINITAB® Release 14 software for Windows®. The chi-square test was used to determine differences in proportion positives between seasons and age groups. Mean rectal temperatures in *Babesia*-positive and negative subjects were compared using the one-way analysis of variance (ANOVA). Significant differences were defined as those with $p \leq 0.05$.

2. Results

Fig. 1 graphically depicts the monthly prevalence of *Babesia* in clinically healthy dogs presented for routine prophylactic procedures from March 2008 to August 2009. The graph shows no clear-cut seasonal pattern in percentage positives but trends towards higher percentage positive (1.7 (1/59)–2.4% (2/84)) during the rainy season (December–March) and the colder months of the year (May and June, 2.1% (1/47) and 1.3% (1/77), respectively). Cross-sectional survey revealed a proportion of *Babesia* positives of 0.5% (1/200) in the dry season and 0.62% (1/161) in the rainy season. There was no significant difference in the two season's proportion positives, ($\chi^2 = 0.024$, $df = 1$). However,

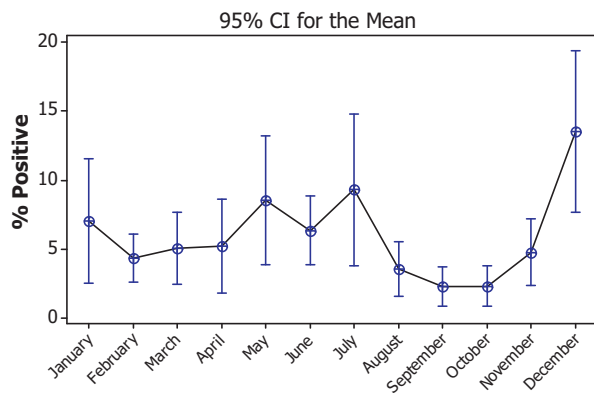


Fig. 2. Interval plot of mean monthly *Babesia* prevalence in laboratory samples from the University of Zambia, School of Veterinary Medicine, Service Laboratory; 1994–2009 ($n = 7197$).

the chi-square approximation may not be reliable due to two values being less than 5 and should thus be taken with caution.

There were significant differences ($p < 0.01$) between the mean rectal temperatures of *Babesia*-positive (mean 39.4°C ; range $38.2\text{--}40.5^{\circ}\text{C}$ ($\text{SD} = 0.66$); $n = 10$) and non-positive dogs (mean 38.7°C ; range $36.9\text{--}40.1^{\circ}\text{C}$ ($\text{SD} = 0.69$); $n = 1103$). Of the dogs examined, 57.2% (684/1196) and 63.4% (229/361) demonstrated lymphadenopathy in the pseudo-longitudinal study and cross-sectional study, respectively, and 90% (9/10) of all the positive cases demonstrated lymphadenopathy. Of animals that were included in the pseudo-longitudinal and cross-sectional survey, 59.3% (923/1557) had single or multiple tick species present either on the ears or on the body.

Morphological characteristics of the *Babesia* in positive samples indicated that all were of the large-sized *B. canis* infection. PCVs in *Babesia* positive dogs ranged from 9% to 56% and differential white cell counts were non-specific in these *Babesia*-positive dogs.

Fig. 2 shows the 95% confidence interval (CI) of the mean monthly prevalence of *Babesia*-positive samples from the retrospective analysis of laboratory blood samples submitted to the University of Zambia Veterinary Teaching Clinics service laboratory from 1994 to 2009. The data show that the mean average monthly prevalence of *Babesia* in routine diagnostic blood samples differs from month to month, and the prevalence is lowest during the months of September and October. There are no significant differences between these two low values with other months of the year other than December, which also has a statistically significantly higher prevalence than August and February. The highest monthly prevalence was 28.6% (8/28) in the month of December 2007. The highest average monthly prevalence over the retrospective study period from 1994 to 2009 was 12.4% (range 1.9–28.6%; $\text{SD} = 8.58$) in the month of December, and the lowest was 1.6% (range 0–6.3%; $\text{SD} = 2.2$) during the month of October (data not shown). The year 2001 demonstrated the highest average prevalence of 10.4% (49/467), while 2006 had the lowest prevalence of 3% (12/383) (data not shown). A chi-square test was performed to determine if *Babesia* presence is associated with

season and the result showed that the relationship between *Babesia* presence in laboratory samples and the three seasons is significantly different ($\chi^2 = 56.1$, $df = 2$, $p < 0.01$). There was a significant difference between the dry hot season and the rainy season and cold dry season ($\chi^2 = 48.2$ and 50.6 , $df = 1$, $p < 0.01$), but no significant differences between the rainy season and the dry cold season ($\chi^2 = 0.6$, $df = 1$, $p = 0.431$). The average annual prevalence rates do not correlate to any weather pattern, such as recorded annual rainfall for the previous rainy season or average yearly temperatures (data not shown).

Retrospective analysis showed that of the *Babesia*-positive patients, 38.7% were dogs younger than 1 year (17.0% 1–2 years; 32.1% >2–5 years; 12.3% >5 years), and the prevalence was significantly different between these dogs and older dogs ($\chi^2 = 7.9\text{--}20.6$, $df = 1$, $p < 0.05$). There were no differences between dogs aged 1–2 years and 2–5 years, 1–2 years and >5 years and 2–5 years and those more than 5 years old, ($\chi^2 = 0.1\text{--}1.0$, $df = 1$, $p > 0.31$). This study also showed that 69% of the positive samples were from male dogs, and the prevalence of *Babesia* in male dogs is higher than that in females (5.6% vs. 4.3%) but the difference is not significant.

3. Discussion

There has been very limited research and few publications on diseases of companion animals other than rabies in Central Africa. This is the first report of the seasonal patterns of *Babesia* in dogs in Central Africa and Zambia, in particular. The results of this study indicate that the percentage of *Babesia* positive dogs in natural populations is quite low and that is similar to findings by Soares and co-workers ((2006) cited by Dantas-Torres and Figueredo, 2006) in Brazil and different to finding by Oduye and Dipeolu (1976) in Nigeria, who found a very high canine *Babesia* prevalence of 41–53%. The differences with the findings in Nigeria may be due to climatic differences with Zambia having a warmer dry climate than Nigeria's warm wetter climate. Zambia also has a longer dry season and the unfavourable climatic conditions that result, such as low relative humidity and high ambient temperatures, may induce ticks to undergo diapause and inhibit reproduction and questing behaviour and, thus, consequently reduce chances of infecting hosts (Maia et al., 2007) and thus lower canine *Babesia* prevalence. Unfortunately, there is no published literature on seasonal canine tick vector biology in Zambia or Africa; and a study designed to correlate vector burdens and species involved with *Babesia* epidemiology would yield useful data.

Findings of annual average *Babesia* prevalence of 5.7% by microscopic examination in laboratory blood samples in this study are lower than findings by other researchers in Australia, who found *Babesia* seroprevalence of 35.7% in hospital populations (Trapp et al., 2006). This could be attributed to the fact that serology is a more sensitive method of detection of previous, current and sub-clinical infection than microscopy and is thus likely to detect more animals as being positive. The prevalence of *Babesia* in clinical blood samples was higher than that found in normal/healthy dog survey samples. This may

be attributed to the fact that these blood samples were submitted to the laboratory to detect disease (that may include babesiosis) and/or any disease may lower an animal's immunity to allow low, sub-clinical parasitaemias to become microscopically evident. Peaks in the proportion of *Babesia*-positive dogs were observed in May and June in the pseudo-longitudinal study and in May, June and July in the retrospective study. This could be attributed to the dog breeding season, which occurs during these months; dogs are more likely to roam in search of mates and this could increase their likelihood of contact with tick vectors as well as being involved in dog fights and contacting the infection (Bashir et al., 2009). This could also be attributed to the fact that during the cold season in Zambia, average daily temperatures would lie between 10 and 20 °C, and this falls within the range (12–17 °C) that has been described as the optimal temperatures for occurrence of babesiosis, by Leschnik et al. (2008).

This study showed that the prevalence of *Babesia* in male dogs in Zambia is higher than in females, but the difference was not significant. This lack of sex bias concurs with the findings by other researchers (Martinod et al., 1986) but differs from findings by others (Bashir et al., 2009), who found that male dogs have a significantly higher prevalence than female dogs. The age distribution of dogs that were positive for *Babesia* in this study were similar to the findings of Oduye and Dipeolu (1976) in Nigeria and Bashir et al. (2009) in Pakistan where both groups found that younger dogs were more likely to have the infection than older ones. The unremarkable differential white cell count found in *Babesia*-positive dogs is similar to findings by other researchers (Niwetpathomwat et al., 2006), but sepsis leucograms would have been expected had there been complicated cases of babesiosis. The higher mean rectal temperature in *Babesia*-positive dogs was as expected.

As transfusion-associated transmission of *Babesia* has been demonstrated (Stegeman et al., 2003), the prevalence of *Babesia* in healthy populations will also have a bearing on the potential risk that blood from apparently healthy donors poses during canine blood transfusions where minimal amount of screening is routinely done for first-time transfusions in Zambia.

This study only demonstrated the large-sized *Babesia*, which is similar to findings by other researchers in Africa (Abdullahi et al., 1990; Jacobson, 2006). This large-sized form, when diagnosed microscopically, is assumed to be *B. canis* although recent evidence suggests that, other than the three known subspecies of *B. canis*, namely *B. canis canis*, *B. canis vogeli* and *B. canis rossii*, there is another distinct large form but yet unnamed subspecies reported in North America (Ayoob et al., 2010). In Africa, small-sized *Babesia* species have been reported in East Africa (Kjemtrup et al., 2000) with the rest of Africa reporting the large-sized *Babesia*, namely *B. canis vogeli* and *B. canis rossii* (Abdullahi et al., 1990; Jacobson, 2006). A *Babesia* seroepidemiological survey would be a useful follow-up study to give an indication of the seroprevalence of *Babesia* in Zambian dogs. However, serology would have the disadvantage of indicating past exposure but not current status (Inokuma et al., 2004). It would thus be desirable to do molecular studies on the archived collected samples to also determine

molecular prevalence as well as the exact subspecies of *Babesia* present in Zambia. A combination of a serosurvey and molecular survey would overcome shortcomings that arise from serosurveys or blood smear examinations surveys alone.

4. Conclusion

In conclusion, this study shows that the prevalence of *Babesia* in dogs in Zambia, as determined by blood smear examination, is quite low and that it seems to increase during the rainy season. Both pseudo-longitudinal and retrospective studies also showed a slight increase of *Babesia* in the cold dry season months of the year.

Conflict of interest

The authors of this article declare that none of them have financial or personal relationships with individuals or organisations that would unacceptably bias the content of this article.

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