INTRODUCTION

Canine babesiosis is a tick-transmitted, haemoparasitic disease of domestic dogs (Canis familiaris)\(^3,5\). The organisms belong to the genus Babesia, family Babesiidae, order Piroplasmida, within the phylum Apicomplexa\(^2\). Over 100 species of Babesia have been identified but only 2 (Babesia canis and B. gibsoni) are known to infect dogs\(^8,15,19\). Previously these organisms were classified according to their morphological appearance into the large species (B. canis) and small Babesia (B. gibsoni)\(^3,12,15\). However, recent molecular analyses and serological surveys have shown these organisms to be more genotypically diverse\(^2,12,15\). Genetic sequencing has confirmed 3 distinct subspecies of B. canis namely B. canis canis, B. canis rossi and B. canis vogeli\(^1,11,12\). In addition, at least 3 subtypes of small Babesia affecting dogs are thought to occur, including B. gibsoni (Asian type), B. conraadi and a B. microti-like organism\(^11,12\).

South African canine babesiosis is a heterogeneous complex of disease presentations caused by Babesia canis rossi, transmitted by Haemaphysalis leachi ticks\(^3,5,15,15\). This strain of babesia is widespread in South Africa and notoriously the most virulent\(^1,15\), costing the dog-owning public an estimated R20 million a year\(^5\). The uncomplicated clinical form of the disease is characterized by anaemia, fever and splenomegaly\(^3,5,10\). Complicated babesiosis presents as one or more syndromes of organ dysfunction, including cerebral, acute renal failure, hepatic dysfunction, myocardial disease, rhabdomyolysis, haemocoagulation, adult respiratory distress syndrome, pancreatitis, dermal necrosis, haemorrhagic diathesis and immune mediated haemolytic anaemia; and this form of the disease can have a high mortality rate\(^5,10,13,15\).

Babesiosis is a common cause of clinical disease in dogs in South Africa. A survey conducted at the Onderstepoort Veterinary Academic Hospital (OVAH) in the Gauteng province indicated that 12 % of sick canines presented were diagnosed with babesiosis\(^5\), while in a country-wide survey\(^2\) 27 % of practitioners considered complicated babesiosis common. About a third of the patients presented to the OVAH were hospitalised due to the severity of the disease and fatalities were common despite treatment\(^2\). Mortality rates vary between studies but range from 5 % in an academic hospital setting\(^7\) to 37 % estimated by practitioners, reaching \(>80 \%\) for cerebral cases\(^5\). The three most common drugs used to treat infections in South Africa are diminazene aceturate, imidocarb and trypan blue\(^5,14\). The diminazene and imidocarb treatments stabilise the infection\(^5,14,15\) which is not ideal in endemic regions where a lack of premunity puts dogs at risk of repeat infections\(^5\). Attempts at developing specific vaccines have proved successful\(^1,3,4\), but the duration of immunity is only 6 months\(^9\) and further research is required\(^14\). Therefore, canine babesiosis remains a major cause of morbidity in domestic dogs in South Africa. In the light of the high incidence of clinical disease, the severity of complicated babesiosis, the absence of premunity development and occurrence of repeat infections and the high mortality rate, prevention of tick transmission of this disease through control of the vector is an important cornerstone on which successful long-term prevention of clinical disease can be built\(^14,16\).

The objective of this study was to evaluate the efficacy of amitraz impregnated tick collars (Preventic–Virbac) as a tick-repellent in the control of South African canine babesiosis caused by B. canis rossi in a population of dogs from KwaZulu-Natal province, exposed to a high tick season from December 2005 to May 2006.

MATERIALS AND METHODS

Trial dog selection

Twenty dogs, which were PCR- and RLB-negative for Babesia canis rossi, were selected to enter the trial. These dogs originated from semi-rural and rural areas of KwaZulu-Natal, where there was...
a known high disease challenge. They all belonged to veterinarians and their signalment and distribution is given in Table 1. Each dog was clinically examined and screened for ticks and blood collected into EDTA, after which an amitraz impregnated tick collar (Preventic-Virbac) was applied. EDTA blood samples were collected from these dogs monthly from December 2005 to May 2006, and the dogs underwent a rudimentary clinical evaluation and screen for tick burdens on each occasion. At the end of the third month, the first Preventic-Virbac collar was replaced by the second according to the stipulated duration of efficacy.

Owing to the high incidence of canine babesiosis in this region, it was considered unethical to deny dogs acaricidal treatment. Therefore, it was decided that dogs presented to the regional welfare organisation (Pietermaritzburg Society for Prevention of Cruelty to Animals), with no history of previous tick control, would be used as control dogs. At each monthly bleed of the treatment group, 5 dogs were randomly selected from the Pietermaritzburg Society for Prevention of Cruelty to Animals (SPCA) to act as a control group. These control dogs had no history of acaricide treatment and a different set of 5 control dogs was used at every bleed. These dogs were not screened for tick burdens because the authors felt it might influence the random nature of selection.

**Babesia DNA Identification**

**DNA extraction**

DNA was extracted from 200 µl of whole blood using the Qiamp blood and tissue extraction kit (Qiagen, Hilden, Germany).

**PCR**

PCR was performed with primers RLB-F2 and RLB-R2 amplifying a fragment of 460–540 bp from the 18S rRNA gene spanning the V4 region. The conditions for the PCR included an initial step of 3 min at 37 °C, 10 min at 94 °C, 10 cycles of 94 °C (20 s) – 67 °C (30 s) – 72 °C (30 s), with lowering of annealing step after every second cycle with 2 °C (touchdown PCR). The reaction was then followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 30 s.

**Reverse line blot hybridization**

PCR-amplified products were tested with the RLB, as previously described. An additional plasmid control was used as an internal positive control to check whether all Babesia species-specific probes were correctly attached to the RLB membrane and functioning properly.

**Statistical analysis**

The total number of dogs that tested positive for *B. canis rossi* by PCR/RLB during the study period, was compared between the treatment and control group using the Fisher exact test. The 95% confidence interval of difference in cumulative PCR/RLB positive rate between groups was also calculated using the Farrington & Manning Score method. Only *P* values <0.05 were considered as significant (type error α = 5%).

**RESULTS**

The treatment group of 20 dogs had Preventic–Virbac collars applied at the beginning of December 2005 and collars were replaced at the beginning of March 2006. These 20 dogs remained negative for *B. canis rossi* throughout the trial period. The monthly control groups of 5 randomly selected dogs, which had no history of acaricide treatment, revealed *B. canis rossi*-positive individuals in each of the monthly groups (Table 2).

All 20 dogs with Preventic–Virbac collars remained PCR/RLB negative for *B. canis rossi* during the study period. In the untreated control group, on the other hand, 8 of 30 dogs tested positive for *B. canis rossi*. The cumulative rate of infection was statistically significantly different (*P* = 0.0155) between the groups. The PCR-positive dog in January 2006 died a week later despite treatment.

**DISCUSSION**

Amitraz is a formamidine pesticide that, depending on the concentration and route of application, can act as an acaricide or tick repellent. As a 9% impregnated collar (Preventic–Virbac) it acts as a tick repellent. Dogs become infected with *Babesia* following the bite of an infected tick and these ticks need to feed for 2–3 days for complete transmission to occur. Therefore, the hypothesis was that if the amitraz-impregnated collars could repel the tick vectors, this would effectively exclude transmission of canine babesiosis and thus prevent clinical disease. The PCR assay is the gold standard for detection of babesiosis infection and is an extremely sensitive technique, being able to detect parasitaemia of 0.0001% and to differentiate between the various *B. canis* and *B. gibsoni* genotypes.

The PCR/RLB analysis of the control group indicated high infection with *B. canis rossi* in this untreated population during the six-month trial period, which coincided with the peak tick season in KwaZulu-Natal. Eight of the 30 control dogs were PCR/RLB positive for *Babesia*.

<table>
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<th>Treatment group</th>
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<th>Feb 06</th>
<th>March 06</th>
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*This dog died from complicated babesiosis a week later.*
REFERENCES