# A field trial evaluation of the prophylactic efficacy of amitraz-impregnated collars against canine babesiosis (*Babesia canis rossi*) in South Africa

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## ABSTRACT

South African canine babesiosis caused by *Babesia canis rossi* is a common clinical disease in dogs in South Africa and remains a significant cause of domestic dog mortality. To determine whether tick-repellent, 9 % amitraz-impregnated tick collars (Preventic–Virbac) could prevent tick-borne exposure to *B. canis rossi,* 50 dogs were assigned to two groups. Group 1 (20 dogs), polymerase chain reaction (PCR)- and reverse line blot (RLB)-negative for *B. canis rossi,* were fitted with amitraz collars and blood samples collected monthly , over a 6-month period, and analysed for *B. canis rossi.* Group 2 (30 dogs) included 5 dogs selected on a month-by-month basis from a population of dogs from the same geographical area as the group 1 dogs, but with no history of previous tick control, which were blood-sampled together with the treatment group and analysed for *B. canis rossi* by PCR and RLB, to serve as the control group. Eight of the 30 control dogs (26.6 %) were PCR/RLB positive for *B. canis rossi,* indicating high pathogen exposure during the trial period. All twenty of the treatment group dogs remained negative for *B. canis rossi* throughout the 6 months of the trial. These results suggest that the use of amitraz-impregnated collars had a significant effect on reducing infection with *B. canis rossi.*


**Key words**: babesiosis control, polymerase chain reaction (PCR), reverse line blot (RLB), tick attachment, tick collar.

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## INTRODUCTION

Canine babesiosis is a tick-transmitted, haemoprotozoal disease of domestic dogs (*Canis familiaris*)[3,15]. The organisms belong to the genus *Babesia*, family Babesiidae, order Piroplasmida, within the phylum Apicomplexa[23]. Over 100 species of *Babesia* have been identified but only 2 (*Babesia canis* and *B. gibsoni*) are known to infect dogs[1–3,5,8,15]. Previously these organisms were classified according to their morphological appearance into the large babesias (*B. canis)* and small babesias (*B. gibsoni*)[3,12,15]. However, recent molecular analyses and serological surveys have shown these organisms to be more genotypically diverse[1,11,12,17]. Genetic sequencing has confirmed 3 distinct subspecies of *B. canis* namely *B. canis canis, B. canis rossi* and *B. canis vogeli*[3,15]. In addition, at least 3 subtypes of small *Babesia* affecting dogs are thought to occur, including *B. gibsoni* (Asian type), *B. conradae* and a *B. microti*-like organism[3,11,12].

South African canine babesiosis is a heterogeneous complex of disease presentations caused by *Babesia canis rossi,* transmitted by *Haemophysalis leachi* ticks[3,13,15]. This strain of babesia is widespread in South Africa and notoriously the most virulent[8,15,19], 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[3,5,8–10]. Complicated babesiosis presents as one or more syndromes of organ dysfunction, including cerebral, acute renal failure, hepatic dysfunction, myocardial disease, rhabdomyolysis, haemoconcentration, 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[3,4,7–10,15,19].

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[22] , while in a country-wide survey[5] 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[22]. Mortality rates vary between studies but range from 5 % in an academic hospital setting[8] 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[5,16]. The diminazene and imidocarb treatments sterilise the infection[16,18] which is not ideal in endemic regions where a lack of premunity puts dogs at risk of repeat infections[18]. Attempts at developing specific vaccines have proved successful[14,20,21], but the duration of immunity is only 6 months[20] 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[24,16].

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 challenge during the peak of the 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

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a known high disease challenge[5]. 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.

Table 1: **Treatment dogs listed by region with breed, sex and age details.**

| Pietermaritzburg | Howick | Paddock | Greytown | Richmond | Mandini |
|---|---|---|---|---|---|
| Lab-x-F-14 | GSD-F-7 | GSH-M-10 | Afric-F-2 | Lab-M-5 | Lab-F-4 |
| GSD-M-6 | ACD-F-9 | GSH-M-9 | AS-M-2 | Dalm-F-7 | Lab-F-6 |
| GSD-x-F-2 | | | AS-F-5 | | JRT-F-4 |
| | | | AS-F-5 | | Cross-F-5 |
| | | | Afric-F-6 | | B/Bul-M-1 |
| | | | | | Point-F-14 |

GSD, German shepherd dog; ACD, Australian cattle dog; AS, Australian shepherd; Lab, Labrador; JRT, Jack Russell terrier; GSH, German shorthair pointer; Afric, Africanus; B/Bul, Boerbul; Point, Pointer; Dalm, Dalmation.

### *Babesia DNA Identification*

*DNA extraction*

DNA was extracted from 200 $\mu\ell$ 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[8,17]. 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[17]. 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[18].

### *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 extract 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 $\alpha = 5$ %).

## 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 *Babesia 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 repellant[6]. As a 9 % impregnated collar (Preventic–Virbac) it acts as a tick repellant[6]. 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[3,13]. 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[1,2,11,12,17].

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 2: **PCR results for *Babesia canis rossi* for dogs with** (treatment group) **and without** (control group) **amitraz-impregnated collars.**

| | Dec 5 | Jan 06 | Feb 06 | March 06 | Apr 06 | May 06 | Total number of dogs tested |
|---|---|---|---|---|---|---|---|
| **Treatment group** | | | | | | | |
| Positive PCR | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Negative PCR | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| **Control group** | | | | | | | |
| Positive PCR | 1 | 1* | 1 | 2 | 2 | 1 | 8 |
| Negative PCR | 4 | 4 | 4 | 3 | 3 | 4 | 22 |

*This dog died from complicated babesiosis a week later.

*canis rossi,* while all 20 of the Preventic-treated group remained negative throughout the trial period. This was a higher incidence rate than that reported by Shakespeare[22] but these dogs were from the SPCA and probably did not have the same degree of owner care as that of the population investigated by Shakespeare[22]. Statistical analysis of results showed significant differences between the treatment and control groups. The death of one of the control dogs due to fulminating complicated babesiosis a week after being sampled confirmed the virulence of this strain of *Babesia.*

These findings suggested that it was significant that the dogs fitted with amitraz-impregnated collars remained PCR negative for *B. canis rossi* during the trial period. Provided that preventive protection is maintained on the animal (regular checks of the collar by the owner and renewal every 3 months), a preventive effect on *Babesia* infection can be expected.

## REFERENCES


1. Ano H, Makimura S, Harasawa R 2001 Detection of babesia species from infected dog blood by polymerase chain reaction. *Journal Veterinary Medical Science* 63: 111–113
2. Birkenheuer A J, Levy M G, Breitschwerdt E B 2003 Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. *Journal of Clinical Microbiology* 41: 4172–4177
3. Boozer A L, Macintire D K 2003 Canine babesiosis. *Veterinary Clinics of North America – Small Animal Practice* 33: 885–904
4. Clark I A, Jacobson L S 1998 Do babesiosis and malaria share a common disease process? *Annals of Tropical Medicine and Parasitology* 92: 483–488
5. Collett M G 2000 Survey of canine babesiosis in South Africa. *Journal of the South African Veterinary Association* 71: 180–186
6. Estrada-Pena A, Remé C 2005 Efficacy of a collar impregnated with amitraz and pyriproxyfen for prevention of experimental tick infestations by *Rhipicephalus sanguineus, Ixodes ricinus,* and *Ixodes scapularis* in dogs. *Journal of the American Veterinary Medical Association* 226: 221–224
7. Jacobson L S 1994 Cerebellar ataxia as a possible complication of babesiosis in two dogs. *Journal of the South African Veterinary Association* 65: 130–131
8. Jacobson L S 2006 The South African form of severe and complicated canine babesiosis: clinical advances 1994–2004. *Veterinary Parasitology* 138: 126–139
9. Jacobson L S, Clark I A 1994 The pathophysiology of canine babesiosis: new approaches to an old puzzle. *Journal of the South African Veterinary Association* 65: 134–145
10. Kettner F, Reyers F, Miller D 2003 Thrombocytopaenia in canine babesiosis and its clinical usefulness. *Journal of the South African Veterinary Association* 74: 63–68
11. Kjemtrup A M, Conrad P A 2006 A review of the small canine piroplasms from California: *Babesia conradae* in the literature. *Veterinary Parasitology* 138: 112–117
12. Kjemtrup A M, Wainwright K, Miller M, Penzhorn B L, Carreno R A 2006 *Babesia conradae,* sp. nov., a small canine *Babesia* identified in California. *Veterinary Parasitology* 138: 103–111
13. Lewis B D, Penzhorn B L, Lopez-Rebollar L M, de Waal D T 1996 Isolation of a South African vector-specific strain of *Babesia canis. Veterinary Parasitology* 63: 9–16
14. Lewis B D, Penzhorn B L, Lopez Rebollar L M 1995 Immune responses to South African *Babesia canis* and the development of a preliminary vaccine. *Journal of the South African Veterinary Association* 66: 61–65
15. Lobetti R G 1998 Canine babesiosis. *Compendium of Continuing Education* 20: 418–430
16. Lobetti R G 2001 Canine babesiosis. In Day M, Mackin A, Littlewood J (eds) *Manual of canine and feline haematology and transfusion medicine.* Iowa University Press, Ames: 85–91
17. Matsuu A, Ono S, Ikadai H, Uchide T, Imamura S, Onuma M, Okano S, Higuchi S 2005 Development of a SYBR green real-time polymerase chain reaction assay for quantitative detection of *Babesia gibsoni* (Asian genotype) DNA. *Journal of Veterinary Diagnostic Investigation* 17: 569–573
18. Penzhorn B L, Lewis B D, de Waal D T, Lopez Rebollar L M 1995 Sterilisation of *Babesia canis* infections by imidocarb alone or in combintion with diminazene. *Journal of the South African Veterinary Association* 66: 157–159
19. Reyers F, Leisewitz A L, Lobetti R G, Milner R J, Jacobson L S, van Zyl M 1998 Canine babesiosis in South Africa: more than one disease. Does this serve as a model for *falciparum* malaria? *Annals of Tropical Medicine and Parasitology* 92: 503–511
20. Scheffers T P, Kleuskens J A, Scholtes N C, van de Crommert J , Krijnen E, Moubri K, Gorenflot A, Vermeulen A N 2006 Onset and duration of immunity against *Babesia canis* infection in dogs vaccinated with antigens from culture supernatants. *Veterinary Parasitology* 138: 140–146
21. Scheffers T P, Strydom T, Crafford D, Kleuskens J A, van de Crommert J, Vermeulen A N 2007 Immunity against *Babesia rossi* infection in dogs vaccinated with antigens from culture supernatants. *Veterinary Parasitology* 144: 10–19
22. Shakespeare A S 1995 The incidence of canine babesiosis amongst sick dogs presented to the Onderstepoort Veterinary Academic Hospital. *Journal of the South African Veterinary Association* 66: 247–250
23. Vial H J, Gorenflot A 2006 Chemotherapy against babesiosis. *Veterinary Parasitology* 138: 147–160
24. Willadsen P 2006 Tick control: thoughts on a research agenda. *Veterinary Parasitology* 138: 161–168