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SHORT COMMUNICATION

Prevalence of vector-borne pathogens *Ehrlichia canis*, *Babesia* spp. and *Dirofilaria immitis* in dogs in Townsville, far north Queensland

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Ehrlichia canis, *Babesia* spp. and *Dirofilaria immitis* are blood-borne pathogens transmitted to dogs by arthropods. The aim of the current study was to investigate the prevalence of *E. canis*, *Babesia* spp. and *D. immitis* in domestic dogs, aged 6 months or older, in Townsville, in far north Queensland, Australia. Dogs were recruited through convenience sampling, with the assistance of local veterinary clinics and James Cook University staff and students. Up to 3 ml of blood was collected per dog, into EDTA vacutainer tubes. Testing for *E. canis* and *Babesia* spp. was performed through qPCR, with a second PCR used to identify the species in *Babesia*-positive cases. Testing for *D. immitis* was performed using a commercial antigen detection kit and the modified Knott's test (MKT); microfilariae identity was confirmed by morphological features and qPCR. Of 301 dogs sampled, none tested positive for *E. canis*, whereas 9 (3.0%, 95% CI 1.1–4.9%) tested positive for *Babesia vogeli*, and 15 (5.0%; 95% CI 2.5–7.5%) tested positive for *D. immitis*, based on the combined antigen and MKT results.

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Ehrlichia canis, *an obligate intracellular, gram-negative bacterium, causes canine monocytic ehrlichiosis (CME) and is transmitted primarily by the brown dog tick* Rhipicephalus sanguineus sensu lato *("tropical strain"), which was recently renamed* R. linnaei.[1,2] *Although common worldwide,* E. canis *was considered exotic to Australia until recently.*[3] Babesia vogeli, *a protozoan parasite that causes a relatively mild form of canine babesiosis, is also transmitted by* R. linnaei, *and was first reported in domestic dogs in Australia in 1966.*[4] *A second species,* B. gibsoni, *was detected in dogs in south-eastern Australia in 2002.*[5] Dirofilaria immitis *(heartworm), a mosquito-borne nematode, causes a life-threatening condition in dogs and is endemic in Australia.*[6] D. immitis *can infect a wide range of hosts, including humans.*[7] *The prevalence and geographical distribution of vector-borne pathogens are influenced by factors such as the presence of vectors, the socio-economic status of owners, host availability, and the presence of a wildlife or free-ranging reservoir species.*[8]

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The aim of the current study was to investigate the prevalence of *Ehrlichia canis*, *Babesia* spp., and *Dirofilaria immitis* in client-owned dogs in Townsville, far north Queensland, Australia.

The study protocol was approved by the James Cook University Animal Ethics Committee (A2919). A sample size of 296 dogs was required, assuming a 26% prevalence of infection with *D. immitis*,[6] with a 95% confidence level. Up to 3 ml of blood was collected via aseptic venipuncture into EDTA vacutainer tubes from healthy dogs aged 6 months or older attending participating veterinary clinics for routine clinical procedures or owned by James Cook University staff or students. Dogs were recruited through convenience sampling.

Samples were separated into aliquots of whole blood as well as plasma, buffy coat fraction, and red cell pellet after centrifugation. Genomic DNA was extracted and purified from whole blood using the QuickGene DNA whole blood kit S (Kurabo Industries Ltd., Osaka, Japan) and from the buffy coat fraction using the QuickGene DNA tissue kit S (Kurabo Industries Ltd.), with the QG-Mini480 (Kurabo Industries Ltd.), following the manufacturer's instructions.

Purified buffy coat DNA was used to detect *E. canis*-specific DNA using two primer sets, and purified whole blood DNA was used to detect *Babesia* genus DNA. All positive *Babesia* DNA extracts were retested using primers specific to *B. vogeli*, *B. canis*, and *B. gibsoni* (Table S1). Quantitative real-time PCR was run in duplicate using the SensifastSYBR Hi-Rox kit (Bioline, Luckenwalde, Germany) as per the manufacturer's instructions on a MIC PCR cycler (GeneTarget Solutions Pty. Ltd., Dural, NSW), with a dog BetaActin gene control, positive control, and a negative nontemplate control. All positive samples were retested twice more, and only those that remained positive were recorded as positive. Positive control DNA for *Babesia* genus was obtained from a dog that had previously tested positive for *B. vogeli* on PCR and had *Babesia* piroplasms observed in its blood. Positive control material for molecular testing of *E. canis* was provided by courtesy of the State of Queensland through the Biosecurity Sciences Laboratory, Department of Primary Industries. Testing for *D. immitis* antigen was performed on whole blood using a commercially available diagnostic test, Anigen rapid CHW Ag Test Kit 2.0 (Bionote, Hwaseong-si, Gyeonggi-do, Korea) as per the manufacturer's instructions. Dogs were checked for microfilariae (Mf) using the modified Knott's test (MKT).[9] The identity of Mf was confirmed by morphological features and morphometry and by qPCR (Qiagen Rotorgene Q) using DNA extracted from whole blood as per Albonico (2014)[10] (Table S1) and gel electrophoresis (1% TAE gel, 100 V).[11]

A total of 301 dogs were sampled in this study. Of these, 131 (43.5%) were males, 165 (54.8%) were females, with no sex data recorded for the remaining 5 (1.7%). The median age of all sampled dogs was 4 years (range 0.5–15.25 years). Sampling occurred during April and May 2024, coinciding with the end of the wet season in Townsville (19°15′ S 146°49′ E), which has a dry tropical climate.

Of the 301 dogs tested, none were positive for *E. canis*. In Australia, *E. canis* was first detected in 2020, in sick dogs from a remote community in Western Australia, and subsequently spread to dogs in the Northern Territory and North Queensland, including Townsville.[3] In immunologically naïve dogs, *E. canis* can cause immune-mediated destruction of platelets and white blood cells, resulting in bleeding and fatal bacterial sepsis. Some dogs may be subclinically infected or recover spontaneously, whereas others will develop chronic ehrlichiosis.[1] Because only healthy dogs were sampled, our results suggest that chronic ehrlichiosis is either not yet endemic in this region or is present at very low levels. The results of this study are supported by the identification of only two cases of *E. canis* in 2024 by the Queensland Biosecurity Laboratory (Dr. Louise Jackson, personal communication). During the 2 months of this study, no cases were detected by one of the private diagnostic laboratories serving the area (Dr. Philippa McLaren, personal communication).

In contrast, 9 (3.0%, 95% CI 1.1–4.9%) of the 301 dogs tested positive for *Babesia* spp., all due to *B. vogeli*. A 2003 study found that *Babesia* 18S small subunit ribosomal RNA (rRNA) gene sequences in dogs in northern Australia were 99% homologous to *B. vogeli*, whereas those in south-eastern Australia were 100% homologous to *B. gibsoni*.[12] *B. vogeli* typically causes subclinical infections in mature dogs, but can cause severe anaemia in puppies.[4]

In the current study, babesiosis was not confirmed by visualisation of the parasites in blood smears due to the low sensitivity of this method in chronic or subclinical cases and unreliable species identification.[13] Typically, *B. vogeli* parasitaemia is very low, with dogs in Australia previously reported as PCR-positive despite the absence of microscopic parasitaemia or clinical illness.[14,15]

Few studies have investigated canine babesiosis in Australia since it was first detected, incidentally, in Townsville.[4] Surveys found *B. vogeli* in 46/215 (21.3%)[15] and 7/92 (7.6%)[16] of dogs in remote Aboriginal communities in the Northern Territory and north-western NSW, with a higher prevalence in central and northern areas compared to southern areas. A similar study found *B. vogeli* in 13/230 (5.6%) Aboriginal community and pound dogs,[17] and 12/238 (5.0%) client-owned dogs presented to veterinary clinics in the Northern Territory and south-east Queensland, again with higher infection rates in the northern tropics.[18] Comparisons of these studies to the current study should be done with caution, given the differences in sampling frames and source populations. Moreover, the current study included only healthy dogs older than 6 months of age, unlike previous studies that included some dogs with clinical signs suggestive of tick-borne disease as well as younger dogs that commonly have higher *B. vogeli* infection rates.[17]

In the current study, 15/301 dogs (5.0%; 95% CI 2.5–7.5%) tested positive for *D. immitis*, based on the combined antigen test and MKT. Seven dogs were positive on both tests, seven on the antigen test only, and one on the MKT only. The proportion of positive tests determined by each assay was not statistically different (P = 0.07), based on an exact McNemar's test (SPSS version 29.0; SPSS Inc., Chicago IL, USA). No coinfections of *D. immitis* and *B. vogeli* were detected.

The prevalence of *D. immitis* in dogs is higher in tropical areas due to high year-round temperature and humidity favoring a high density of mosquito vectors.[19] In the 1970s, an estimated 77% of dogs in Townsville were infected with *D. immitis*.[20] By the early 2000s,this had dropped to 15% in shelter dogs,[21] as a result of the introduction of macrocyclic lactones for the prevention of heartworm disease in 1994.[22] More recently, a re-emergence of infection with *D. immitis* has been reported in shelter and pig hunting dogs from Queensland (5.8% in southern QLD, up to 21% in central QLD and up to 32.1% in northern QLD).[6,19,23] Furthermore, two cases of infection with *D. immitis* were reported in NSW in June 2024.[24,25]

The obvious difference in the prevalence of infection between shelter (25.8%)[6] and owned (5%, present study) dogs from Townsville is likely to be due to high levels of compliance with the use of preventative treatments in owned dogs. This is supported by the retrospective information obtained on heartworm prevention in the dogs that were positive for *D. immitis* in the current study. The owner of only one dog claimed that they had consistently treated their dog to prevent HW infection; one dog had not been on any preventative treatment, and the remaining dogs (13) had not received consistent preventative treatments.

Anecdotally, local veterinarians report an increased incidence of heartworm disease in client-owned dogs in Townsville in recent times, in comparison to the near negligible incidence observed a decade earlier. The 5% prevalence of heartworm in the current study confirms the apparent increase in prevalence reported by local veterinarians. Reduced owner compliance with preventive treatments,[6] a high reservoir of infection represented by wild dogs in the area,[21] and an apparent loss of efficacy of chemicals used in heartworm prevention[6] might explain the re-emergence of heartworm in dogs from Townsville. The high prevalence of infection with *D. immitis* in dogs from Townsville represents a risk of infection for susceptible hosts, including humans, in the area.[7] Furthermore, infected dogs from Townsville can spread heartworm to other areas of Australia where the mosquito vectors are present.

In conclusion, 3% of healthy dogs In Townsville tested positive for *B. vogeli* and 5% for *D. immitis* in the current study, despite the availability of preventative treatments. Further research is needed to determine if positive cases are due to a lack of owner awareness and diligence, or parasite chemoresistance.

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Conflicts of interest and sources of funding

The authors declare no conflicts of interest or sources of funding for the work presented here.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/avj.13450/suppinfo.

Table S1. Primers used to detect *Ehrlichia canis*, *Babesia* species and *Dirofilaria immitis*.

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