AUSTRALIAN VETERINARY JOURNAL

AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT

ORIGINAL ARTICLE

Estimated seroprevalence of *Ehrlichia canis* in dogs in a remote community in the Northern Territory, Australia

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Background The emergence of *Ehrlichia canis* in northern Australia in 2020 has reshaped the landscape of tick-borne diseases in dogs, particularly in rural and remote communities where the brown dog tick (*Rhipicephalus linnaei*) is endemic. Despite the rapid spread of ehrlichiosis and reported impacts on dog health, its prevalence remains poorly understood. This study aims to provide baseline data on the epidemiology of *E. canis* in Australia by determining its seroprevalence in dogs from a remote Northern Territory community.

Methods In a cross-sectional study, an enzyme-linked immunosorbent assay (ELISA) assessed the point seroprevalence of *E. canis* in community dogs. A door-to-door census was undertaken to quantify resident dogs and obtain information on signalment and clinical signs. Canine serum samples were evaluated for seroreactivity to *E. canis* at the state reference laboratory.

Results Of the 48 dogs present in the community, 44 (91.2%) were included in the serosurvey. ELISA testing found a point seroprevalence of 52.3% (95% CI: 36.7% to 67.5%) for *E. canis*. Seropositive dogs were mostly asymptomatic and had similar body condition scores to seronegative dogs.

Conclusions This study documents a high point seroprevalence of *E. canis* in dogs from a remote Australian community. There was no significant correlation between serostatus and gender, body condition or the presence of clinical signs. These results underscore the need for further research to understand the clinical significance of seropositivity in asymptomatic dogs and highlight the need for a locally validated diagnostic test to support field-based surveillance and management of ehrlichiosis in Australia.

Keywords Australia; Dog; *Ehrlichia canis*; ELISA; prevalence; vector-borne-disease

Abbreviations BCS, body condition score; CME, canine monocytic ehrlichiosis; ELISA, enzyme-linked immunosorbent assay; NSW, New South Wales; NT, Northern Territory; QLD, Queensland; qPCR, quantitative polymerase chain reaction; SA, South Australia; WA, Western Australia

Aust Vet J 2025 doi: 10.1111/avj.70005

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Canine monocytic ehrlichiosis (CME) is a serious and potentially life-threatening disease in domestic dogs and wild canids caused by the Gram-negative intracellular bacterium *Ehrlichia canis*.[1,2] The bacterium was first described in 1935 as a cause of fever and anaemia in Algerian dogs[3] and has since achieved an almost global distribution, with the greatest prevalence observed in tropical and subtropical regions.[4,5] These warmer climates favour the maintenance of the brown dog tick, *Rhipicephalus linnaei* (formerly *R. sanguineus sensu lato* "tropical lineage"),[6] the primary vector of CME.[7,8]

In dogs, CME progresses through three phases: acute, subclinical and chronic.[9–11] In the acute phase, patients may present with fever, lethargy, lymphadenopathy, anorexia, abnormal bleeding tendencies such as petechiae, ecchymoses and epistaxis, thrombocytopaenia and/or uveitis.[12–14] If left untreated, the infection may enter a subclinical phase, lasting months to years, during which time dogs may be asymptomatic or show mild clinical signs but continue to harbor the pathogen.[9,12,15,16] In some dogs, subclinical infection progresses to a chronic phase characterised by a more severe clinical presentation, including pancytopaenia, severe bleeding tendencies and death.[17] Treatment is most effective when administered during the acute phase of infection, while chronic cases carry a grave prognosis,[18,19] underscoring the importance of early diagnosis and treatment. Despite nearly global endemicity, there is a limited understanding of CME's clinical course and community-level impacts in naïve populations. This is particularly true in rural and remote communities with restricted access to veterinary and animal health services, given limited resources and capacity for diagnostic testing.

Australia had historically been free of *E. canis* until the first case was reported in 2020 in a small town in Western Australia's Kimberley region.[20] Although the origins of the incursion are unknown, whole genome sequencing has revealed that the Australian *E. canis* strain closely aligns with the Taiwan genotype, which is common throughout Asia and the Middle East.[21] The emergence of *E. canis* in 2020 marked a significant change in the country's canine tick-borne disease landscape. Despite the pathogen's swift designation as a nationally notifiable disease and the introduction of movement restrictions,

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E. canis spread rapidly across northern Western Australia (WA) and the Northern Territory (NT).[22] Polymerase chain reaction (PCR) prevalence in tick populations rose dramatically from 2.8% in November–December 2020 to 62.9% by February 2021.[23]

Although not well captured in the published literature, the disease has had devastating impacts on dogs living in rural and remote communities,[22,24] where limited access to veterinary services and animal health products predisposes dogs to overpopulation,[25] high tick burdens and other comorbidities. Coupled with a free-roaming lifestyle and the potential for an expanded interface with local wildlife, such as dingoes, these dogs face an increased risk of exposure to tick-borne diseases.[26–28] Studies evaluating other tick-borne infections have found high rates of tick-borne disease in dogs living in remote areas.[27,29–31] Despite the reported impacts of CME in dogs living in rural and remote parts of Australia, no studies have investigated the epidemiology of *E. canis* in dogs since the incursion, and the prevalence of the disease in dogs in areas where *E. canis* has become endemic has not yet been evaluated.

The emergence of *E. canis* in Australia poses challenges for animal health and welfare, with the potential for adverse public health impacts, given reports of human ehrlichiosis infection in humans from Latin America.[32–34] However, human infections with the Taiwan genotype have not yet been reported.[22] Although most cases of CME in Australia have been identified in northern parts of the country, the current distribution of the CME's primary vector, *R. linnaei*, extends through the NT, Queensland (QLD), and parts of New South Wales (NSW), WA and South Australia (SA),[35] with considerable southward expansion over the past 60 years.[23,36–38] Of concern is the fact that ongoing impacts of climate change may continue to drive the further southward expansion of *R. linnaei*,[38] increasing the risk of *E. canis* in susceptible animals in more southern parts of the country.

Current epidemiological data are essential for planning and evaluating strategies to minimise the impact of CME in Australia. Globally, serological surveys of dogs evaluating exposure to tick-borne pathogens have been used to evaluate the infection risk in people and animals.[39–43] At the time of writing, no published studies have assessed the seroprevalence of CME in dogs in parts of northern Australia where the disease has become endemic. This study aimed to improve current knowledge of the prevalence of *E. canis* in dogs in a remote community in the NT.

Materials and methods

Context and study area

The study was conducted in a remote community northeast of Katherine in the Roper Gulf region of the NT. Sample and data collection were performed over 3 days between the 13th and 15th of April 2023, in conjunction with the provision of an animal health and veterinary programme undertaken by the local veterinary service provider. Funding for the veterinary programme was provided by the local government to support animal and public health in the region's geographically and socioeconomically disadvantaged communities. These population-level animal health and management programmes are typically delivered across 3–4 visits annually.

Ethics

This study was approved by the Murdoch University Animal Research Ethics Committee, permit number #R3440/23, Protocol ID 989.

Data collection

The research team collected data on dog demographics as part of a routine, community-wide, door-to-door dog census. Prophylactic flea, tick and worming treatments were administered during the census as part of the veterinary programme. All dogs were given a preventative tick collar containing imidacloprid and flumethrin (Seresto®, Elanco Animal Health), an oral all-wormer (Sentinel™ Spectrum, Elanco Animal Health) and an isoxazoline-based oral parasite preventative containing fluralaner (Bravecto®, MSD Animal Health). Informed verbal consent was obtained from each dog owner or caregiver in the community before participation, using a predefined script that provided a detailed explanation of the study. Dog size, breed, gender, reproductive status, estimated age group, body condition score (BCS) and any significant clinical findings observed were recorded (Table 1). Body condition scoring followed the American Animal Hospital Association Canine BCS guidelines.[44] Dog age was estimated using owner history, previous veterinary records, physical examination findings and dentition.

Serology

Of the 48 dogs identified across the community during the animal census, 44 (91.7%) were included in the serology study. Three of the remaining four dogs were excluded from the study due to temperament, as they were not amenable to handling, and one additional dog was absent from the home at the time of sample collection. For the participating dogs, blood samples were obtained via peripheral venipuncture from the jugular or cephalic vein with the assistance of the local veterinary service provider. Where possible, samples were

Table 1. Signalment and health information obtained from participating dogs

Parameter	Classification
Size	• Small 0–10 kg • Medium 10–20 kg • Large 20 kg+
Breed	• Mixed • Purebred • Dingo
Sex	• Male • Female
Reproductive status	• Desexed • Entire
Age	• Puppy <1 year • Adult 1–6 years • Geriatric 6+ years
Body condition score	• 1–3 Underweight • 4–6 Ideal • 7–9 Overweight
Clinical signs	For example, petechiae/ecchymoses, hyphaema or corneal oedema, epistaxis

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collected from dogs undergoing routine surgical desexing procedures to minimise stress. Then, 1–5 mL of blood was collected from each participating dog. Whole blood samples preserved in ethylenediaminetetraacetic acid (EDTA) were sent to the state laboratory (Berrimah Veterinary Laboratory) for evaluation. Samples were analysed for the presence of circulating IgG antibodies to *E. canis* using commercial enzyme-linked immunosorbent assay (ELISA) (Euroimmun®, Medizinische Labordiagnostika AG, Lübeck, Germany), which has a reported sensitivity of 92% and specificity of 100% for *E. canis* based on a European study.[45] The ELISA was performed according to the manufacture's test instruction[46] using a dilution of 1:101 in sample buffer. Results were interpreted based on the ratio of the extinction value for the control sample to that of the calibrator. Ratios <0.8 were considered negative, those between ≥0.8 and 1.1 were considered borderline, and those ≥1.1 were considered positive. Once sample results were available, seropositive dogs were offered a 28-day course of doxycycline (10 mg/kg orally every 24 h for 28 days) free of charge by the regular veterinary service provider to address potential active infection and carrier status in the absence of complementary antigen testing.

Triplex quantitative polymerase chain reaction (qPCR)

Additional *E. canis* qPCR testing was performed by the Berrimah Veterinary Laboratory on samples from two dogs presenting with signs of systemic illness at the time of sampling. Funding constraints precluded broader sampling of the asymptomatic dog population using qPCR; therefore, the selection of dogs for PCR testing was based solely on clinical concerns identified by the attending veterinarian and the need for further diagnostic testing to inform treatment decisions. DNA extraction was performed using a MagMax CORE extraction kit and AgPath ID (Thermo Fischer Scientific, Australia) for triplex qPCR, including *E. canis*, *Anaplasma platys* and *Babesia vogeli*. *A. platys gltA* gene fragment was amplified using the *gltA84-F* (5′-GACCTACGATCCGGGATTCA-3′) and *gltA84-R* (5′-TGGCGCAGTATACCCTTTTCTC-3′) primers as previously described,[47] and a PLATYSp (5′-*VIC*-TCTACCGCGGCATGCA-GCTCTG-*MGBNFQ*-3′) probe, at concentrations of 600, 600 and 250 nM, respectively. *E. canis* 16S rRNA gene fragment was amplified using the *Ec16S-F* (5′-TATAGCCTCTGGCTATAGGAAATTG TTA-3′) and Ec16S-R (5′-ACCATTTCTAATGGCTATTCCGTAC TA-3′) primers as previously described,[48,49] along with the *Ec16S-P* probe (5′-*FAM*-TGGCAGACGGGTGAGTGGCAGACGGGTGAG-TAATGCGTAGG-*BHQ1*-3′), at concentrations of 900, 900 and 250 nM, respectively. qPCR amplification of the *B. vogeli* heat shock protein *hsp70* utilised the Bchsp70-F (5′-GTCATCACTGTGCCT-GCGTACT-3′) and Bchsp70-R (5′-GCATGACGTTGAGACCGG-CAAT-3′) primers as previously described,[49] and the *Bchsp70-P* (5′-*ROX*-AGCGCCAGGCCACCAAGGACGCT-*BHQ2*-3′) probe, at concentrations of 900, 900 and 250 nM, respectively.

Previously identified qPCR-positive samples for each pathogen were included in the assay as positive controls. Nuclease-free water was used as the negative extraction control with a no-template qPCR control. qPCR reactions were carried out using 20 μL of commercial qPCR master mix and 5 μL of DNA, with the following thermocycling conditions: 45°C for 10 min for reverse transcription and 95°C for 10 min for the hot-start Taq polymerase activation. This was followed by 45 cycles of 15 s at 95°C and 45 s at 60°C for target amplification on the Rotor-Gene 6000 thermal cycler (Qiagen, Venlo, Netherlands).

Data analysis

For the purpose of this study, point seroprevalence was defined as the proportion of dogs that tested positive for *E. canis* serology at a specific point in time (i.e., sampling period). Statistical analyses were conducted using R software (version 2024.04.2+764, R Core Team, 2024). The Shapiro–Wilk test was used to assess normality, and differences in BCS between seropositive and seronegative groups were evaluated using a two-sample t-test for normally distributed data. The association between *E. canis* serostatus and the presence or absence of clinical signs was assessed using Pearson's Chi-squared test with Yates' continuity correction in R.

Results

Study population demographics

A total of 44 mixed-breed community dogs were enrolled in the study and underwent blood sampling. The study population was comprised of nearly equal proportions of male (56.8%) and female (43.2%) dogs, with nearly 60% of all the dogs desexed before or during the veterinary programme. Nearly three-quarters (n = 32) of all dogs included in the study were classified as adults (1–5 years of age), with an average BCS of 3.89 out of 9 (SD 1.1; range 2–7). Aside from body condition, only five dogs in the study population exhibited clinical signs suggestive of ehrlichiosis. Two of these dogs showed evidence of unilateral corneal oedema, whereas another had petechiae noted at the time of sampling.

Seroprevalence of E. canis

Of the 44 samples assessed using ELISA, 23 returned a positive result, 1 was borderline and 20 were negative, with a serological point prevalence of 52.3% (95% CI: 36.7% to 67.5%) for *E. canis*. The borderline result was excluded from the analysis as the recommended protocol for resampling and retesting after 14 days[50] could not be instituted under the sampling constraints imposed by veterinary service delivery. The gender, age, desexing status and BCS of seropositive dogs were similar to those of the sample population (Table 2). Three of the seropositive dogs, 13.0% (3/23) had a BCS of 2/9 compared with 11.4% (5/44 dogs) in the overall study population. Two of the dogs with low BCS were also described as being cachexic, both of which were found to be seropositive for *E. canis*. Among the seropositive dogs, one dog was observed to have high tick burdens, whereas another had evidence of corneal oedema. However, there was no statistically significant difference in mean BCS between the seronegative and seropositive groups (P = 0.48). Similarly, there was no significant association between *E. canis* serostatus and the presence or absence of clinical signs (P = 0.26), nor between *E. canis* serostatus and sex (P = 0.6).

qPCR analysis

Two dogs with clinical signs of illness at sampling were additionally evaluated for CME using qPCR. One of the dogs presented with a low BCS (2/9), evidence of petechiae and observations of subjectively

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Table 2. Demographics of the study population and *Ehrlichia canis* positive dogs

	Study population (n = 44)	*E. canis* positive (n = 23)
Gender		
Male	25 (56.8%)	14 (60.9%)
Female	19 (43.2%)	9 (39.1%)
Desexing status		
Desexed	26 (59.1%)	11 (47.8%)
Entire	18 (41.0%)	12 (52.2%)
Age		
Puppy (<1 year)	7 (16.0%)	1 (4.3%)
Adult (1–5 years)	32 (72.7%)	18 (78.3%)
Geriatric (6+ years)	5 (11.4%)	4 (17.4%)
BCS		
Mean	3.9 (SD 1.1)	4 (SD 1.2)
Minimum	2	2
Maximum	7	7

increased haemorrhage perioperatively and was found to be negative for *E. canis* on both ELISA and qPCR testing. The other had a low BCS (2/9) and a large tick burden and was found to be positive for *E. canis* on both ELISA and qPCR. Both dogs were also positive for *Anaplasma platys* based on qPCR results.

Discussion

The introduction of CME into Australia has had a devastating impact on dogs living in remote communities.[22] However, these impacts have not been objectively quantified due to limited resources and logistical challenges in undertaking diagnostic evaluations in a remote, field-based setting. To the authors' knowledge, this is the first study to assess the seroprevalence of CME in dogs in Australia since its detection in 2020, focusing on dogs living in remote communities where access to veterinary services and preventive care treatments is often limited. Results indicated that over half of all dogs within the community were seropositive for *E. canis*, with a point seroprevalence of 52.3%, suggesting widespread exposure of dogs to *E. canis* in areas where the disease is established. This figure is similar to the prevalence of other endemic tick-borne diseases previously reported in community dogs in the NT, compared with only 3% in pound dogs from southeast QLD.[51]

The prevalence of tick-borne diseases is influenced by several geographic, environmental, anthropogenic, socioeconomic and dog-specific factors.[52] A study by Hii et al.[27] demonstrated an overall prevalence rate of 16.4% for tick-borne diseases in dogs and found that dogs from the NT were 3.6 times more likely to have a tick-borne pathogen than those from southeast QLD. This finding was attributed to differences in the prevalence of the vector *R. linnaei*. Male dogs were also found to be 2.3 times more likely to have a tick-borne pathogen than female dogs,[27] although international studies from countries where CME is endemic have produced conflicting results regarding gender as a risk factor for infection.[53,54] Although the high seroprevalence of dogs to *E. canis* in this NT community is consistent with other studies investigating tick-borne diseases in community dogs, the present study showed no significant difference in the serostatus of dogs in terms of gender. However, as sampling for this study was undertaken in 2023, seroprevalence may have changed, and additional research is needed to better understand the temporal variations in seropositivity in dogs.

In a field environment with limited diagnostic capacity and the financial constraints experienced by animal owners, clinical examination and syndromic case definitions are often used to obtain a presumptive diagnosis and guide therapeutic management. A recent study revealed that lethargy, anorexia, weakness and weight loss were the most common clinical signs reported in dogs diagnosed with ehrlichiosis, with epistaxis, petechiae, seizures, anorexia and cough being associated with seropositivity.[55] However, the current study found no significant difference in serostatus based on the dog's BCS and the presence or absence of clinical signs. Similar findings were reported in a study by Fung et al.,[56] who reported no significant difference in BCS and the presence or absence of zoonotic parasites in dogs in Panama. In another study from Costa Rica, Rojas et al.[57] revealed that over half of all dogs identified as PCR-positive for tick-borne illness were asymptomatic, including 58% of dogs that were positive for *E. canis*. As such, loss of body condition and the presence of clinical signs suggestive of CME do not appear to be reliable predictors of *E. canis* seroprevalence in dogs.

Heavy tick infestations are associated with a higher prevalence of CME,[53,57] whereas veterinary care and regular antiparasitic treatment have been shown to significantly reduce the prevalence of this disease in dogs.[53] This underscores the importance of regular access to veterinary services and the local physical and financial availability of antiparasitic treatments in reducing the risk of CME and other canine vector-borne diseases in rural and remote Australian communities. In these areas, the lack of regular veterinary service delivery and limited access to animal health products pose an ongoing challenge and are likely to influence the prevalence of tick-borne diseases. For approximately 7 years, council animal management programmes have provided visiting veterinary services to over 15 Indigenous communities across the present study region. Despite an effort to deliver three visits annually, seasonal factors such as flooding and inclement weather during the wet season (December to April) can impact access to remote communities, complicating parasite control and increasing the risk of tick-borne disease exposure. Movement restrictions and logistical challenges brought about by the COVID-19 pandemic also disrupted treatment efforts, delaying visits and limiting the availability of antiparasitic treatments. The free-roaming nature of the community dog population and migration of dogs between communities may have also contributed to lapses in parasitic treatment coverage for individual dogs, contributing to the maintenance of tick populations within the region. Furthermore, although the prevalence of *E. canis* in Australian dingo populations is unknown,[35] interactions with free-roaming dogs could facilitate the spread and maintenance of the brown dog tick vector and CME in dogs in remote communities.[35,58] Although tick burden was not assessed as a risk factor for serostatus in the dogs included in this study, further research to understand and quantify the relationship

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between tick burden and infection status in dogs in remote Australian communities may provide additional insights into the epidemiology and risk factors for canine ehrlichiosis in Australia.

Coinfection with *A. platys* was identified in one of the two symptomatic dogs participating in the current study, with the other testing positive for *A. platys* only. This is consistent with results from another Australian study which identified *A. platys*, *B. vogeli* and haemotrophic mycoplasmas from dogs in an Aboriginal community in the NT, with 22 of the 130 dogs evaluated having evidence of coinfection.[51] Although *B. vogeli* was not identified in either of the dogs evaluated by qPCR in the current study, coinfection with *A. platys* is significant, as coinfection with *E. canis* can exacerbate disease severity and impact the effectiveness of treatment[59] The immunosuppressive nature of *E. canis* may also increase susceptibility to other infections, increasing the likelihood of coinfection and contributing to the severity of disease progression.[57,59] In Grenada, Lanza-Perea et al. (2014) reported that seropositivity to both *A. platys* and *E. canis* in apparently healthy, free-roaming dogs was linked to increased intraoperative bleeding tendencies, even when haemostatic parameters appeared normal.[60] Veterinarians working in remote Australian communities have also anecdotally reported concerns about perioperative bleeding in clinically normal dogs in communities where CME is already established. An increased risk of surgical complications, including a greater likelihood of perioperative bleeding, has important implications for patient safety and, by association, community trust and the uptake of dog desexing procedures as part of animal health and management programmes. This poses challenges for veterinarians working in remote community animal health programmes, particularly in light of the fact that clinical signs and body condition scoring may not be a reliable indicator of *E. canis* status. A shared tick vector, overlapping clinical signs and the potential for cross-reactivity in serological testing can also complicate diagnosis,[61,62] particularly in low-resource settings.

Although this is the first study to assess the seroprevalence of *E. canis* infection in Australian dogs, relying solely on serology to determine its prevalence has notable limitations. Findings from previous studies suggest that antibodies to *E. canis* can be detected in the blood as early as 2 days after infection,[63] typically peak 2 to 5 months after infection[16,18,63] and can remain detectable in the blood and tissues for extended periods, even after the pathogen has been eliminated from the body.[18,63–66] As such, serology cannot readily distinguish between past and current infection.[67] Potential cross-reactivity with other closely related tick-borne pathogens known to be endemic in remote Indigenous communities in Australia, particularly *Anaplasma platys*, can also result in false-positive results.[29,51,59]

Furthermore, although the serological test used in this study has demonstrated a specificity of 0.98 in dogs from Israel, the sensitivity of ELISA is highest when antibody levels exceed a titre of 1:320 as determined by an immunofluorescent assay test (IFAT).[68] Therefore, a false-negative ELISA result may be seen in individuals with low antibody titres and does not definitively exclude the presence of infection. To overcome this limitation in test sensitivity, repeated testing 1–2 weeks after the initial assay is recommended.[68] Despite published reports on test specificity, local validation of serologic assays—including point-of-care kits for *E. canis* developed overseas—is essential, as geographic variations in test reactivity may occur due to genetic diversity and differences in host immune responses across various populations.[69] There is also the potential for variability of antigens among pathogen strains, with subsequent impacts on the sensitivity and specificity of diagnostic testing.[70] Guidance from the World Organisation for Animal Health (WOAH) supports the importance of local test validation in animals of unknown disease status due to the challenges in determining true infection status, particularly where reference tests are imperfect and can be further enhanced by Bayesian latent class analysis to estimate diagnostic accuracy.[71] Given these considerations, validation of tests in the Australian context is essential. Furthermore, the development of accurate, cost-effective point-of-care *E. canis* antigen tests would be invaluable in supporting prompt diagnosis and effective management of *E. canis* infections in remote areas, particularly in the very acute stages of the disease when antibody levels may not yet be detectable, but early intervention can significantly improve prognosis.

Despite these limitations, the current study offers valuable insights into *E. canis* exposure in dogs living in remote communities and the limitations of clinical signs and BCS in predicting serostatus. Further investigation is required to develop a better understanding of the true infection status and the potential health impacts on dogs with subclinical infection.

Conclusion

This study provides valuable insights into the point seroprevalence of *E. canis* in dogs in Australia. Our results suggest a high seroprevalence of *E. canis* in dogs living in a remote community in the NT, with the majority of seropositive animals being asymptomatic. No significant relationships were observed between serostatus and body conditions, clinical signs or gender, which can complicate the identification of seropositive dogs. However, the use of serostatus as a sole indicator of infection rates presents certain limitations, as it cannot differentiate between active and past infection and may fail to detect infections in their early stages. Further research is needed to better understand the clinical significance of seropositivity in guiding animal health and management programmes in remote communities. The development of a locally validated serological and molecular diagnostic tests for *E. canis*, particularly point-of-care tests, would expand diagnostic capacity in field-based ehrlichiosis surveillance programmes and assist in the effective management of this emerging threat.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest. Laboratory fees associated with this project were funded by the Roper Gulf Regional Council and in-kind veterinary and veterinary nursing support to assist with the project. Doxycycline for the treatment of seropositive dogs was donated by Animal Management in Rural and Remote Indigenous Communities (AMRRIC). Dr. Emily Shepherdson undertook this research study as part of Murdoch University's Veterinary

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Professional Investigation unit of the Doctor of Veterinary Medicine degree.

Acknowledgments

We acknowledge the Traditional Owners of the lands on which the study took place, with special thanks to the community residents and their dogs. We extend our gratitude to David Hurst and Roper Gulf Regional Council for their invaluable support throughout the study. We would also like to acknowledge Dr. Samantha Phelan for her veterinary contributions during fieldwork and to AMRRIC, particularly Dr. Bonny Cumming, for assisting with project development and review of the manuscript. Finally, the authors wish to acknowledge Berrimah Veterinary Laboratory for providing the testing and assisting with the methods section of the manuscript. Open access publishing facilitated by Murdoch University, as part of the Wiley - Murdoch University agreement via the Council of Australian University Librarians.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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(Accepted for publication 22 July 2025)

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