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Original Article

Molecular detection by PCR of *Rickettsia rickettsii* in canines in Ciudad Obregón, Sonora, México

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Abstract

Introduction: *Rickettsia* is an intracellular, Gram-negative, zoonotic bacterium that is transmitted by arthropods, such as ticks, fleas and lice. *Rickettsioses* have been reported in Mexico since the 1930s, with a higher prevalence in the northern states. Infections affect both dogs and humans in marginalized areas, with *Rickettsia rickettsii* being the most pathogenic species and associated with high mortality rates.

Methodology: This study aimed to detect *R. rickettsii* in dogs from Ciudad Obregón, Sonora, using the *ompA* gene PCR. Between July and August 2024, 110 EDTA whole blood samples were collected: 95 from dogs with a presumptive clinical diagnosis of *rickettsiosis*, and 15 from stray dogs with active tick infestations. DNA extraction, PCR amplification and product purification were performed at the Veterinary Molecular Biology Laboratory of the Instituto Tecnológico de Sonora. Sanger sequencing, Snap Gene Viewer, and BLASTn against GenBank were used to confirm the identity of the sequences.

Results: Sequencing of the *ompA* fragment confirmed 99–100% homology with *R. rickettsii*. One positive case (6.7%) was detected, corresponding to a stray dog from a disadvantaged area of the municipality of Cajeme, indicating the presence of the pathogen in a localized area.

Conclusions: The detection of *R. rickettsii* in a stray dog in Ciudad Obregón confirms the presence of the pathogen in the area, highlighting the importance of free-roaming canines and environmental conditions in sustaining vectors. Early diagnosis and control of ectoparasites are critical to reducing zoonotic transmission, underlining the importance of continuous molecular surveillance in public health.

Key words: *Rickettsia rickettsii*; Tick; PCR; Sonora; dogs; Mexico.

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Introduction

Rickettsia is a genus of strictly intracellular, Gram-negative, zoonotic bacteria with coccobacillary morphology. These bacteria are responsible for various epidemics worldwide and are transmitted by blood-sucking arthropods such as ticks, fleas, lice, and mites. Direct human-to-human transmission does not occur. Infection in susceptible hosts occurs through the inoculation of vector salivary secretions during feeding or self-inoculation with infected feces deposited on the host's skin or fur [1].

Rickettsioses were first recognized in Mexico in the mid-1930s, primarily in the northern states, with recurrent outbreaks reported in Sonora, Sinaloa, Durango, and Coahuila [2]. Subsequent studies have confirmed the presence of the pathogen in various northern regions of Mexico, demonstrating frequent infections in dogs from these communities [3]. Furthermore, vectors have been found in walls, dirt floors, and mattresses inside homes, which explains the high incidence among women and children. This results in an epidemiological profile that differs from that observed in the United States [4]. Not all *Rickettsia* species are equally pathogenic: *R. rickettsii* can cause severe clinical manifestations with high mortality, whereas *R. parkeri* typically results in milder disease with no confirmed lethal cases to date [5].

Ticks are the principal natural reservoir for *R.*

rickettsii, encompassing various genera and species within the Ixodidae family [6]. The hosts include domestic vertebrates, such as dogs and rodents, as well as other mammals. Humans are considered accidental (dead-end) hosts, becoming infected through bites by infected arthropod vectors. During the feeding process, the tick adheres to the host's skin, employing its chelicerae to penetrate the dermis and capillaries to ingest blood. This process is facilitated by the presence of anticoagulant compounds in the tick's saliva [5].

Once the infection has taken hold, *R. rickettsii* invades endothelial cells, altering vascular permeability and causing systemic damage that may manifest as pulmonary oedema, interstitial pneumonia, neurological alterations and seizures [7]. In a smaller proportion of cases, infection with *R. rickettsii* can present as conjunctival inflammation, diarrhea, weight loss, dehydration, a capillary refill time of more than two seconds and pale mucous membranes [8].

Rickettsia DNA is detected using the polymerase chain reaction (PCR) technique on blood or tissue samples from infected dogs. This method enables a rapid, sensitive and specific diagnosis, regardless of the clinical phase of the disease. To ensure DNA integrity, samples must be preserved under optimal conditions before extraction [9].

R. rickettsii OMP-A is an immunodominant outer membrane autotransporter protein that is conserved in the spotted fever group (SFG). Previous studies and genomic comparisons suggest that *ompA* gene is involved in adhesion and may be critical for virulence. A variable region within this gene has been identified that differs between *Rickettsia* species, and this has been exploited for primer and probe design to develop highly specific molecular assays. Molecular detection relies on primers that amplify a fragment of approximately 380–397 base pairs for *Rickettsia* species [10]. The *ompA* gene is preferred over other targets, such as the *gltA* or *ompB* gene, due to its higher interspecies variability and exclusive presence in SFG *rickettsiae*. This specificity reduces the likelihood of false positives from the typhus group of *Rickettsia* (TG), such as *R. typhi*. Furthermore, the discriminatory power of the *ompA* gene makes it particularly valuable for epidemiological studies and species-level identification in endemic regions. Despite its variability, careful primer design enables robust detection while maintaining high sensitivity and specificity. Consequently, the targeting of the *ompA* gene is a reliable strategy for molecular detection of *R. rickettsii* and other closely related SFG species [11].

Methodology

Project location

The study was conducted at the Veterinary Molecular Biology Laboratory at the Instituto Tecnológico de Sonora (ITSON) in Ciudad Obregón, Sonora. The city's geographical location is specified by its latitude of 27°29′ north and its longitude of 109°59′ west (27°29′11″N 109°56′27″W), with an altitude above sea level of 40.8 m.

Study Population

A total of 110 whole blood samples were collected from dogs between July and August 2024. This corresponds to the summer season in Ciudad Obregón, Sonora, when the average ambient temperature typically ranges between 30 and 40 °C. Of these, 95 were dogs with a presumptive clinical diagnosis of rickettsiosis, and 15 were stray dogs captured by municipal animal control services, all of which showed active tick infestations.

As samples were obtained through routine diagnostic submissions from a commercial laboratory and from municipal animal control services, detailed individual-level metadata such as age, sex, breed, ownership status, prior antibiotic treatment, quantified tick burden, and housing or peridomestic conditions were not systematically available for all animals. Consequently, the analysis was limited to the available categorical classification of dogs (clinically suspected to be owned versus stray with active tick infestation).

Samples collection

Samples were obtained from a commercial diagnostic laboratory in Ciudad Obregón, Sonora. The blood samples were then transported to the Veterinary Molecular Biology Laboratory (ITSON) and stored at 4°C for a maximum of 24 hours prior to processing. Chilled samples were centrifuged at 3,500 rpm for 15 minutes, and 200 µL of the buffy coat was collected for DNA extraction.

DNA Extraction

Bacterial DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN®, Hilden, Alemania), following the manufacturer's protocol. DNA quality and purity were assessed using a BioSpec-nano spectrophotometer (Shimadzu®), and DNA integrity was confirmed by 1.5% agarose gel electrophoresis stained with ethidium bromide.

Positive Control Design

The NCBI GenBank BLASTn tool was used to

Table 1. Primers used for the detection of Rickettsia rickettsii in canine blood samples.

Agent	Primer	Sequence (5′–3′)	Gene	Amplicon size (bp)	Annealing temperature (°C)
Rickettsia rickettsii	Forward (Rr190.70p) Reverse (Rr190.602n)	ATGGCGAATTCTCCAAAA AGTGCAGCATTCGCTCCCCCT	ompA	576	58

retrieve the full sequence of the *ompA* gene. This sequence was aligned with primers *Rr190.70p* (ATG GCG AAT TCT CCA AAA) and *Rr190.602n* (AGT GCA GCA TTC GCT CCC CCT), covering a 576 bp region [2]. The primer sequences used in this study are detailed in Table 1.

PCR Amplification and Analytical Sensitivity

Prior to the processing of biological samples, the PCR protocol was standardized using triplicates of synthetic positive controls. The GoTaq® Flexi DNA Polymerase Kit (Promega), which includes Green GoTaq® Buffer, was used in 25 µL reactions: 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM primers, 1.25 U polymerase, 2 µL DNA, and nuclease-free water. The PCR cycling conditions consisted of an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 5 minutes, after which the samples were stored at 4°C until further analysis. Amplified products were visualized on 1.5% agarose gels stained with ethidium bromide. A product of 576 bp was considered a positive result.

To determine the analytical sensitivity of the assay, synthetic positive control DNA was initially tested at 5 ng, then at 1 ng, followed by serial 1:10 dilutions down to 0.01 fg. Each dilution was tested in triplicate to confirm the minimum detectable concentration capable of producing a visible amplicon under the described PCR conditions. Finally, with the optimized protocol and established analytical sensitivity, the detection of *R. rickettsii* was performed on all biological samples included in this study.

Sequencing and In Silico Analysis

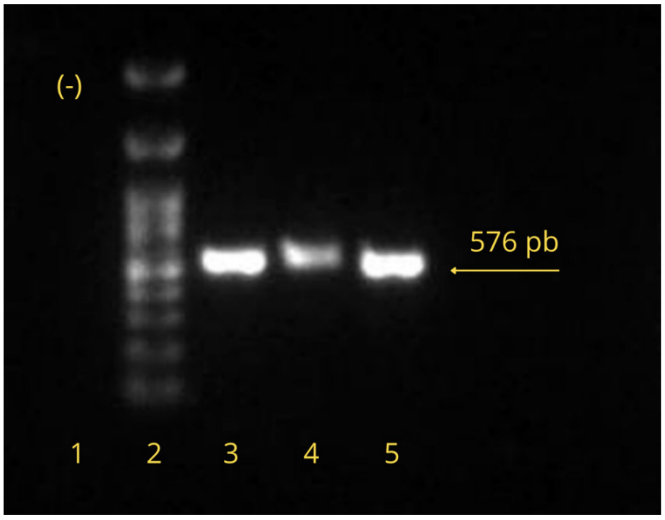
To confirm the identity of the amplified fragment, the product was purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Sanger sequencing was performed at Langebio-CINVESTAV, Irapuato. The sequence was edited with SnapGene Viewer (Insightful Science) and analyzed with the BLASTn algorithm to confirm its identity through homology with GenBank entries.

Results

PCR standardization was achieved using 0.5 ng/µL of synthetic positive control DNA, successfully producing the expected amplicons (Figure 1). This phase ensured optimal efficiency and specificity of the PCR conditions for *R. rickettsii.* Subsequently, a minimum analytical sensitivity of 0.1 pg was established, thereby demonstrating the method's high capacity to detect low quantities of DNA (Figure 2).

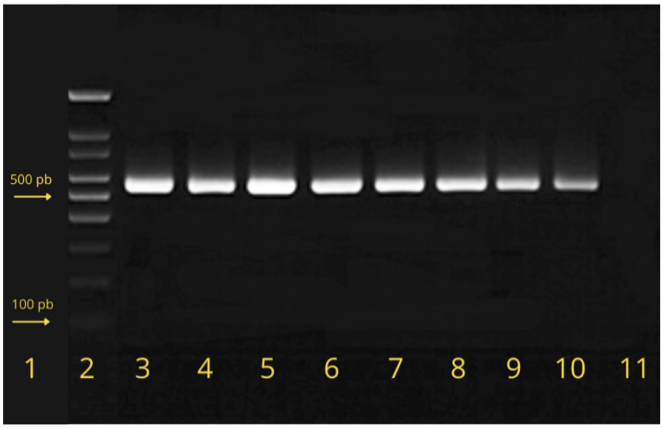
Overall, one positive sample was detected among

Figure 1. 1.5% agarose gel electrophoresis in triplicate with a synthetic positive control for *Rickettsia rickettsii*.



C1: negative control, C2: DNA ladder ranging 100 to 500 base pairs (bp), C3, C4, and C5: positive control located at 576 bp.

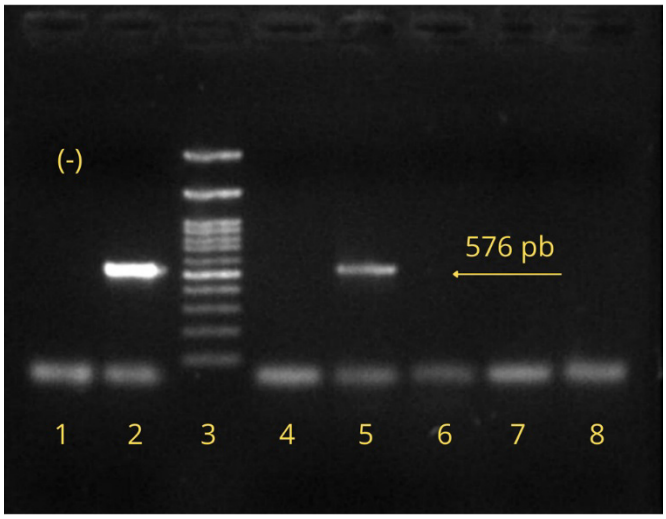
Figure 2. Analytical sensitivity of PCR targeting the *ompA* gene of *Rickettsia rickettsii*.



Lane 1: negative control; lane 2: DNA ladder (molecular weight marker); lane 3: 25 ng; lane 4: 1 ng; lane 5: 0.1 ng; lane 6: 0.01 ng; lane 7: 1 pg; lane 8: 0.1 pg; lane 9: 0.01 pg; lane 10: 1 fg; lane 11: 0.01 fg.

the 110 dogs analyzed (1/110), corresponding to an overall prevalence of 1.1% (95% confidence interval (CI): 0.03–5.78%). When the data were stratified by subgroup, no positive cases were detected among clinically suspected owned dogs (0/95; 0%, 95% CI: 0.00–3.83%), whereas one positive case was identified among stray dogs (1/15), corresponding to a subgroup-specific prevalence of 6.7% (95% CI: 0.17–31.95%). These estimates reflect the high uncertainty associated with small sample sizes and should be interpreted with caution (see Figure 3 and Table 2). They indicate the presence of the pathogen in underprivileged areas of the municipality of Cajeme, Sonora. The positive sample was sequenced to confirm the identity of the amplicon. High-quality chromatograms were obtained, and BLASTn analysis showed 99–100% homology with *R. rickettsii* sequences in GenBank.

Figure 3. PCR result targeting the *ompA* gene (570–580 bp) for *Rickettsia rickettsii* on a 1.5% agarose gel.



C3: 100 bp DNA ladder. C1: negative control, C2: synthetic positive control, C5: positive sample from the 15 additional samples of stray dogs captured at the pound.

Discussion

The persistence of this bacterium is linked to the increasing presence of the brown dog tick (*Rhipicephalus sanguineus*), whose proliferation is favored by warm and humid environmental conditions. These conditions promote aggregation among stray dogs or among dogs infested with ticks [12]. Although only 1–5% of ticks may be infected in low-incidence regions, this frequency can vary depending on the local persistence of hemoparasites. The disease is commonly associated with inadequate sanitary conditions in marginalized or rural areas, as evidenced by the positive stray dog that was captured at the pound. This animal showed evident tick infestation, and humans, as accidental hosts, may develop severe disease or even die following infection [13].

Additionally, the absence of systematically collected animal-level metadata is an important limitation of the present study, restricting a more detailed epidemiological interpretation of the findings. Known influencing factors include variables such as age, sex, breed, intensity of tick infestation, prior antimicrobial treatment, and housing or peridomestic conditions. In particular, prior antibiotic treatment may reduce the load of the pathogen in the dog to below the limit of PCR detection, as rickettsial DNA in blood tends to be short-lived and decreases rapidly with antimicrobial therapy [14,15]. This limitation could partially explain the lack of positive results among the clinically suspected owned dogs included in this study.

A key component of this study was the utilization of the GenBank database via BLASTn, which enabled the retrieval and alignment of reference sequences for *R. rickettsii*, thereby facilitating the standardization of the PCR protocol. The *ompA* gene (outer membrane protein A) was chosen as the molecular target due to its conserved nature among members of the spotted fever group (SFG) *rickettsiae*, including *R. rickettsii.* This gene encodes a heat-stable, surface-exposed outer membrane protein that plays a critical role in host cell adhesion, making it a highly suitable marker for molecular diagnostics [16,17]. Importantly, the *ompA* gene is known to be highly expressed and present in multiple copies during infection, enhancing the analytical sensitivity of PCR-based detection methods [18]. The partial region commonly amplified in diagnostic protocols exhibits sufficient sequence variability to distinguish *R. rickettsii* from other closely related species, while maintaining conserved regions that ensure efficient primer binding and reliable amplification [19].

Similar strategies that combine *in silico* analysis and PCR amplification have been previously employed with success in detecting other pathogens from the order *Rickettsiales* in veterinary samples [2]. Molecular

Table 2. Number of cases and incidence of *Rickettsia rickettsii* in canines in Ciudad Obregón, Sonora, by PCR (n = 110).

Group	Total (n)	Positive (n)	Prevalence (%)
Veterinary clinic dogs	95	0	0%
Stray dogs	15	1	6.7%
Total	110	1	1.1%

detection of *Ehrlichia canis*, *Anaplasma platys* and *R. rickettsii*, with the latter being the most pathogenic and clinically relevant to both canine and human health. The rationale behind the selection of *ompA* gene as a molecular target is twofold: its specificity and diagnostic utility, and its epidemiological relevance in endemic regions such as northwestern Mexico. In such regions, timely detection of *R. rickettsii* is imperative for public health surveillance and disease control.

A study conducted along the U.S.-Mexico border detected *Rickettsia spp*. in blood and ticks by PCR [20]. In Sonora, a 14% positivity rate was reported (3/38), while Baja California reported 65% (23/35) and Coahuila 21% (12/58). Notably, 25% of tested dogs had active tick infestations. Most dogs sampled were owned by their respective owners, were in good health, and practiced proper hygiene. However, it should be noted that a good physical condition does not necessarily guarantee immunity against tick infestation or subsequent infection.

In the present study, 110 blood samples were analyzed from dogs residing in Cajeme, Sonora, including both clinical and stray dogs. Overall, one sample tested positive for R. rickettsii, corresponding to an overall prevalence of 1.1%. When the results were analyzed by subgroup, the positive case was identified exclusively among stray dogs (1/15, 6.7%), whereas no positive cases were detected among clinically suspected owned dogs (0/95). A similar study by Aragón López *et al.* (2021) examined 170 domestic dogs in the same region, and two positive samples were identified, corresponding to an approximate prevalence of 1.2%. These findings highlight a consistent and low-level circulation of *R. rickettsii* among canine hosts in Cajeme, independent of housing or care conditions. However, in contrast to the present study, in which the positive samples were derived from a stray dog, Aragón López *et al.* exclusively detected positive cases in domestic dogs. This discrepancy may be attributable to several factors, including variations in sample populations, differences in environmental exposure, or potential biases in sampling strategies. It is important to note that the prevalence observed in the subgroup of stray dogs should not be interpreted as equivalent to the overall prevalence, given that these estimates are derived from markedly different sample sizes and epidemiological contexts. Due to the small number of stray dogs sampled, there is a high level of uncertainty around this estimate, and it should be interpreted with caution. It is hypothesized that stray dogs may have different patterns of contact with tick vectors compared to domestic dogs, potentially influenced by roaming behavior and habitat use. Consequently, the detection of *R. rickettsii* in both populations underscores the pervasive presence of infected vectors across diverse ecological settings in Cajeme. These findings reinforce the need for comprehensive surveillance encompassing both domestic and free-roaming dogs to better understand transmission dynamics and effectively design control measures. In addition, the performance and reproducibility of PCR-based diagnostics can be influenced by the stability of protein-based assay components (e.g., polymerases), which are sensitive to storage temperature, buffer conditions, and repeated freeze–thaw cycles [21].

Conclusions

The PCR-based molecular technique detected *Rickettsia rickettsii* in a stray dog captured by municipal authorities in Ciudad Obregón, Sonora. Given that only one PCR-positive case was identified, the results should be interpreted with caution and considered primarily as evidence of pathogen circulation rather than as a precise estimate of prevalence. These findings underscore the importance of continued molecular surveillance in both stray and owned dog populations to support animal welfare and mitigate potential public health risks in the region.

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Conflict of interest

No conflict of interest is declared.

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