Short Note [Nota Corta]

Epidemiological factors associated with the distribution of *Rickettsia* hosts in Ucú, Yucatan, Mexico †

[Factores epidemiológicos asociados con la distribución de hospederos de *Rickettsia* en Ucú, Yucatán, México]

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SUMMARY

Background. Rickettsioses are zoonoses caused by pathogenic bacteria of the genus *Rickettsia* transmitted through bites or feces of ectoparasites (ticks, fleas, lice, and hematophagous mites). Rodents and dogs play a role in the transmission of pathogenic species. However, little is known about the factors associated with the chances of infections and their spatial distribution at the local scale. **Objective.** To determine the frequency of *Rickettsia* Spotted Fever group (SFG) and Typhus group (TG) in dogs and rodents, their spatial distribution, and the factors associated with the *Rickettsia* infection at the local scale. **Methods.** DNA was obtained from 64 dog blood samples and 36 rodent spleens from 48 dwellings in Ucú, Yucatan, Mexico. *Rickettsia* was determined by multiplex semi-nested PCR (snPCR). The predictor variables for infection in dogs and rodents were analyzed using generalized linear models (GLM) ($p<0.05$). **Results.** SnPCR revealed *Rickettsia* in 28.1 % (18/64, 95 % CI 17.6 %-40.8 %) of the dogs and 27.8 % (10/36, 95 % CI 14.2 %-45.2 %) of the rodents. *Rickettsia* SFG was detected in dogs; rodents hosted both *Rickettsia* TG and SFG. According to the corresponding GLM, dogs living in the interior area of Ucú had a lower chance of infection by *Rickettsia* SFG (OR = 0.14) than those living in the other regions. The presence of opossums (*Didelphis* spp.) in dwelling premises increases the chances of *Rickettsia* TG infection in rodents (OR = 14), and the presence of SFG-infected dogs in dwelling premises increases the chances of *Rickettsia* SFG infection in rodents (OR = 21). **Implications.** The spatial distribution of *Rickettsia* hosts was assessed at Ucú. The specific *Rickettsia* species were not identified, nor were the probable vectors evaluated. While the results provide a local overview, they do not accurately represent the epidemiological conditions at other sites. The information is valuable and should be considered in developing prevention measures for Rickettsiosis. **Conclusions.** Statistical modelling revealed that the spatial location

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of the dwelling, the presence of infected dogs, and the presence of opossums in the same dwelling serve as proxies for the *Rickettsia* infection in the captured rodents.
Key words: dogs; *Rickettsia* infections; Rodentia; Siphonaptera; ticks; Vector-borne diseases.

RESUMEN

Antecedentes. Las rickettsiosis son zoonosis ocasionadas por bacterias patógenas del género *Rickettsia* transmitidas por picaduras o heces de ectoparásitos (garrapatas, pulgas, piojos y ácaros hematófagos). Los roedores y perros participan en la transmisión de especies patógenas. Sin embargo, se conoce poco sobre los factores asociados con la posibilidad de infección y su distribución espacial a escala local. **Objetivo.** Determinar la frecuencia de *Rickettsia* del grupo fiebre manchada (SFG) y del grupo tifo (TG) en perros y roedores, su distribución espacial y los factores asociados con la infección con *Rickettsia* a escala local. **Metodología.** Se obtuvo ADN de 64 muestras de sangre de perros y 36 bazos de roedores de 48 viviendas de Ucú, Yucatán, México. Se determinó ADN de *Rickettsia* por PCR semianidada multiplex (snPCR). Las variables predictoras de la infección en perros y roedores se analizaron mediante modelos lineales generalizados (GLM) ($p<0.05$). **Resultados.** La snPCR reveló *Rickettsia* en 28.1 % (18/64, IC 95 % 17.6 %-40.8 %) de los perros y 27.8 % (10/36, IC 95 % 14.2 %-45.2 %) de los roedores. Se detectó *Rickettsia* SFG en perros; los roedores hospedaron tanto *Rickettsia* TG como SFG. Según el GLM correspondiente, los perros que vivían en el área interior de Ucú tuvieron menor posibilidad de infección por *Rickettsia* SFG (OR = 0.14) que los que vivían en otras regiones. La presencia de zarigüeyas (*Didelphis* spp.) en las viviendas aumenta las posibilidades de infección por *Rickettsia* TG en roedores (OR = 14), y la presencia de perros con SFG en las viviendas aumenta la posibilidad de infección por *Rickettsia* SFG en roedores (OR = 21). **Implicaciones.** Se evaluó la distribución espacial de hospederos de *Rickettsia* en Ucú, Yucatán, México. No se identificaron las especies de *Rickettsia* ni se evaluaron los vectores. Los resultados ofrecen una visión local, pero no representan las condiciones epidemiológicas en otros sitios. La información es valiosa y debe considerarse al desarrollar medidas de prevención de rickettsiosis. **Conclusiones:** El modelo estadístico reveló que la ubicación espacial de la vivienda, la presencia de perros infectados y la presencia de zarigüeyas en la misma vivienda son indicadores de la infección con *Rickettsia* en los roedores capturados.
Palabras clave: enfermedades transmitidas por vector; garrapatas; infecciones; perros; *Rickettsia*; Rodentia; Siphonaptera

INTRODUCTION

Rickettsioses are infectious diseases caused by bacteria of the genus *Rickettsia*. They are transmitted through the bites or feces of hematophagous ectoparasites (ticks, lice, fleas, and mites) (Sánchez-Montes *et al*., 2021a). Rymaszewska and Piotrowski (2024) propose the most recent classification for the genus, which includes the Spotted Fever groups (SFGI and SFGII), the Typhus group (TG), the Canadensis group (CG), and the Bellii group (BG).

Rickettsioses have different prevalence rates across Mexico. According to Sánchez-Montes *et al*. (2021a) and Torres-Castro *et al*. (2024), the prevalence of *Rickettsia* is higher in the northern and southeastern states of the country, including Yucatan, where species of the SFG, such as *R. rickettsii*, *R. parkeri* (Torres-Castro *et al*., 2022a), and species of the TG, such as *R. typhi*, have been reported (Torres-Castro *et al*., 2022b). Additionally, antibodies against *Rickettsia* SFG and *Rickettsia* TG species have been identified in residents of this region. Therefore, they are considered public health problems (Aguilar-Tipacamú *et al*., 2025).

Certain biotic and abiotic factors increase the risk of *Rickettsia* infection in residents of some localities in Yucatan, Mexico. These factors include younger people living in rural areas, peridomestic family orchards, and free-living ectoparasites in the peridomicile (Torres-Castro *et al*., 2020, 2024). Similarly, animals were recorded as *Rickettsia* SFG and TG-infected in dwellings with at least one case of human Rickettsiosis (Dzul-Rosado *et al*., 2021). These findings suggest the potential role of dogs and rodents in transmitting *Rickettsia* (Torres-Castro *et al*., 2024). The transmission of SFG and TG species is generally associated with environments characterized by overcrowding, unsanitary conditions, and a high degree of marginalization. These conditions contribute to the proliferation of vectors, primarily ticks, lice, and fleas, which cause infestations in humans (Álvarez-Hernández *et al*., 2010; Sánchez-Montes *et al*., 2021)

Dogs and rodents harbor pathogenic *Rickettsia* species, contributing to the maintenance of epidemiological cycles (Sánchez-Montes *et al*., 2021a; Arroyo-Ramírez *et al*., 2023; Torres-Castro *et al*., 2024). In Yucatan, Mexico, these animals host *R. typhi* (Martínez-Ortiz *et al*., 2016; Torres-Castro *et al*., 2018), *R. parkeri* (Arroyo-Ramírez *et al*., 2023), and *R. felis* (Panti-May *et al*., 2015); as well as several ectoparasite species, including ticks and fleas (Martínez-Ortiz *et al*., 2019; Arroyo-Ramírez *et al*., 2022; Torres-Castro *et al*., 2022c).

The spatial distribution of these hosts is crucial for understanding the risk of *Rickettsia* infection, particularly in inhabited areas (Torres-Castro *et al*., 2020, 2022c; Arroyo-Ramírez *et al*., 2023). Studies that explored the spatial distribution of ectoparasites and rickettsial hosts revealed that infection tends to occur in clusters where vectors and hosts converge (Dzul-Rosado *et al*., 2021). However, the spatial distribution of dogs and rodents in human settlements makes understanding the risk of *Rickettsia* infection a challenging task. This study aimed to determine the frequency of *Rickettsia* SFG and TG infections in the animals studied (dogs and rodents), their spatial distribution, and to identify the factors associated with local *Rickettsia* infections.

MATERIALS AND METHODS

Ethical statement

The study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine, *Universidad Autónoma de Yucatán*, Mexico (CB-CCBA-M-2021-003). The Ministry of Environment and Natural Resources of Mexico authorized the extraction of rodents for scientific collection purposes (31/K5-0108/12/20).

Study site

The locality of Ucú (22°01′55″ N, 89°44′47″ W), Yucatan, Mexico, has an area of 130.8 km^2 and an average altitude of 8 m above sea level. It has a population of approximately 4,049 residents in 1,139 dwellings and a warm-subhumid climate with summer rainfall. The average annual temperature is 26°C, and the rainfall is 800 mm (INAFED, 2021).

For locality selection, a couple of preliminary field trips were conducted to identify the dog population, rururban conditions, and interest in the study among the inhabitants.

Study design

The study type was descriptive and cross-sectional. Due to sanitary restrictions (COVID-19), the sample size was obtained based on the number of occupied dwellings (1,139) and not based on a previous prevalence (dogs) or with infinite populations (rodents). The sample size was obtained using the formula to calculate sample size to estimate a frequency (Thrusfield, 2007) using a 95 % confidence level, 80 % precision, and an expected proportion of 6 % of animal captures according to previous studies in peridomiciles with conditions similar to those of the present study (Panti-May *et al*., 2016, 2018; Torres-Castro *et al*., 2022c).

To cover the extension of Ucú, two imaginary lines that crossed its center were drawn to divide it into three concentric areas (interior, central, and exterior). For a homogeneous distribution of dwellings, 16 per area, totaling 48 dwellings throughout Ucú, were considered (Figure 1). The dwellings were conveniently selected, and the sample included those whose owners signed informed consent (Suárez-Galaz *et al*., 2024).

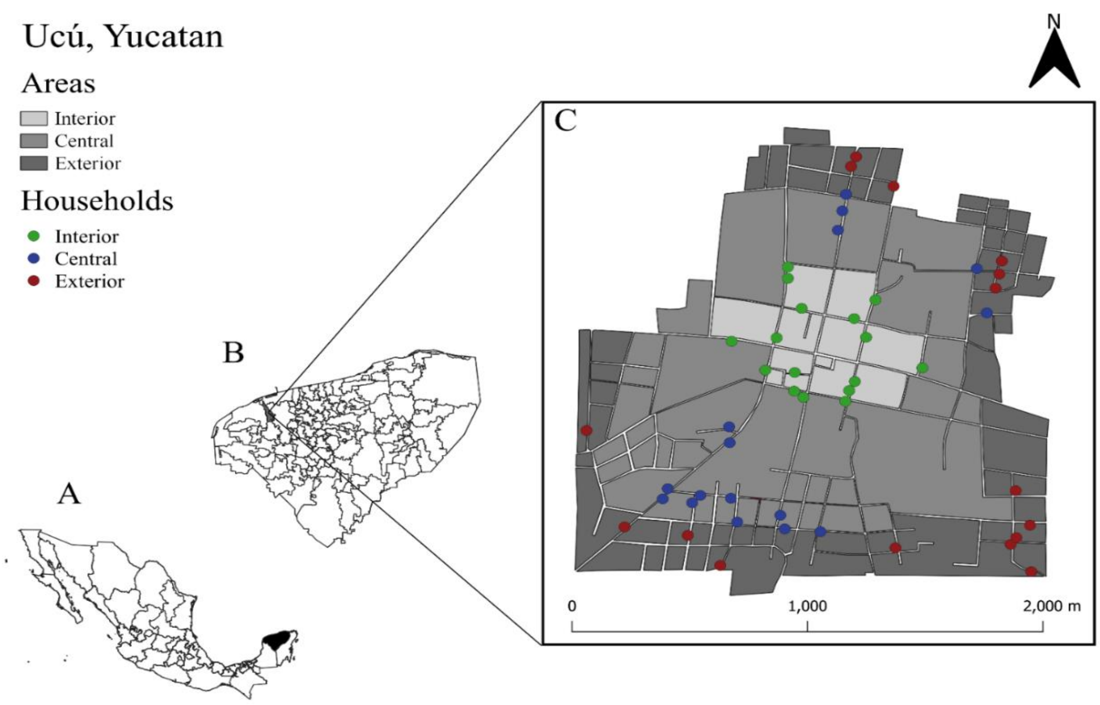

Figure 1. Maps of Mexico (A), Yucatan (B), and Ucú (C) showing the three areas (interior, central, and exterior) and the distribution of dwellings studied in Ucú, Yucatan, Mexico.

Biological samples

Between August and October 2021, a single visit was conducted to each dwelling. Puppies (< 2 months) and dogs that received antibiotics at least two months before the visit (information provided by the owner) were excluded.

Before blood sampling, the venipuncture area was sanitized to prevent cross-contamination with ectoparasite feces. Peripheral blood samples (up to 5 mL) were collected from each dog through femoral or cephalic vein puncture, using 5 mL sterile syringes (BD Plastipak; Mexico) and placed in tubes with heparin (BD Vacutainer® PST; USA).

Blood samples were stored in portable refrigerators, transported to the laboratory, and centrifuged (1,500 g for 10 minutes at 26°C) to separate the plasma. The plasma was stored in 1.8 mL microcentrifuge tubes (Eppendorf®; USA) at -80°C until diagnostic procedures were performed.

Ten Sherman traps (8 × 23 × 9 cm; HB Sherman Traps, Inc., USA) were used per dwelling to capture rodents. The traps were baited with oat flakes and vanilla flavoring and placed in the peridomicile for two consecutive nights. The total capture effort was 960 nights/trap.

The captured rodents were transported to the laboratory for further processing. The species were identified using field guides (Reid, 2009). All rodents were anesthetized with isoflurane (Piramal Enterprises Limited®; United Kingdom) and euthanized with an overdose (90-210 mg/kg) of pentobarbital sodium (Aranda®; Mexico). After euthanasia, the spleen was collected and stored in 1.8 ml microcentrifuge tubes at -80°C.

Animal data collection

Dog data were collected by a questionnaire from each animal's owner. Additionally, dogs were visually inspected on the day of sampling. The data included sex (male or female) and age (puppy: < 1 year, adult: 1-6 years, or geriatric: ≥ 6 years) (Torres-Castro *et al*., 2022c). The study examined whether dogs were infested with ticks or fleas (positive or negative, regardless of the number of ectoparasites) and the level of infestation (regardless of the type of ectoparasite). Infestation levels were classified as negative (no presence), mild (≤ 10 ectoparasites), moderate (11 to 30 ectoparasites), or severe (> 30 ectoparasites) (Torres-Castro *et al*., 2022c). The study also considered whether dogs had access to roam outside the dwelling (positive or negative, regardless of time). All dogs that were in the dwelling on the day of the visit were included.

Rodent data were collected on sex (male or female), age (adult or juvenile), weight (≤ 10 g or > 10 g), and reproductive conditions (active or inactive) (Panti-May *et al*., 2016).

Dwelling data collection

The questionnaire also collected information on dwelling characteristics: area (interior, central, and exterior), the presence/absence of 1) companion animals other than dogs (e.g., cats, rabbits, birds, etc.), 2) production animals (e.g., cattle, horses, poultry, etc.), and 3) synanthropic fauna (rodents or opossums).

To monitor the presence of opossums (*Didelphis* spp.), a Tomahawk trap (23 × 23 × 66 cm, Tomahawk Live Trap, Inc.; USA) was placed per dwelling. Traps were baited with pineapple and kept open during the same nights of rodent trapping, resulting in a total capture effort of 96 nights/trap. All captured opossums were released in the nearest forested area to Ucú.

Genomic DNA extraction and identification of *Rickettsia* groups

DNA was extracted from blood samples and spleens using the Wizard Genomic DNA Purification Kit® (Promega®; USA). The quality (A260/280) and concentration (ng/µl) of the extracted DNA were assessed using a Nanodrop (Thermo Fisher Scientific™; USA) and the samples were stored at -20°C until diagnostic procedures.

To identify *Rickettsia* SFG and TG, a semi-nested multiplex PCR (snPCR) targeting a fragment of the *sca5* gene in all *Rickettsia* species was performed. In the first round, the oligonucleotides rOmpB OF (5’-GTAACCGGAAGTAATCGTTTCGTAA-3’), rOmpB OR (5’-GCTTTATAACCAGCTAAACCACC-3’) (Choi *et al*., 2005), and rOmpB-804 Fw TG (5’-GAATCAACTGATACAGCAGC-3’) were used. In the second, the oligonucleotides rOmpB SFG IF (5'-GTTTAATACGTGCTGCTAACCAA-3’), rOmpB SFG/TG IR (5'-GGTTTGGCCCATATACCATAAG-3’) (Choi *et al*., 2005), and rOmpB TG IF (5'-AAGATCCTTCTGATGTTGCAACA-3’) were used (Lugo-Caballero *et al*., 2021).

This snPCR amplifies fragments of 420 bp for SFG and 237 bp for TG (Choi *et al*., 2005). Characterized positive controls were used to confirm all reactions (Torres-Castro *et al*., 2020, 2022c; Lugo-Caballero *et al*., 2021). The negative control was the PCR mixture without template DNA. Electrophoresis was performed in 1.5 % agarose gels stained with ethidium bromide. A gel photo documentation system (Biorad®, California, USA) was used for visualization. The sequencing and analysis of positive products to identify *Rickettsia* species were not conducted.

Statistical analysis

Descriptive statistics were used to analyze the snPCR results and determine the frequency of *Rickettsia* infection in the animals studied. The Clopper-Pearson procedure was used to estimated 95 % confidence intervals (CI) with the Quantitative Parasitology package for R (Reiczigel, 2019).

Fisher's exact test determined the statistical association between the *Rickettsia* infection frequency (rodents and dogs) and the surveyed characteristics of the studied animals and dwellings (Katz, 2011). Associations $p<0.3$ were significant for inclusion as independent variables in the generalized linear models (GLM) analyses (Katz, 2011).

A GLM with a binomial error distribution was used to select predictor variables for the chances of *Rickettsia* infection in the dogs studied. According to the snPCR results, the dependent variable for the dog's data was 1: positive or 2: negative for *Rickettsia* SFG.

The rodent’s data were analyzed by a multinomial GLM using the *Rickettsia* infective group as a dependent variable at three levels (1: negative, 2: SFG-infected, 3: TG-infected). The "negative" level was used as a reference.

In both cases, Akaike’s information criterion (AIC) was used to select the best-fit models. The statistical significance was $p<0.05$. The Odds Ratio (OR) and CI of 95 % were estimated. R software version 3.9.0 used within the RStudio programming environment, helped with the statistical analyses (R Core Team, 2024).

RESULTS AND DISCUSSION

We studied 64 dogs and 36 rodents. Of the dogs studied, 57.8 % (37/64) were males and 42.2 % (27/64) were females; 21.8 % (14/64) were puppies, 67.2 % (43/64) were adults, and 10.9 % (7/64) were geriatric; 29.7 % (19/64) were infested with fleas and 75 % (48/64) with ticks. The infestation levels were 7.8 % (5/64) negative, 67.2 % (43/64) mild, 20.3 % (13/64) moderate, and 4.7 % (3/64) severe; 73.4 % (47/64) of the dogs had access to roam outside the dwelling (Table 1).

Regarding the characteristics of the dwellings, 29.7 % (19/64) of the dogs were from dwellings in the interior area, the same amount from the central area, and 40.6 % (26/64) were from dwellings in the exterior area; 62.5 % (40/64) of the dogs coincided with at least one production animal; 87.5 % (56/64) coincided with companion animals different from dogs; 46.9 % (30/64) were sampled from dwellings with rodents; and 25 % (16/64) were sampled from dwellings with opossums (Table 1).

Table 1. Frequency of *Rickettsia* SFG regarding the characteristics of studied dogs and dwellings of Ucú, and its respective p-value from the Fisher’s exact test for the selection of predictor variables.

Variables	Dogs (%) N = 64	SFG (%) N = 18	p-value
Dogs’ characteristics			
Sex			
Male	37 (57.8)	9 (50)	0.6
Female	27 (42.2)	9 (50)	
Age			
Puppy	14 (21.8)	6 (33.3)	0.18*
Adult	43 (67.2)	9 (50)	
Geriatric	7 (10.9)	3 (16.7)	
Flea infestation			
Presence	19 (29.7)	3 (16.7)	0.22*
Absence	45 (70.3)	15 (83.3)	
Tick infestation			
Presence	48 (75)	15 (83.3)	0.52
Absence	16 (25)	3 (16.7)	
Infestation level			
Negative	5 (7.8)	1 (5.6)	0.96
Mild	43 (67.2)	12 (66.7)	
Moderate	13 (20.3)	4 (22.2)	
Severe	3 (4.7)	1 (5.6)	
Access to roam			
Yes	47 (73.4)	15 (83.3)	0.41
No	17 (26.6)	3 (16.7)	

Variables	Dogs (%) N = 64	SFG (%) N = 18	p-value
Dwellings' characteristics			
Location			
Interior	19 (29.7)	2 (11.1)	0.11*
Central	19 (29.7)	6 (33.3)	
Exterior	26 (40.6)	10 (55.6)	
Production animals			
Presence	40 (62.5)	11 (61.1)	1
Absence	24 (37.5)	7 (38.9)	
Companion animals			
Presence	56 (87.5)	17 (94.4)	0.52
Absence	8 (12.5)	1 (5.6)	
Synanthropic animals			
Presence	36 (56.2)	12 (66.7)	0.17*
Absence	28 (43.8)	6 (33.3)	
Rodents			
Presence	30 (46.9)	10 (55.6)	0.55
Absence	34 (53.1)	8 (44.4)	
Opossums			
Presence	16 (25)	3 (16.7)	0.52
Absence	48 (75)	15 (83.3)	

*p<0.3

Among the rodents, 88.9 % (32/36) were *Mus musculus*, 8.3 % (3/36) were *Peromyscus yucatanicus*, and 2.8 % (1/36) were *Ototylomys phyllotis*; 66.7 % (24/36) were males and 33.3 % (12/36) were females; 86.1 % (31/36) were adults and 13.9 % (5/36) were juveniles (Table 2).

According to the dwellings' characteristics, 27.8 % (10/36) of the rodents were captured in dwellings in the interior area, 16.7 % (6/36) in the central area, and 55.6 % (20/36) in the exterior area; 58.3 % (21/36) were captured from dwellings with at least one production animal; and 80.6 % (29/36) were captured from dwellings with companion animals. Opossums were captured for 16.7 % (6/36) of the rodents studied (Table 2).

SnPCR revealed *Rickettsia* DNA in 28.1 % (18/64, 95 % CI 17.6 %-40.8 %) of dogs and 27.8 % (10/36, 95 % CI 14.2 %-45.2 %) of rodents. The amplifications for both groups of *Rickettsia* (SFG and TG) are presented in Figure 2.

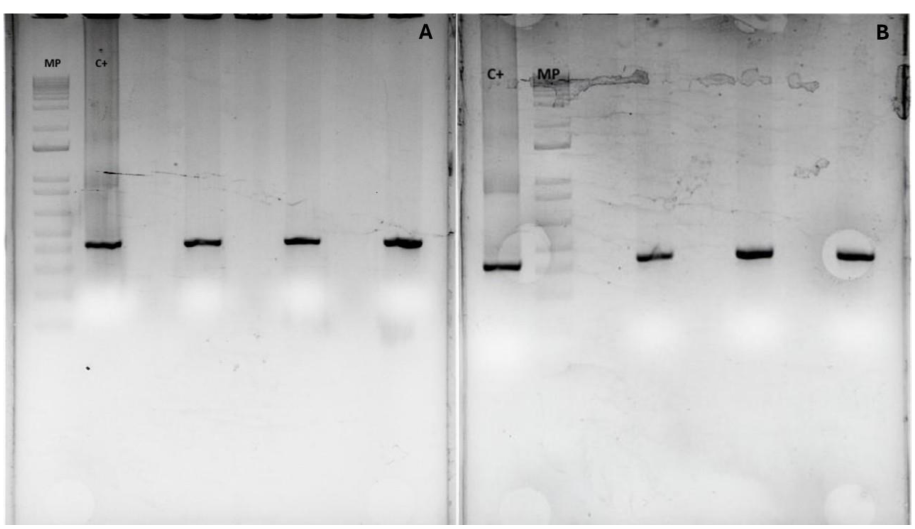

Figure 2. A) A 2 % agarose gel stained with ethidium bromide showing some *Rickettsia* SFG-positive products obtained from the animals studied in Ucú, Yucatan, Mexico. B) A 2 % agarose gel stained with ethidium bromide showing some *Rickettsia* TG-positive products obtained from the animals studied in Ucú, Yucatan, Mexico. MP: 100-base pair molecular weight marker; C+: positive control.

All *Rickettsia*-infected dogs were SFG. There were equal numbers of females and males among these dogs (50 %, 9/18); 50 % (9/18) were adults, 33.3 % (6/18) were puppies, and 16.7 % (3/18) were geriatric (Table 1).

Among the *Rickettsia*-infected rodents, 90 % (9/10) were *M. musculus*, and 10 % (1/10) were *P. yucatanicus*; 70 % (7/10) were SFG-infected, including the *P. yucatanicus*, and 30 % (3/10) were TG-infected; 80 % (8/10) were male and 20 % (2/10) were female; 80 % (8/10) were adults and 20 % (2/10) were juveniles (Table 2). Finally, 16.7 % (6/36) of the captured rodents coincided with at least one *Rickettsia* SFG-infected dog.

The bivariate analyses revealed statistical associations between *Rickettsia* SFG-infected dogs and age, flea infestation, dwelling location, and the presence of synanthropic animals (Table 1). The best predictor variables for the *Rickettsia* infection in rodents were 1) for *Rickettsia* SFG, dogs with *Rickettsia* SFG infection, and 2) for *Rickettsia* TG, weight, and opossums (Table 2).

The binomial GLM with the smallest AIC value (76.33) for *Rickettsia* SFG-infected dogs included the predictor variables dwelling location and companion animals. The model showed that dogs from the "interior area" had a lower chance (OR = 0.14; 95 % CI 0.02 – 0.65) of *Rickettsia* SFG infection than those from other areas (central and exterior) (Table 3).

Table 2. Frequency of *Rickettsia* SFG and TG infection regarding the characteristics of positive rodents and dwellings of Ucú, Yucatan, Mexico, and their respective p-value from Fisher's exact test for the selection of predictor variables.

Variables	SFG (%) N = 7	*P*-value	TG (%) N = 3	p-value
Rodents' characteristics				
Sex				
Male	6 (85.7)	0.38	2 (66.7)	1
Female	1 (14.3)		1 (33.3)	
Age				
Juvenil	1 (14.3)	1	1 (33.3)	0.37
Adult	6 (85.7)		2 (66.7)	
Weight (gr)				
< 10	1 (14.3)	0.49	1 (33.3)	0.23*
> 10	6 (85.7)		2 (66.7)	
Reproductive condition				
Male				
Active	6 (85.7)	--	2 (66.7)	--
Inactive	0		0	
Female				
Active	0		1 (33.3)	--
Inactive	1 (14.3)		0	
Dwellings' characteristics				
Dwelling location				
Interior	1 (14.3)	0.54	0	--
Central	2 (28.6)		0	
Exterior	4 (57.1)		3 (100)	
Production animals				
Presence	5 (71.4)	0.67	2 (66.7)	1
Absence	2 (28.6)		1 (33.3)	
Companion animals				
Presence	6 (85.7)	1	2 (66.7)	0.49
Absence	1 (14.3)		1 (33.3)	
Opossums				
Presence	1 (14.3)	1	2 (66.7)	0.07*
Absence	6 (85.7)		1 (33.3)	
Dogs with *Rickettsia* SFG infection				
Presence	4 (57.1)	0.007*	0	--
Absence	3 (42.9)		3/3 (100)	

*p<0.3

Table 3. Results of the GLM with binomial error distribution to identify the Odds Ratio (OR) and its 95 % confidence intervals (CI) of *Rickettsia* SFG infection in the dogs of Ucú.

Variables	p-value	OR	95 % CI
Dwelling location			
Exterior	--		
Central	0.32	0.51	0.13-1.88
Interior	0.02*	0.14	0.02-0.65
Companion animals			
Absence	--		
Presence	0.14	5.57	0.77-115.2

*p<0.05

The multinomial model with the smallest AIC value (53.68) showed more chances of *Rickettsia* TG infection (OR = 14; 95 % CI 0.95-206) in rodents captured in dwellings with "presence of opossums" and more chances of *Rickettsia* SFG infection (OR = 21; 95 % CI 2.3-196) in rodents captured in dwellings with "presence of *Rickettsia* SFG-infected dogs" (Table 4).

Studies in rodents and dogs (Dzul-Rosado *et al*., 2021; Arroyo-Ramírez *et al*., 2023; Torres-Castro *et al*., 2024) in southeastern Mexico have reported *Rickettsia* TG and SFG infections (Sánchez-Montes *et al*., 2021a). However, knowledge about the biotic or abiotic factors that influence infection in these animals is limited (Torres-Castro *et al*., 2024). Therefore, the results of this research contribute to the understanding of epidemiological elements and the transmission dynamics of these bacteria (Torres-Castro *et al*., 2020; Dzul-Rosado *et al*., 2021; Sánchez-Montes *et al*., 2021a).

The results for the dogs studied revealed *Rickettsia* SFG infection, which differs from that reported by Torres-Castro *et al*. (2022c) in dogs from Maxcanú, Yucatan, infected with *Rickettsia* TG. However, the sampling months differ, influencing the abundance, richness, and diversity of vector ectoparasites, including ticks (Rodríguez-Vivas *et al*., 2023, 2024). Contact between dogs and *Rickettsia* SFG has been documented in some countries. For example, antibodies against *Rickettsia* SFG were found in dogs from areas with human Rickettsiosis in Brazil (Neves *et al*., 2023), Colombia (Arroyave *et al*., 2020), and the USA (Nicholson *et al*., 2010).

In Mexico, Foley *et al*. (2019) and Palacios-Santana *et al*. (2023) demonstrated the presence of antibodies against *Rickettsia* SFG in dogs from Baja California and Reynosa, Tamaulipas, respectively. Furthermore, Beristain-Ruiz *et al*. (2022) detected *R. rickettsii* (SFG) in dogs from Chihuahua. At the regional level, evidence of antibodies against *Rickettsia* SFG has been obtained in dogs from Mérida (Dzul-Rosado *et al*., 2021). Using PCR and bioinformatics tools, Ojeda-Chi *et al*. (2019) and Arroyo-Ramírez *et al*. (2023) identified *R. parkeri* (SFG) in dogs from municipalities of Yucatan.

Our findings reinforce the hypothesis that dogs are sentinels for the epidemiological risk of pathogens transmitted by arthropod vectors (including *Rickettsia* SFG) in humans who coexist with them (Nicholson *et al*., 2010; Foley *et al*., 2019; Beristain-Ruiz *et al*., 2022). This study was not designed to prove or refute this hypothesis. Additional research is necessary to understand the risk posed to people by having contact with *Rickettsia* SFG-infected dogs. In this sense, it has been demonstrated that dogs serve as amplifying hosts of some SFG species, thereby transmitting bacteria to their ectoparasites and facilitating zoonotic transmission to humans and domestic animals, as documented by Binder *et al*. (2021).

Table 4. Results of the multinomial stratified model to identify the Odds Ratio (OR) and its 95 % confidence intervals (CI) of *Rickettsia* SFG and TG infection in the rodents of Ucú

Variables	p-value	OR	95 % CI
Presence of opossums			
Negative	--		
TG	0.05*	14	0.95-206
SFG	0.36	3.5	0.24-51.5
Presence of *Rickettsia* SFG-infected dogs			
Negative	--		
TG	0.93	0.002	$3e^{-62}$-$1.6e^{+56}$
SFG	0.008*	21	2.3-196

*p<0.05

The present study did not register the ectoparasite species that infest dogs. Dogs contract *Rickettsia* SFG through ticks, including *Rhipicephalus sanguineus* sensu lato (Rodríguez-Vivas *et al*., 2016). This tick exhibits endophilic behavior and has a wide distribution, affecting dogs in tropical regions of Mexico, such as the Yucatan Peninsula (Sánchez-Montes *et al*., 2021b). Therefore, it is found infesting dogs from localities similar to Ucú, Yucatan, Mexico (Martínez-Ortiz *et al*., 2019; Rodríguez-Vivas *et al*., 2023). This tick has been previously implicated as a vector of *R. rickettsii* (SFG) (Bermúdez and Troyo, 2018).

Other findings that explain *Rickettsia* SFG transmission include Vázquez-Guerrero *et al*. (2023), who reported *R. amblyommatis* and *R. bellii* (SFG members) in ticks *Amblyomma auriculatum*, *A. ovale*, and *A. mixtum* collected on dogs from Yucatan, Mexico. Finally, Martínez-Ortiz *et al*. (2019) found *Rickettsia* in *A. mixtum*, *R. sanguineus* s. l., and *Ixodes keiransi*, collected from dogs of a Yucatan locality with a history of Rickettsiosis. The authors suggest that ticks may have a role in the zoonotic transmission of *Rickettsia*. The distribution of diseases transmitted by ticks, such as SFG-Rickettsiosis, is influenced by demographic, ecological, and social factors, such as the population density of ticks and their hosts, such as dogs (Szabó *et al*., 2013).

According to the multinomial model, dogs from dwellings in the interior area have less chance (OR = 0.14, 95 % CI 0.02–0.67) of *Rickettsia* SFG infection than those from other areas (central and exterior). Obtaining background information for comparison with this research is challenging due to the lack of studies using a similar sampling design. We collected biological samples and analyzed data on the risk of *Rickettsia* infection, dividing Ucú into concentric areas with a specific number of dwellings per area. This spatial system found that some regions had different chances of *Rickettsia* infection in susceptible hosts from the study site (local scale).

The higher dwelling density in the interior area partially explains this finding, because owners were likelier to keep dogs indoors, contrary to dwellings from the central and exterior areas, where frequent wandering dogs were observed (field observation). In this sense, wandering outside the dwelling has been associated with *Rickettsia* SFG transmission scenarios (vacant lots, vegetated areas, and unpaved roads), where other dogs, synanthropic and wild fauna, and *Rickettsia* SFG vectors also circulate (Foley *et al*., 2019). Szabó *et al*. (2013) reported a positive correlation between dog seropositivity against *Rickettsia* SFG and access to vegetated areas around dwellings.

Another factor that helps explain the lower chances of *Rickettsia* SFG infection in dogs in the interior area was veterinary care (vaccination, periodic deworming, and ectoparasite control). In contrast, dogs from dwellings in the central and exterior areas frequently present infestations. The deficiency of veterinary care is associated with ectoparasite infestation (Ojeda-Chi *et al*., 2019), which increases the chances of *Rickettsia* SFG infection. Forshey *et al*. (2010) documented that dogs that stay in their homes longer have veterinary care, reducing the probability of being parasitized by ticks and the chances of *Rickettsia* SFG infection.

The snPCR confirmed the presence of *Rickettsia* TG and SFG in the rodents. Detecting *Rickettsia* in *M. musculus* and *R. rattus* has been identified as an epidemiological factor in the transmission of these bacteria to humans (Forshey *et al*., 2010). Only one species of *Rickettsia* TG, *R. typhi*, has been reported in rodents from Yucatan, Mexico (Torres-Castro *et al*., 2018; Sánchez-Montes *et al*., 2021a). On the other hand, positive serological reactions for *Rickettsia* SFG have been detected in rodents from Mérida, Yucatan, Mexico (Dzul-Rosado *et al*., 2021).

In its “classic” transmission cycle, the most important vector of *Rickettsia* TG is *Xenopsylla cheopis* (Leulmi *et al*., 2014). Contact between rodents and *R. typhi* has been confirmed in endemic areas. For example, Lledó *et al*. (2003) reported antibodies against *R. typhi* in rodents from Spain, and Chiang *et al*. (2022) reported the same in rodents from Taiwan, where murine typhus is endemic. Additionally, a positive correlation was described between the incidence rate of murine typhus in humans and the seropositivity rate of *R. typhi* in rodents. In Mexico, López-Pérez *et al*. (2022) identified *R. typhi* in rodents from Baja California.

The multinomial analysis revealed that rodents from dwellings where opossums were captured had a higher chance of *Rickettsia* TG infection. These results provide new insights into the importance of opossums in the transmission cycle of *Rickettsia* TG (Santoyo-Colín *et al*., 2021). The opossums captured in this study were not evaluated to identify the *Rickettsia* groups. However, in the region, there is serological evidence of *Rickettsia* TG in these animals (Dzul-Rosado *et al*., 2021).

As mentioned, *X. cheopis* is the main reservoir of *Rickettsia* TG. However, evidence indicates that *Ctenocephalides felis* is the vector of an “alternative” cycle of *R. typhi* (Santoyo-Colín *et al*., 2021; Bezerra-Santos *et al*., 2021). This flea's indirect life cycle allows it to infest several hosts, including rodents (Peniche-Lara *et al*., 2015) and opossums (Bezerra-Santos *et al*., 2021). This ecological factor explains the positive correlation between *Rickettsia* TG-infected rodents and the presence of opossums.

Rodents acquire *Rickettsia* SFG through ticks, including *Amblyomma*, *Ixodes*, and *Rhipicephalus* (Carrasquilla *et al*., 2013; López-Pérez *et al*., 2022). Records of rodents with *Rickettsia* SFG are numerous worldwide. For example, Milagres *et al*. (2013) found antibodies against *Rickettsia* SFG in rodents from Brazil. In Mexico, *R. rickettsii* was identified in rodents from several regions (Sosa-Gutiérrez *et al*., 2014).

The multinomial GLM showed more significant higher chances (OR = 21; 95 % CI 2.3-196) of *Rickettsia* SFG infection in rodents captured in dwellings where an SFG-infected dog resides. This finding suggests that rodents and dogs participate in the same *Rickettsia* SFG transmission cycle. However, bioinformatic analyses of *Rickettsia* populations are necessary to prove it.

The connection between rodents and SFG-infected dogs may involve tick species that use several hosts during their life cycles. In the *Amblyomma* complex, immature ticks infest rodents (Fernandes-Martins *et al*., 2016), and adults infest larger mammals such as dogs (Carrasquilla *et al*., 2013). In this context, horizontal transmission maintains some members of *Rickettsia* SFG; therefore, larvae, nymphs, and adults participate in the transmission of *Rickettsia* SFG in susceptible hosts (Moraes-Filho *et al*., 2018).

Previously, antibodies against *Rickettsia* SFG were detected in rodents and dogs from the same dwellings (Dzul-Rosado *et al*., 2021). Similarly, our analysis revealed that these animals share *Rickettsia* transmission cycles by exchanging vector ectoparasites.

CONCLUSIONS

This study provides new evidence regarding the role of dogs and rodents in the epidemiological cycles of *Rickettsia* SFG and TG. Our findings suggest that these hosts participate in the same transmission cycle by exchanging ectoparasites (fleas and ticks). Statistical modelling demonstrated that the dwelling location, the presence of *Rickettsia*-infected dogs, and the presence of opossums serve to assess the risk of *Rickettsia* infection in synanthropic rodents. While the results provide a local overview, they do not accurately represent the epidemiological conditions at other sites in the study region.

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Compliance with ethical standards. The study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine of the *Universidad Autónoma de Yucatán*, Mexico (CB-CCBA-M-2021-003). The Ministry of Environment and Natural Resources of Mexico authorized the extraction of rodents for scientific collection purposes (31/K5-0108/12/20).

Data availability. All data is included in the present paper.

Author contribution statement (CRediT). A. Arroyo-Ramírez – Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation., **C. Lugo-Caballero** – Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Original Draft Preparation., **E. Reyes-Novelo** – Data Curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – Review & Editing., **H. Noh-Pech** – Formal Analysis, Investigation, Methodology, Validation., **A. Suárez-Galaz** – Formal Analysis, Investigation, Resources, Writing – Review & Editing., **J.A. Panti-May** – Investigation, Methodology, Resources, Writing – Review & Editing., **R. I. Rodríguez-Vivas** – Formal Analysis, Methodology, Writing – Original Draft Preparation, **T. Osorio-Primo** – Investigation, Methodology, Resources., **H. Ruiz-Piña** – Methodology, Resources., **M. Torres-Castro** – Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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