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Original article

Ticks and tick-borne rickettsiae from dogs in El Salvador, with report of the human pathogen *Rickettsia parkeri*

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ABSTRACT

Twelve tick species have been reported in El Salvador; however, information regarding ticks infesting domestic dogs is lacking, and pathogenic tick-borne *Rickettsia* species have never been reported in El Salvador. This work evaluated ticks infesting 230 dogs from ten municipalities in El Salvador from July 2019 to August 2020. A total of 1,264 ticks were collected and identified into five species: *Rhipicephalus sanguineus* sensu lato (s.l.), *Rhipicephalus microplus, Amblyomma mixtum, Amblyomma ovale*, and *Amblyoma* cf. *parvum*. The tick *R. sanguineus* s.l. was the most frequent species in all localities (81.3% of sampled dogs), followed by *Amblyomma mixtum* (13.0%), *Amblyomma ovale* (10.9%) and *Amblyomma* cf. *parvum* (10.4%). The overall mean intensity of tick infestation was 5.5 ticks/dog. The highest specific mean intensity value was for *R. sanguineus* s.l. (4.8 ticks/dog), varying from 1.6 to 2.7 ticks/dog for the three *Amblyomma* species. From a random sample of 288 tick specimens tested molecularly for the presence of rickettsial agents, three spotted fever group *Rickettsia* were detected: *Rickettsia amblyommatis* in 90% (36/40) *A. mixtum,* 46% (11/24) *A.* cf. *parvum,* 4% (7/186) *R. sanguineus* s.l., and 17% *Amblyomma* spp.; *Rickettsia parkeri* strain Atlantic rainforest in 4% (1/25) *A. ovale*; and an unnamed rickettsia agent, designated as '*Rickettsia* sp. ES-A.cf.parvum', in 4% (1/24) *A.* cf. *parvum.* Our finding of *R. parkeri* strain Atlantic rainforest in *A. ovale* is highly relevant because this agent has been associated to spotted fever illness in other Latin American countries, where *A. ovale* is implicated as its main vector. These findings suggest that spotted fever cases caused by *R. parkeri* strain Atlantic rainforest could be occurring in El Salvador.

1. Introduction

Ticks are considered a major group of arthropods responsible for transmitting important pathogens to animals and humans, second only to mosquitoes as vectors. The transmission risks of tick-borne pathogens to humans increase when interacting with forest ecosystems or with free-roaming animals (Bermúdez et al., 2016; Estrada-Peña, 2015). Many factors influence the presence of ticks in a region: climate, vegetation, vertebrate diversity and human activities (e.g., forest degradation, agricultural activities), which increase the probability of interaction between parasites from rural and urban areas (Szabó et al., 2007).

Despite of having a relatively narrow territorial area, Central America has an abundant tick fauna. For example, there have been at least 40 species in Costa Rica and Panama (Guglielmone et al., 2021; Springer et al., 2018). However, in El Salvador the reported tick fauna comprises only 12 species; two of them belonging to family Argasidae (soft ticks), *Ornithodoros dyeri* and *Ornithodoros yumatensis*, and 10 species belonging to the family Ixodidae (hard ticks), *Amblyomma dissimile, Amblyomma mixtum, Amblyomma ovale, Amblyomma* cf. *parvum, Amblyomma sabanerae, Amblyomma scutatum, Dermacentor dissimilis, Dermacentor nitens, Rhipicephalus microplus* and *Rhipicephalus sanguineus* sensu lato (s.l.) (Romero et al., 2021).

Dogs are common pets in Central America, where they may serve as hosts for several hard tick species. The species *R. sanguineus* s.l. is recognized as the most common and widely distributed tick on dogs. There are also records of *Amblyomma maculatum, A. mixtum, A. ovale, Amblyomma triste, Amblyomma pecarium, R. microplus* and *Ixodes*

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boliviensis affecting dogs in Central America (Bermúdez et al., 2016; Campos-Calderón et al., 2016; Ferrel et al., 2017; Pacheco-Solano et al., 2019; Springer et al., 2018; Troyo et al., 2012; Vogel et al., 2018;).

Bacteria of the genus *Rickettsia* (Rickettsiales: Rickettsiaceae) are obligate intracellular organisms that multiply within eukaryotic cells of invertebrates and occasionally mammals, including humans, who may develop rickettsiosis (Parola et al., 2013). Currently, *Rickettsia* species are phylogenetically divided into four major groups: the spotted fever group (SFG), primarily associated with ticks; the typhus group (TG), associated with fleas and lice; the transitional group (TRG), associated with ticks, fleas, and mites; and the ancestral group (AG), associated with a great variety of organisms, including ticks, several insect orders, leeches, and unicellular organisms (Diop et al., 2019; Gillespie et al., 2007). Most of the pathogenic *Rickettsia* species belong to the SFG; consequently, ticks are recognized as the most important vectors of agents that cause human rickettsioses worldwide.

Currently in Latin America, only three tick-borne rickettsiae, all belonging to the SFG, are recognized as human pathogens: (i) *Rickettsia rickettsii,* the agent of Rocky Mountain spotted fever (RMSF), with reported cases in Mexico, Costa Rica, Panama, Colombia, Argentina and Brazil (Parola et al., 2013); (ii) *Rickettsia parkeri,* with human clinical cases reported in Brazil, Argentina, Uruguay, Colombia, and Mexico (Peniche-Lara and Lara-Perera, 2022; Silva-Ramos et al., 2021); and (iii) *Rickettsia massiliae,* with a single human case reported in Argentina (García-García et al., 2010). Although human infection due to *R. parkeri* is yet to be confirmed in Central America, this agent has been reported infecting *A. ovale* and *A. maculatum* in Belize (Polsomboon Nelson et al., 2022) and *A. ovale* in Nicaragua (Vogel et al., 2018). In the later country, although the agent was reported as *Rickettsia africae,* further analysis of the DNA sequences revealed that it was in fact *R. parkeri* strain Atlantic rainforest (Sevá et al., 2019).

Several *Rickettsia* species of unknown or unproven pathogenicity have been reported infecting ticks in Central America, which include the SFG agents *Rickettsia amblyommatis,* '*Candidatus* Rickettsia colombianensi', '*Candidatus* Rickettsia nicoyana' and *Rickettsia monacensis*-like agents (Bermúdez and Troyo, 2018; Springer et al., 2018), the TRG agents *Rickettsia felis* and *Rickettsia asembonensis*, and the AG agent *Rickettsia bellii* (Pacheco-Solano et al., 2019; Troyo et al., 2016). Regarding El Salvador, only three *Rickettsia* species have been reported in ticks: *R. amblyommatis* in *A. mixtum, A.* cf. *parvum* and *D. nitens*; '*Ca.* R. colombianensi' in *A. dissimile* and *A. scutatum*; and *R. bellii* in *A. dissimile, A. ovale* and *A. sabanerae* (Barbieri et al., 2012; Romero et al., 2021).

It is important to know the tick species affecting dogs and their distribution in a region, since dogs increase the transmission risk of ticks to the owners enabling an interaction with several tick-borne pathogens, including *Rickettsia* spp. (Ferrel et al., 2017; Jones et al., 2018). In El Salvador there is lack of information regarding tick species and *Rickettsia* spp. affecting dogs. Thus, the present study aimed to report tick species infesting domestic dogs in El Salvador, and the *Rickettsia* species infecting the canine ticks.

2. Materials and methods

2.1. Collection sites

El Salvador is located in Central America, bordering to the west with Guatemala, to the north with Honduras, to the east with Honduras and Nicaragua and to the south with the Pacific Ocean. Country extension is approximately 21,040.70 km^2, with 296 km of Pacific coastline that extends throughout the country. Its geographic coordinates are between 13° and 14° North latitude and between 87° and 90° West length. The annual precipitation is about 1200 to 2800 mm with an annual temperature between 12.7 °C and 26.9 °C. Two parallel mountains from west to east separate the country in two regions: mountains and central valley and coastal plains. The southern mountain chain is made up of 20 volcanoes. Its political division includes three geographic zones: West Zone, Central Zone and East Zone.

Tick samples were collected from dogs of 10 municipalities: Agua Caliente, Apopa, Chalatenango, Ilopango, Metapán, Nueva Concepción, San José las Flores, San Marcos, Santa Rita and Santa Tecla. These municipalities are located among northern mountain chain and central valley in the West and Central zones. Dogs were selected by convenience sampling getting at least 21 tick-infested dogs from each municipality, in coordination with city hall authorities and local residents. The selected zones have population nucleus close to forest areas and were characterized by having reports of dengue suspected cases, according to Ministry of Public Health from El Salvador between years 2017 to 2018. A total of 230 mongrel dogs infested with ticks were selected from July 2019 to August 2020. These animals were known to wander outside in the landscape surrounding their households. For each dog, the owner was requested to give permission to work with the animal. Dogs were checked by physical exploration through visual inspection and palpation to detect the presence of ticks. The approach focused on the predilection anatomic sites for ticks on dogs: ears, around the eyes, neck, nose, armpits and between toes. Few samples were obtained through two veterinary clinics. Each batch of ticks from each dog was recorded with an identification code and preserved in absolute ethanol until morphological and molecular analysis. The sites of collection were georeferenced for spatial distribution of tick species.

2.2. Morphological and molecular identification of ticks

Collected ticks were firstly separated according to life stage: adults (males or females), nymphs, and larvae. Adults were morphologically identified to species following Barros-Battesti et al. (2006) and Nava et al. (2014). Nymphs and larvae of the genus *Amblyomma* were morphologically identified only to genus level due to the lack of appropriated taxonomic keys for *Amblyomma* subadult stages of the Central American tick fauna. For molecular identification, at least one tick of each morphotype and some immature stages were analyzed by Polymerase Chain Reaction (PCR). DNA extraction was performed individually through DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's instructions of complementary protocol for ticks. Extracted DNA was submitted to conventional PCR targeting a 460 base pairs (bp) fragment of the tick mitochondrial 16S rRNA gene, according to Mangold et al. (1998). PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific Inc) and sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit and an ABI 3500 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA). Generated sequences were edited with BioEdit software, version 7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html) and submitted to BLASTn analyses (www.ncbi.nlm.nih.gov/blast) in order to infer closest identities in GenBank.

2.3. Data analyses of canine ticks

The following tick parameters were calculated according to Bush et al. (1997): prevalence of infestation: number of infested dogs / total number of sampled dogs x 100; mean intensity: total number of ticks / number of infested dogs. For each sample site, the georeferencing data was recorded. Layers were loaded to retrieve administrative borders using cartographic information available in the Ministry of Environmental and Natural Resource (MARN) and the National Center of Registers (CNR) from El Salvador. The maps were created using ArcGIS version 9.0, available at the Laboratory of Geographic Information Systems in the Post graduate School and Continuing Education of the Faculty of Agronomic Sciences of the University of El Salvador.

2.4. Rickettsial infection in canine ticks

A random sample of 288 ticks from dogs were selected for testing for rickettsial infection. Extracted DNA samples were tested by the same

conventional PCR protocol targeting the tick mitochondrial 16S rRNA gene described above, in order to certify successful DNA extraction. Thereafter, DNA samples were initially tested by a Taqman real-time PCR assay targeting the rickettsial *gltA* gene, as described (Labruna et al., 2004; Soares et al., 2012). Positive samples by this Taqman real-time PCR (cycle threshold $\leq$ 35) were tested by two protocols of conventional PCR, one targeting a 401 bp fragment of the rickettsial citrate synthase gene (*gltA*) (Labruna et al., 2004), and a heminested PCR assay targeting the rickettsial 190-kDa outer membrane protein gene (*ompA*); the latter protocol consisted of a first reaction targeting a 631 bp fragment, and a second targeting a 532 bp fragment, as described (Eremeeva et al., 2006). In each set of reactions, negative control tubes containing water and a positive control tube containing *Rickettsia vini* DNA were included. PCR products were DNA-sequenced and submitted to BLASTn analyses as described above.

Partial DNA sequences obtained from the amplified PCR products (*gltA* and *ompA*) were aligned with the corresponding sequences of other SFG *Rickettsia* species and one TRG species (*Rickettsia australis*) available in GenBank using the CLUSTAL algorithm and adjusted manually by GeneDoc. Phylogenetic trees were inferred by Bayesian (B) and maximum parsimony (MP) methods. The concatenated alignment (*gltA* + *ompA*) was analyzed by B and MP methods. MP trees were constructed using the PAUP* v4.0b10 program (Swofford 2002) via a heuristic search with 100 replicates of random additions of the terminals followed by branching (RAS-TBR branch-breaking). Bootstrap support analyses were performed on 100 replicates with the same parameters used in the search. Bayesian analyses were performed in the MrBayes v.3.1.2 program (Ronquist and Huelsenbeck, 2003); 1000,000 generations were employed using GTR as a substitution model and four range categories plus an invariant proportion of sites. In the Bayesian analyses, "posteriori" probability values were obtained for branch support verification by using the MrBayes program. *Rickettsia australis* was designated as outgroup.

3. Results

3.1. Canine infestations by ticks

Among 230 tick-infested dogs enrolled in this study, a total of 1264 ticks were collected and identified into five species, as follows: *R. sanguineus* s.l. (899 specimens; 71.1% of all ticks), *A. ovale* (68; 5.4%), *A.* cf. *parvum* (61; 4.8%), *A. mixtum* (48; 3.8%), and *R. microplus* (1; 0.08%); other 187 specimens (184 nymphs and 3 larvae; 14.8% of all ticks) were retained as *Amblyomma* spp. because they could not be identified to species level through morphological examinations (Table 1).

Besides morphological analyses, the following 28 ticks were molecularly identified to species following individual analysis of a $\approx$ 400 bp fragment of the mitochondrial 16S rRNA gene: *A. mixtum* (1 adult, 16 nymphs and 5 larvae), *A. ovale* (1 adult and 1 nymph), *A.* cf. *parvum* (2 adults and 1 nymph) and *R. microplus* (1 adult). For *A. mixtum,* two 16S rRNA haplotypes were generated, which were 99.7–100% identical to a 16S rRNA partial sequence of *A. mixtum* from Ecuador (GenBank accession number KT820359). For *A. ovale,* also two haplotypes were generated, which were 99.8–100% identical to *A. ovale* from Belize (KU001155). There was a single haplotype for the specimens of *A.* cf. *parvum*, which was 100% identical to *A.* cf. *parvum* from Panama (KT820312). The single specimen of *R. microplus* generated a haplotype 100% identical *R. microplus* from Colombia (MN650726). Sequences were submitted to GenBank and haplotype accession numbers are OP198636 and OP198637 for *A. mixtum,* OP198638 and OP198639 for *A. ovale*, OP198640 for *A.* cf. *parvum*, and OP198641 for *R. microplus*.

The most prevalent tick species on dogs was *R. sanguineus* s.l., collected on 81.3% of the sampled dogs (Fig. 1). Moreover, it was the most frequent tick species in all 10 municipalities, except for Santa Tecla. Dogs from Apopa and San José las Flores had no tick species other than *R. sanguineus* s.l. (Table 2). This tick was found in elevations between 264 and 1689 m among urban and rural locations.

The second most prevalent tick species was *A. mixtum,* present in 13.0% of the dogs, followed by *A. ovale* (10.9% of the dogs) and *A.* cf. *parvum* (10.4% of the dogs). *Amblyomma mixtum* was collected in six municipalities (elevations between 264 and 1130 m); *A.* cf. *parvum* in five municipalities (elevations between 264 and 825 m), and *A. ovale* in only two municipalities (elevations of 451 and 1801 m). The areas of these three *Amblyomma* species were characterized chiefly by a mix of production systems (crops, livestock) and secondary forest fragments. Geographical overlap of *A. mixtum* with *A.* cf. *parvum* was observed in two municipalities, and *A.* cf. *parvum* with *A. ovale* in one municipality; there was no overlap between *A. mixtum* and *A. ovale* (Fig. 2). A single dog (0.4% of all dogs) from Chalatenango municipality was found infested by *R. microplus*, whereas undetermined species of *Amblyomma* spp. nymphs and larvae were recorded on 11.7% of the dogs from three municipalities (Table 2).

The overall mean intensity of tick infestations on dogs was 5.5 ticks/dog. Considering each tick species separately, the highest value was for *R. sanguineus* s.l. (4.8 ticks/dog). Mean intensity values of the three *Amblyomma* species varied from 1.6 to 2.7 ticks/dog (Table 1). The 6.9 mean intensity value for unidentified nymphs and larvae of *Amblyomma* spp. might represent more than one *Amblyomma* species.

Overall, 36 dogs were co-infested with two tick species; among these, 35 were co-infested with *R sanguineus* s.l. and one *Amblyomma* species (*A. mixtum* on 15 dogs, *A.* cf. *parvum* on 12 dogs, *A. ovale* on 8 dogs), and one dog was co-infested with *A. mixtum* and *A.* cf. *parvum.* A single dog was co-infested by three tick species (*R sanguineus* s.l., *A. mixtum, A.* cf. *parvum*). The remaining dogs were infested by a single tick species.

3.2. Rickettsial infection in canine ticks

The 288 tested ticks encompassed 186 (65%) specimens of *R. sanguineus* s.l., 40 (14%) *A. mixtum,* 25 (9%) *A. ovale,* 24 (8%) *A.* cf. *parvum*, 1 (0.3%) *R. microplus*, and 12 (4%) *Amblyomma* spp. Real-time PCR assay revealed rickettsial DNA in 58 (20%) ticks, which yielded amplicons by the two conventional PCR assays targeting fragments of the *gltA* and *ompA* rickettsial genes (Table 3). The highest rickettsial

Table 1
Ticks infesting 230 domestic dogs in El Salvador from July 2019 to August 2020. Data presented as total number (No.) of tick specimens according to tick species and life stages; prevalence (P: no. infested dogs / no. sampled dogs x 100); mean intensity (MI: total number of tick specimens / number of infested dogs); and range of canine infestations (minimum and maximum number of tick specimens per dog).

Tick species	No. tick specimens					No. infested dogs (P)	MI (range)
	males	females	nymphs	larvae	total		
Rhipicephalus sanguineus s.l.	396	415	87	1	899	187 (81.3)	4.8 (1–20)
Amblyomma mixtum	13	14	16	5	48	30 (13.0)	1.6 (1–6)
Amblyomma ovale	34	33	1		68	25 (10.9)	2.7 (1–13)
Amblyomma cf. *parvum*	30	30	1		61	24 (10.4)	2.5 (1–9)
Rhipicephalus microplus		1			1	1 (0.4)	1 (1)
Amblyomma spp.			184	3	187	27 (11.7)	6.9 (1–25)
Total	473	493	289	9	1264	230 (100)	5.5 (1–27)

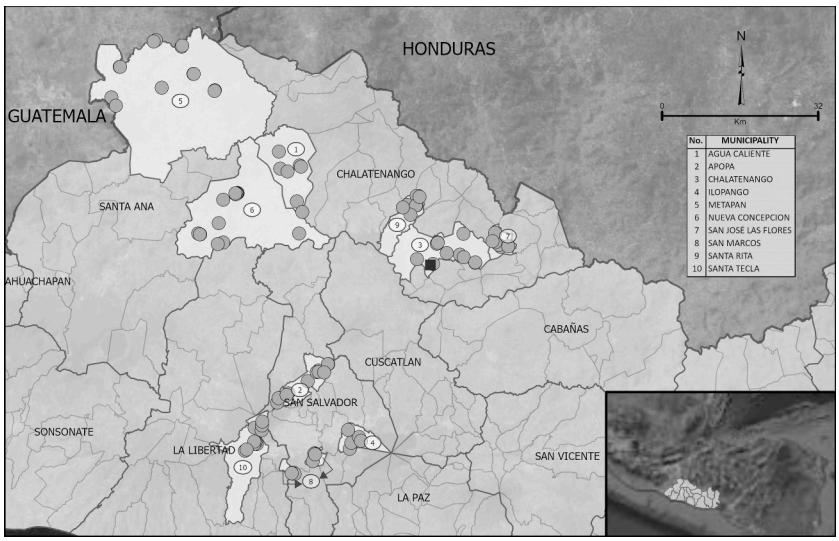

Fig. 1. Map of distribution of *Rhipicephalus* ticks infesting 230 domestic dogs in El Salvador from July 2019 to August 2020. Circles indicate *R. sanguineus* s.l.; square indicates *R. microplus*. The municipalities with sampled ticks are indicated by numbers according to the map legend.

Table 2
Number of infested dogs (n) and prevalence of infested dogs (P) according to tick species and the 10 municipalities where dogs were sampled in El Salvador.

Municipalities	No. sampled dogs	*Rhipicephalus sanguineus* s.l.		*Rhipicephalus microplus*		*Amblyomma mixtum*		*Amblyomma ovale*		*Amblyomma* cf. *parvum*		*Amblyomma* spp.	
		n	P	n	P	n	P	n	P	n	P	n	P
Agua Caliente	22	16	72.7			2	9.1			13	59.1		
Apopa	21	21	100										
Chalatenango	23	19	82.6	1	4.3	9	39.1					10	43.5
Ilopango	23	21	91.3			1	4.4			3	13.0		
Metapán	25	19	76.0			7	28.0					8	32.0
Nueva Concepción	23	21	91.4			7	30.4			4	17.4	9	39.1
San José Las Flores	25	25	100										
San Marcos	24	16	66.7					10	41.7	1	4.2		
Santa Rita	21	16	76.2			6	28.6			3	14.3		
Santa Tecla	23	13	56.5					15	65.2				
TOTAL	230	187	81.3	1	0.4	32	13.9	25	10.9	24	10.4	27	11.7

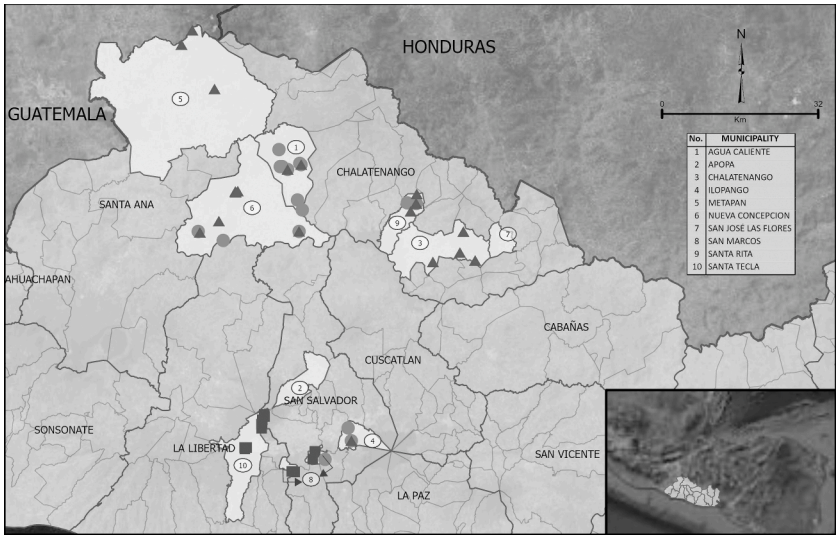

Fig. 2. Map of distribution of *Amblyomma* ticks infesting 230 domestic dogs in El Salvador from July 2019 to August 2020. Triangles indicate *A. mixtum*; squares indicate *A. ovale*; circles indicate *A.* cf *parvum*. The municipalities with sampled ticks are indicated by numbers according to the map legend.

Table 3
Rickettsial infection (determined by molecular analyses) in ticks collected from domestic dogs in El Salvador from July 2019 to August 2020.

Tick species	No. infected ticks / No. tested ticks (% infection)				*Rickettsia* species
	Adults	Nymphs	Larvae	Total	
Rhipicephalus sanguineus s.l.	7/170 (4)	0/1 (0)	0/15 (0)	7/186 (4)	*R. amblyommatis*
Amblyomma mixtum	13/17 (76)	17/17 (100)	6/6 (100)	36/40 (90)	*R. amblyommatis*
Amblyomma ovale	1/24 (4)	0/1 (0)		1/25 (4)	*R. parkeri*
Amblyomma cf. *parvum*	11/23 (48)	1/1 (100)		12/24 (50)*	*R. amblyommatis* *Rickettsia* sp.
Rhipicephalus microplus	0/1 (0)			0/1 (0)	
Amblyomma spp.		2/10 (20)	0/2 (0)	2/12 (17)	*R. amblyommatis*
Total	32/235 (14)	20/30 (33)	6/23 (26)	58/288 (20)	

* Among the 12 *A.* cf. *parvum*-infected ticks, 11 (46%) were infected with *R. amblyommatis* and 1 (4%) with an unnamed *Rickettsia* sp. (designated as '*Rickettsia* sp. ES-*A.* cf.*parvum*').

infection rate was found in *A. mixtum* (90%), followed by *A.* cf. *parvum* (50%), and then 4% for *R. sanguineus* s.l. and *A. ovale*. The single *R. microplus* specimen was not infected, whereas 17% of the *Amblyomma* spp. immatures contained rickettsiae.

Rickettsial DNA in seven *R. sanguineus*, 36 *A. mixtum*, 11 *A.* cf. *parvum,* and two *Amblyomma* spp. ticks were identified as *R. amblyommatis* (Table 3), since their *gltA* (350 bp) and *ompA* (587 bp) gene fragments were 100% identical to corresponding sequences of *R. amblyommatis* from GenBank (JF694089 and MT712882, respectively). Rickettsial DNA in one *A. ovale* specimen was identified as *R. parkeri* strain Atlantic rainforest, since its *gltA* (350 bp) and *ompA* (587 bp) gene fragments were 100% identical to corresponding sequences of *R. parkeri* strain Atlantic rainforest from Colombia (CP040325). Finally, one adult of *A.* cf. *parvum* yielded *gltA* and *ompA* partial sequences that were 100% (350/350 bp) and 99.8% (462/463 bp) identical to *Rickettsia* sp. ApBA2 (MH445973) and ApBA1 (MH445972), respectively, previously reported in *A. parvum* from Brazil (Maia et al., 2018); the *ompA* partial sequence from this *A.* cf. *parvum* specimen was at the same time 100% identical to *Rickettsia* sp. Gu263, associated to *Amblyomma auricularium* from Guatemala (JF523328).

Regarding the municipalities, *R. amblyommatis* was found in *A. mixtum* ticks from Agua Caliente, Chalatenango, Ilopango, Metapán,

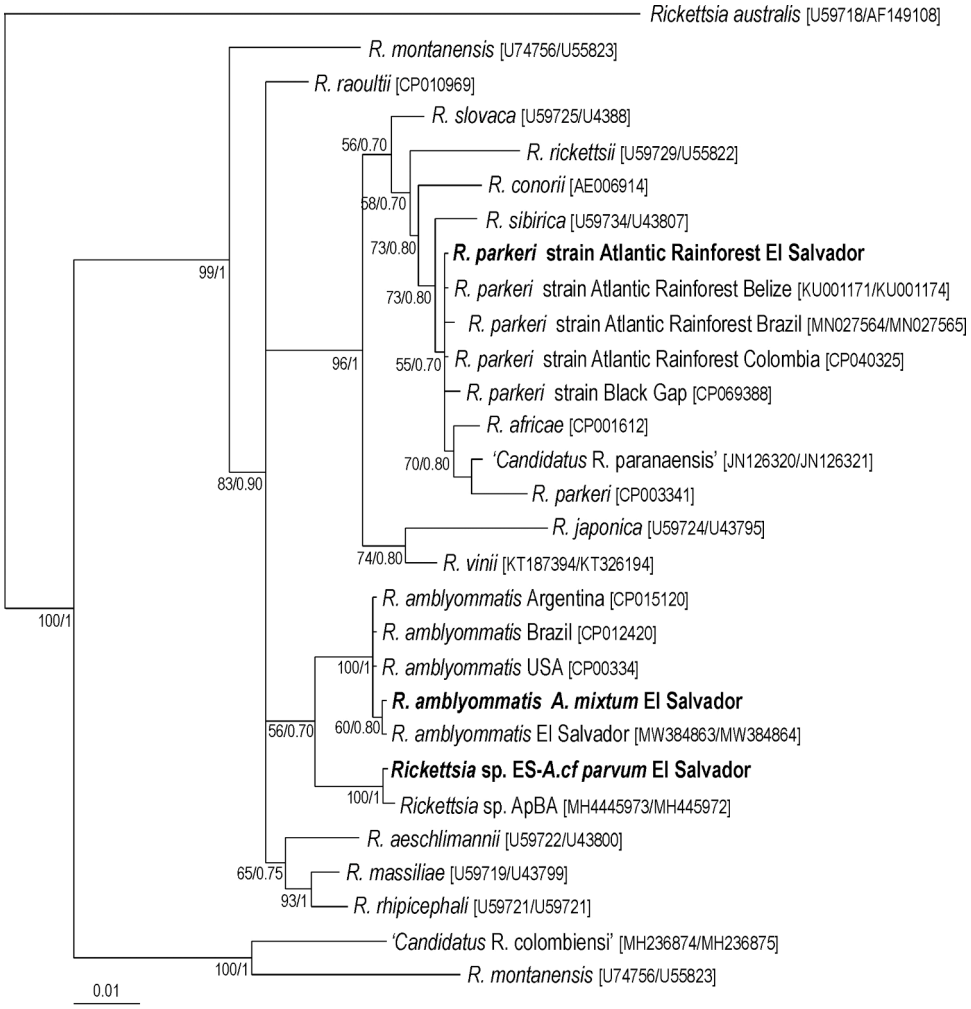

Fig. 3. Molecular phylogenetic analysis of spotted fever group rickettsiae. A total of 961 nucleotide sites (136 variable characters) of the rickettsial genes *gltA* and *htrA* were concatenated and subjected to analysis for Maximum Parsimony inferences. Bootstrap values are shown at the nodes. GenBank accession numbers for the sequences included in this analysis are shown within brackets. The rickettsial agents detected in El Salvador in the present study are written in bold.

Nueva Concepción and Santa Rita, *A.* cf. *parvum* from Agua Caliente, Ilopango, Nueva Concepción and Santa Rita, *R. sanguineus* s.l. from Metapán, Nueva Concepción and San Marcos, and *Amblyomma* spp. from Chalatenango and Nueva Concepción. The *R. parkeri*-infected *A. ovale* tick was from Santa Tecla, and the *Rickettsia* sp.-infected *A.* cf. *parvum* was from Agua Caliente.

The phylogenetic analysis inferred included a concatenated alignment (total length: 961 bp) of the rickettsial *gltA* (350 bp) and *ompA* (≈490 bp) partial sequences. The consensus sequences of *R. amblyommatis* and *R. parkeri* strain Atlantic rainforest generated in this study were each one part of a clade with its conspecific sequences, confirming their taxonomic identities (Fig. 3). On the other hand, the rickettsial sequences generated from a specimen of *A.* cf. *parvum,* here designated as '*Rickettsia* sp. ES-*A.*cf.*parvum*', formed a distinct clade with '*Rickettsia* sp. ApBA *A. parvum* Brazil' [100; 1 (B; MP) node support], which was sister to the *R. amblyommatis* clade.

The DNA sequences of *Rickettsia* spp. generated in this study were deposited in GenBank under the following accession numbers (*gltA* and *ompA* genes, respectively): *R. amblyommatis* (OP375579, OP375580), *R parkeri* (OP375581, OP375582), and *Rickettsia* sp. ES-*A.*cf.*parvum* (OP375583, OP375584).

4. Discussion

This study reports five tick species infesting dogs in El Salvador. These species are within the list of eight tick species that have been reported infesting dogs in other Central American countries (Bermúdez et al., 2016; Campos-Calderón et al., 2016; Ferrel et al., 2017; Pacheco-Solano et al., 2019; Springer et al., 2018; Troyo et al., 2012; Vogel et al., 2018). Indeed, our findings are congruent to most of these previous studies, which also reported *R. sanguineus* s.l., *A. mixtum*, and *A. ovale* as the most frequent tick species on dogs (Campos-Calderon et al., 2016; Ferrel et al., 2017; Pacheco-Solano et al., 2019; Springer et al., 2018; Vogel et al., 2018).

All stages of *R. sanguineus* s.l. prefer the domestic dog as host; nevertheless, this tick can parasitize several other host species in Central America, including humans (Bermúdez et al., 2022; Ojeda-Chi et al., 2019). The high prevalence of *R. sanguineus* s.l. on dogs in the present study corroborates previous studies that reported high canine prevalence of this tick species, as for example, the presence of *R. sanguineus* s.l. on 160 (97%) out of 165 tick-infested dogs in Costa Rica, and on 158 (99%) out of 159 infested dogs in Nicaragua (Campos-Calderón et al., 2016; Springer et al., 2018). In addition, *R. sanguineus* s.l. infestation is usually found under high loads on dogs (Szabó et al., 2007). In fact, among five tick species, *R. sanguineus* s.l. showed the highest mean intensity of tick infestations on dogs of the present study. This tick has been recorded in elevation up to 1200 m in Panama, in rural and urban areas (Bermúdez et al., 2016); however, Fairchild et al. (1966) reported it up to 1500 m, similarly to the present study (up to 1600 m). *Rhipicephalus sanguineus* s.l. is a tick adapted to anthropogenic environment, with endophilic habits, taking advantage of interiors furniture and cracks in the walls (Bermúdez et al., 2016). Domestic dogs can carry ticks to the inside of the houses from *peri* domestic environment, increasing the risk contact between humans and ticks (Álvarez-Hernández et al., 2017). The predominance of this tick on dogs in El Salvador warns to an important risk of pathogen transmission between dogs and owners.

Ticks of the genus *Amblyomma* are usually found on free-roaming dogs because they go into vegetation areas, increasing the risk of acquiring ticks associated to wildlife (Szabó et al., 2007). This was observed here with detection of three tick species on dogs from rural areas and periphery of urban sites surrounded by vegetation. In Central America, *Amblyomma* is the most common tick genus on humans from forested areas (Bermúdez et al., 2022).

The tick *A. mixtum* has a wide geographic distribution ranging from Texas to Ecuador (Nava et al., 2014). Humans and several domestic and wild animals are usually parasitized by this tick (Bermúdez et al., 2016; Bermúdez y Troyo, 2018; Ojeda-Chi et al., 2019). In Central America, low frequency detection has been observed on dogs from Nicaragua, Costa Rica, and Panama (Bermúdez et al., 2016; Campos-Calderón et al., 2016; Pacheco-Solano et al., 2019; Vogel et al., 2018). *Amblyomma mixtum* inhabits paddocks, deciduous forests, and riparian vegetation, taking advantage of pastures, parasitizing several domestic and synanthropic hosts. It is a common tick on cattle and horses, exposing livestock workers to frequently bites (Bermúdez et al., 2016; Bermúdez y Troyo, 2018). This tick was found in Panama in elevations under 800 m, with wide distribution around rural towns in shrub areas on the Pacific slope, but also in fields with introduced cultivated grass (Bermúdez et al., 2016). In the present study, *A. mixtum* was detected over a wide elevation range in areas with production systems. The wide distribution, associated to the aggressiveness to humans, make *A. mixtum* a species with potential public health concern in El Salvador.

Amblyomma ovale is a parasite of wide range of hosts, including Carnivora, large mammals and humans for adult ticks, and birds and rodents for immature stages (Guglielmone et al., 2021; Murgas et al., 2013). Its geographic range goes through Mexico to Argentina (Murgas et al., 2013). In Central America this tick is found in variable frequency on dogs, being influenced by environmental conditions as well as forested areas. The recovering of *A. ovale* on hosts corresponds to rural zones or periphery of urban sites with nearby forested areas (Bermúdez et al., 2016; Murgas et al., 2013). In Central America, canine prevalence of *A. ovale* has been recorded from 0.9% in Nicaragua to 4.8% in Costa Rica; meanwhile, in a village with dense rainforest in a biologic reserve in Nicaragua, *A. ovale* corresponded to around 86% of the ticks collected from dogs (Pacheco-Solano et al., 2019; Vogel et al., 2018). In Panama, *A. ovale* was reported as the second most common tick on dogs nationwide (Ferrel et al., 2017). *Amblyomma ovale* has been recorded in elevations up to 900 m in Panama (Bermúdez et al., 2016). Herein, *A. ovale* was found in elevation up to 1600 m, in towns with forested areas and periphery of urban sites. Dogs usually maintain low load of this tick, about 2 – 5 specimens per host (Sánchez-Montes et al., 2019). In El Salvador, it was common to observe dogs with a single specimen of *A. ovale,* especially around the eye, behind ear or in the nostril; however, in some dogs the load with *A. ovale* was more than five ticks per dog.

An unexpected result was related to *A.* cf. *parvum* on dogs, since this tick has been usually absent from tick surveys on domestic dogs in Central America (Ferrel et al., 2017; Polsomboon Nelson et al. 2022; Springer et al., 2018). At the same time in Central America, *A.* cf. *parvum* has been recorded from several wild hosts in Belize and El Salvador (Lopes et al., 2016; Romero et al., 2021), from the environment in Costa Rica, from cattle and horses in Nicaragua, but was considered rare in Panama (Düttmann et al., 2016; Montenegro et al., 2021). It is relevant to determine the taxonomy and ecology of this tick because *A.* cf. *parvum* from Central America should be considered a species distinct from *A. parvum* from South America, as they might represent cryptic species, referring to Central America species with the term "confer", abbreviated as "cf" (Lado et al., 2016). *Amblyomma* cf. *parvum* has been reported in seasonally dry tropical forest of Central America in elevations under 300 m (Fairchild et al., 1966; Lado et al., 2016; Montenegro et al., 2021). In the present study, *A.* cf *parvum* was detected in elevations up to 800 m in rural towns in areas with production systems. Records of this tick on humans from Central America are scarce, with records from Panama and Guatemala. In this research, infestation with *A.* cf. *parvum* was variable ranging from low to mild, some dogs having up to nine ticks, as observed for *A. parvum* in Brazil (Szabó et al., 2007). This is the first record of *A.* cf. *parvum* on dogs from El Salvador, since previous records in the country were restricted to wildlife (Romero et al., 2021).

The species *R. microplus* was found on only one dog. This tick is common on cattle worldwide in tropic and subtropic environments but, it is possible to find it on several hosts, including dogs and humans. However, its recovering in a place is due to the presence of cattle (Guglielmone et al., 2006, 2004). There have been only occasional

records of *R. microplus* infesting dogs and humans in Central America (Bermúdez et al., 2022; Ferrel et al., 2017; Romero et al., 2021; Troyo et al., 2012).

Survey of rickettsial DNA in ticks infesting domestic dogs in El Salvador revealed the presence of three SFG rickettsial agents: *R. parkeri* strain Atlantic rainforest, *R. amblyommatis,* and an unnamed rickettsial agent, here designated as *Rickettsia* sp. ES-*A*.cf.*parvum.* Indeed, our finding of *R. parkeri* strain Atlantic rainforest in *A. ovale* is highly relevant because this agent has been associated to spotted fever illness in other Latin American countries, where *A. ovale* is implicated as its main vector (Sevá et al., 2019; Silva-Ramos et al., 2021). These findings suggest that spotted fever cases caused by *R. parkeri* strain Atlantic rainforest could be occurring in El Salvador, as well as the other Central American countries (Belize and Nicaragua) where this emerging pathogen has been reported in *A. ovale* ticks (Polsomboon Nelson et al., 2022; Vogel et al., 2018). This statement is supported by the fact that *A. ovale* is among the most frequent human-biting ticks in Central America (Bermúdez et al., 2022).

Rickettsia amblyommatis was the most common SFG agent, detected in the three tick species. This finding corroborates the previous studies that reported *R. amblyommatis* as the most frequent tick-borne rickettsia infecting Central American ticks (reviewed by Bermúdez and Troyo, 2018). Indeed, most of these records were from *A. mixtum,* sometimes reported with high infection rates [e.g., 68% in Costa Rica (Troyo et al., 2016); 91% in Panama (Bermúdez et al., 2021)], similarly to the 80% infection rate found in the present study. These findings indicate that *R. amblyommatis* is primarily associated with *A. mixtum* in Central America, similarly to its primary association with *Amblyomma americanum* in the United States, where this rickettsia is successfully maintained in the tick populations through transovarial and transstadial perpetuations (Karpathy et al., 2016). This rickettsial agent was also reported in 46% of the *A.* cf. *parvum* ticks, also suggesting a primary association. On the other hand, our finding of *R. amblyommatis* in 4% of the *R. sanguineus* s.l. ticks could be a result of horizontal transmission via dogs, since some of the *A. mixtum*-infested dogs were co-infested with *R. sanguineus* s.l.. Despite the wide occurrence in ticks, *R. amblyommatis* is not recognized as a human pathogen, although there have had some reports implicating it as a possible cause of mild or subclinical infection in humans in the United States (Karpathy et al., 2016; Parola et al., 2013).

Rickettsia sp. ES-*A*.cf.*parvum* had DNA sequences 99.8 - 100% identical to *Rickettsia* sp. ApBA from *A. parvum* from Brazil (Maia et al., 2018), and *Rickettsia* sp. Gu263 from *A. auricularium* from Guatemala (available in GenBank #JF523328), what suggest conspecificity of these three agents. This assumption indicates that this unnamed SFG agent, closely related to *R. amblyommatis* (Fig. 3), is associated with *A. parvum* sensu lato, which includes *A. parvum* and *A.* cf. *parvum* (Lado et al., 2016), and possibly Central American *A. auricularium,* since Romero et al. (2021) reported that ticks previously reported as *A. auricularium* from El Salvador were genetically identified as *A.* cf. *parvum*. Because *A.* cf. *parvum* is also a human-biting tick in Central America (Bermúdez et al., 2022), the role of *Rickettsia* sp. ES-*A*.cf.*parvum* as a human pathogen needs to be further evaluated. Finally, further studies employing additional molecular markers are needed to elucidate if *Rickettsia* sp. ES-*A*.cf.*parvum* is a new *Rickettsia* species or a novel strain of *R. amblyommatis.*

This research shows the presence of several species of ticks on dogs from El Salvador, with potential of parasitizing humans. The canine ticks were infected by three SFG rickettsial agents, including the human pathogen *R. parkeri.* Indeed, SFG rickettsiosis should be included in differential diagnosis among suspected cases of arboviruses (e.g., Dengue) in El Salvador, where many cases have remained with uncertain confirmatory diagnostic (unpublished data, Ministry of Public Health of El Salvador). Knowledge of the distribution of tick species and tick-borne pathogens is crucial for implementation of preventive measures both, in veterinary medicine and public health.

CRediT authorship contribution statement

Luis E. Romero: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Lina C. Binder:** Formal analysis, Investigation, Validation, Writing – review & editing. **Arlei Marcili:** Data curation, Investigation, Methodology, Resources, Writing – review & editing. **Marcelo B. Labruna:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

Authors state no conflict of interest.

Data availability

Data will be made available on request.

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