EcoHealth
DOI: 10.1007/s10393-016-1118-8

ECOHEALTH

Original Contribution

Distribution of Spotted Fever Group Rickettsiae in Hard Ticks (Ixodida: Ixodidae) from Panamanian Urban and Rural Environments (2007–2013)

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Abstract: Tick-borne rickettsiosis is an important emerging disease in Panama; to date, there have been 12 confirmed cases, including eight fatalities. To evaluate the distribution of rickettsiae in Panamanian ticks, we collected questing and on-host ticks in urban and rural towns in elevations varying between 0 and 2300 m. A total of 63 sites (13 urban and 50 rural towns) were used to develop models of spatial distributions. We found the following tick species: *Rhipicephalus sanguineus* s.l. (present in 54 of 63 towns and cities), *Amblyomma mixtum* (45/63), *Dermacentor nitens* (40/63), *A. ovale* (37/63), *Rhipicephalus microplus* (33/63), *A. oblongoguttatum* (33/63), *Ixodes affinis* (3/63), and *Ixodes boliviensis* (2/63). *Rhipicephalus sanguineus* s.l. was present in urban and rural towns, and other species were present only in rural towns. DNA was extracted from 408 *R. sanguineus* s.l., 387 *A. mixtum*, 103 *A. ovale*, and 11 *A. oblongoguttatum* and later tested for rickettsiae genes using PCR. Rickettsia DNA was detected in ticks from 21 of 63 localities. *Rickettsia rickettsii* was detected in five *A. mixtum* (1.29%), and *Candidatus* "Rickettsia amblyommii" was found in 138 *A. mixtum* (35%), 14 *R. sanguineus* (3.4%), and one *A. ovale* (0.9%). These results suggest that much of rural Panama is suitable for the expansion of tick populations and could favor the appearance of new tick-borne rickettsiosis outbreaks.

Keywords: ixodidae, spatial distribution model, *Rickettsia* spp., molecular surveillance, Panama

Introduction

Ticks are a group of blood-feeding arachnids that parasitize all classes of terrestrial vertebrates (Labruna et al. 2005). Although ticks are not naturally associated with humans, they constitute the second most important group of arthropods in relevance to human health. Ticks are transmitters of a diverse array of zoonotic agents, including Lyme disease, tick-borne hemorrhagic fevers, ehrlichiosis, or rickettsiosis (TBR) (Telford and Goethert 2008; Williamson et al. 2010). The ecology of this zoonosis depends

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Published online: 11 April 2016

on the distribution of ticks with the capacity to acquire the pathogen from an infected host, maintain the infection through transstadial transmission, and later transmit the pathogen to a new host. The presence of vertebrates to serve as pathogen reservoirs is also necessary to continue zoonosis circulation in the environment (Dantas-Torres 2007; Minniear and Buckingham 2009; Szabó et al. 2013).

The risks associated with transmission of the tick-borne pathogens increase with human interaction with forest ecosystems, including eco-tourism, hunting, logging, as well as the expansion of human settlements into adjacent wilderness areas, or presence of ticks on wandering animals in urban cities (Padmanabha et al. 2009; Bayles et al. 2013). Tick monitoring in a variety of habitats is a critical component of evaluating tick-borne pathogen infection risk to humans (Pinter 2013). In order to develop successful surveillance and prevention programs, it is necessary, as a first step, to measure the ecological parameters that limit the distribution of the potential vectors, including the climatic habitat (Randolph 2004; Estrada-Peña et al. 2014; Illoldi-Rangel et al. 2012; Szabó et al. 2013).

In Latin America, the TBR caused by *Rickettsia rickettsii* is the most significant tick-borne zoonoses because of its high mortality rate (20–80%) in untreated cases (Labruna et al. 2011; Parola et al. 2013). This pathogen has been identified since the early to mid-20th century in several countries in the Americas (Childs and Paddock 2007). After decades of few new records, there has been a recent two- to fourfold increase in positive cases (Openshaw et al. 2010; Labruna et al. 2011). Besides *R. rickettsii*, emerging pathogens, such as *Rickettsia parkeri* or *R. massiliae*, have been identified in recent years in the US, Brazil, Argentina, and Uruguay, adding to the importance of rickettsial diseases in the Americas (Mediannikov et al. 2007; Labruna et al. 2011).

In Panama during 1950–1953, *R. rickettsii* infection was confirmed in five patients, two of which were fatalities (Rodaniche and Rodaniche 1950; Calero et al. 1952; Rodaniche 1953). No new cases were confirmed until beginning of this century when seven people were diagnosed with this disease, six of which were fatal; an emerging pattern that is similar to what was has been seen in other countries (Estripeaut et al. 2007; Tribaldos et al. 2011; De Luca et al. 2013). It is noteworthy that no mild fever cases of TBR have been reported or identified in Panama. However, after the mentioned outbreaks, surveillance studies revealed a national seroprevalence of spotted fever rickettsiosis reaching 5.4–15.2% in 1408 volunteers (Silva-Goytia and Calero 1956), and recently a seroprevalence of 39% (n = 97 volunteers) was shown in three non-endemic TBR areas (Bermúdez et al. 2013). Yet, in this country there are no data to know the real extent of the disease.

The aim of this paper is to present data regarding the distribution and hosts of the principal Ixodid ticks from urban and rural sites in Panama and to identify *Rickettsia* spp. in ticks from varying environments.

Materials and Methods

Collection Sites

Panama is a narrow isthmus of 78,200 km^2 located in southern Central America and northeastern South America. Most towns and cities in Panama are located below 400 m above sea level, in a tropical climate characterized by warm temperatures (20–35°C) and high humidity (70–98%), while villages in higher elevations 1000–2300 m above sea level maintain subtropical temperatures (5–20°C) and high humidity. Because Panama has an equatorial longitude, it does not have great variation in day length throughout the year.

Samples for this study were collected from 2007 to 2013 as part of the activities from various investigations throughout Panama including localities with previous records of human rickettsiosis (Fig. 1). Data were collected by convenience sampling. Tick collection included sampling domestic animals from urban and rural settlements, and collection of questing ticks in vegetation in plots of 100 m^2, in kennels of dogs, and in other places around dwellings. In addition, ticks found parasitizing humans were collected. The sites of collection were georeferenced and environmental characteristics were recorded. Rural areas were classified according to dominant economic activity (e.g., livestock and crops) or proximity to forested areas. All ticks were preserved in 95% ethanol. Distribution data and host records were paired with reference specimens and deposited in the Ectoparasites Compendium in the "Dr. Eustorgio Méndez" Zoological Collection at Instituto Conmemorativo Gorgas de Estudios de la Salud (CoZEM-ICGES, by Spanish acronym).

Tick Identification

Adult ticks were identified using taxonomic keys (Fairchild et al. 1966). Nymphs of the genus *Amblyomma* were identified following Martins et al. (2010, 2014). To identify

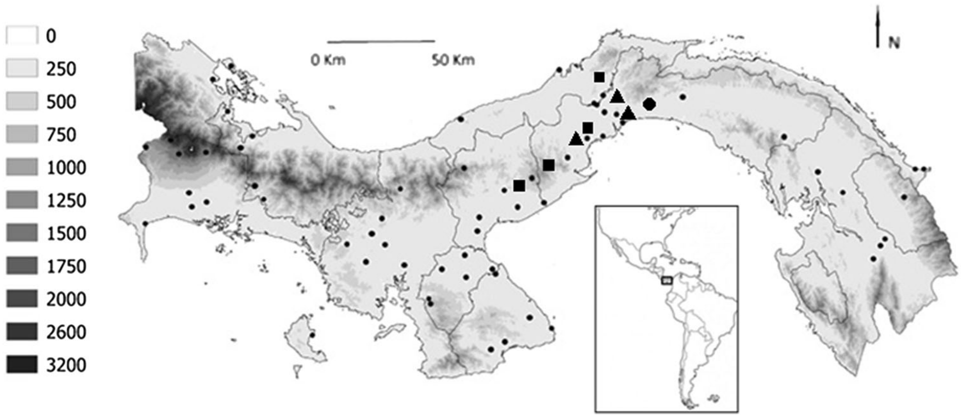

Figure 1. Map of Panama showing the geographical sites (*black points*) when the ticks were collected and the variations of elevations. *Black triangles* indicate 3 TBR cases from 1950 to 1952; *black squares* indicate 4 TBR cases from 2004 to 2014; *big black circles* indicate a TBR cluster (3 cases) in 2007.

the Panamanian species in the *Amblyomma cajennense* group complex, we followed the taxonomic criteria of Nava et al. (2014). We treated the brown dog tick group *Rhipicephalus sanguineus* as a taxonomic complex (Moraes-Filho et al. 2011). Ticks were placed in CoZEM-ICGES and the "G.B. Fairchild" Museum of Invertebrates at the Universidad de Panamá.

Geographic Analysis

We used an initial dataset of 19 bioclimatic layers to a resolution of 30 arc seconds obtained from the WorldClim database (Hijmans et al. 2005) for the selection of variables with higher loads, low correlation, and ecological importance in a Principal Component Analysis (PCA). Layers were loaded on the region of interest in order to retrieve bioclimatic information associated with each point where ticks were reported. Quantum GIS 1.8 was used for the spatial analysis and geographic projection of the scenopoetic potential distribution models (SDM). Georeferenced data and the selected variables were used in potential distribution modeling using a Maximum Entropy algorithm developed by Phillips et al. (2004) and implemented in the MaxEnt 3.3.3 k program. To create the potential distribution model, we ran 30 replicates of 500 iterations in order to obtain an average model per species; the importance of variables was estimated using Jackknife analysis. The evaluation of the average model was performed using all available records in order to obtain an overall sensitivity (points correctly predicted/total points * 100). The probabilities (Habitat Suitability Index, HS) from the average potential distribution models were plotted against the altitudinal records of the sampled ticks to explore how HS is affected by the elevation. All potential distribution models were converted into probabilistic-ranked models and the probabilistic threshold used was selected by the minimum training presence (an average of all 30 models) criteria.

Detection of Spotted Fever Group *Rickettsia*

Ticks were separated by species, developmental stages, and later processed in pools of 5–10 individuals (immature ticks) or individually (adults). DNA was extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany), following the manufacturer's instructions for animal tissues. PCR was initially performed with primers CS-78 and CS-323, targeting a 401-bp portion of the rickettsial citrate synthase gene (*glt*A) (Labruna et al. 2004). Samples yielding visible amplicons were also tested with primers CS-239 and CS-1069 (targeting an overlapping 821-bp fragment of the *glt*A gene), and also with primers Rr190.70 and Rr190.701, targeting 632-bp portion of the rickettsial outer membrane protein A gene (*omp*A) (Roux et al. 1997). All reactions were performed as previously described (Labruna et al. 2004), and used *Rickettsia rhipicephali* DNA as a positive control, and water as a negative control. PCR products were purified using ExoSap (USB) and sequenced in an automatic sequencer (Applied Biosystems/Perkin Elmer, model ABI Prism 3130xl Genetic, California, US). Partial sequences were subjected to BLAST analysis (Altschul et al. 1990) to determine similarities to other *Rickettsia* species.

Results

Geographic Distribution of the Ticks

Information was compiled from 63 towns, including 13 urban cities and 50 rural sites, located at altitudes between 0 and 2300 m. In these locations, questing ticks were collected and the following animals were found infested with ticks: 523/950 dogs, 234/354 horses, and 3/10 cats. In total, we found 54 towns with presence of *R. sanguineus* s.l., 45 *A. mixtum*, 40 *Dermacentor nitens*, 37 *Amblyomma ovale*, 33 *Rhipicephalus* (*Boophilus*) *microplus*, and 11 *A. oblongoguttatum*. Because of the monoxenous cycle of *D. nitens* and *R. microplus*, they are infrequently found on humans and were therefore excluded from this analysis. Other species were not included in the present analysis, such as *Ixodes boliviensis* and *Ixodes affinis*, since their presence was minimal (e.g., three *I. affinis* in two localities) or highly localized (e.g., *I. boliviensis* only over 2000 m of elevation). In both cases, these data will be present in further studies.

Current distribution of *A. mixtum* is seemingly limited to areas below 800 m, particularly in shrub areas on the Pacific slope, and less frequently in fields with introduced cultivated grass (Fig. 2a). During this study, there were no established populations of *A. mixtum* found above 1200 m, in areas with combined low average temperature (below 15°C) and high relative humidity (above 85%). In addition, there were no *A. mixtum* collected in urban cities. Horses were the principal host for immature and adult *A. mixtum*, and other groups of vertebrates were parasitized on a smaller scale. Immature and adult forms were found to parasitize humans in large numbers. Table 1 reflects the most common hosts for *A. mixtum* in Panama.

Rhipicephalus sanguineus s.l. was found at altitudes between 0 and 1200 m, including urban cities and rural towns in both coastal slopes (Fig. 2c). We found engorged females and several immature ticks on exterior of dwellings (walls and windows) and inside dwellings (on furniture). As is expected, dogs are the main host to *R. sanguineus* s.l. and few specimens were found parasitizing other mammals or humans (Table 1).

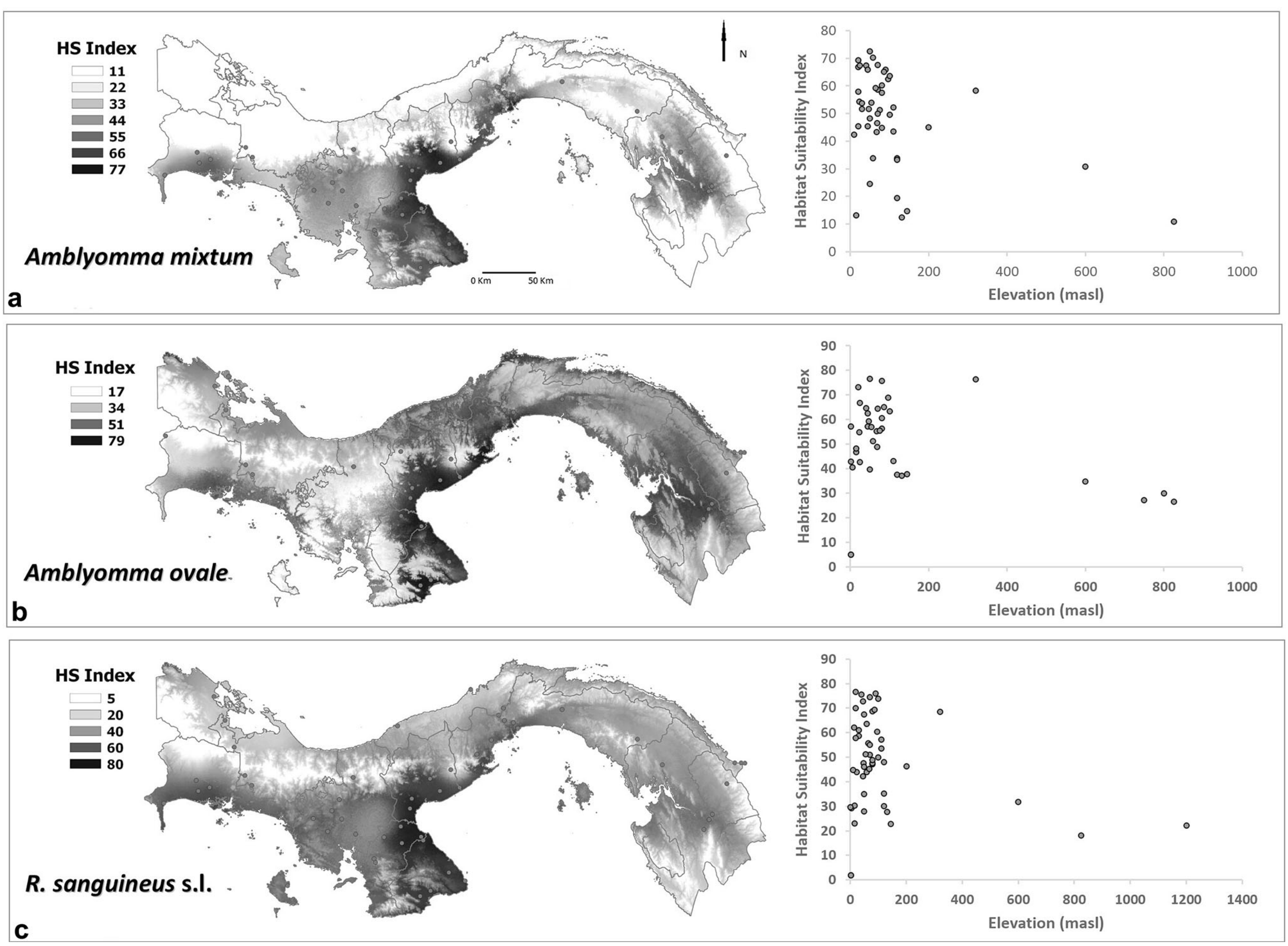

Figure 2. Potential distributions of hard ticks in rural and urban environments in Panama. *Color ranks* in the scale represent the Habitat Suitability Index and the records are shown in a *white circle*. Altitudinal distribution and Habitat Suitability Index. **a** *Amblyomma mixtum*, **b** *Amblyomma ovale*, and **c** *Rhipicephalus sanguineus* s.l.

Table 1. Checklist of Hosts Parasitized by *Amblyomma mixtum* and *Rhipicephalus sanguineus* s.l. in Panama.

Species	Host	Number[a]	Environment
A. mixtum	Horse	234	Rural towns
	Mule	5	Rural towns
	Cow	4	Rural towns
	Chicken (*Gallus gallus*)	1	Rural towns
	Hawk (*Buteo magnirostri*)	–	Cited in Fairchild et al. (1966)
	Dogs	41	Rural towns
	Coyote (*Canis latrans*)	1	Road kill
	Poncho (*Hydrochoerus isthmius*)	5	García et al. (2014)
	Tapir (*Tapirus bairdii*)	9	Captive animals in Zoo
	"Deer"	3	Road kill
	Tayassu tajacu	–	Cited in Fairchild et al. (1966)
	Domestic pig	3	Rural towns
	Armadillo (*Dasypus novemcinctus*)	–	Cited in Fairchild et al. (1966)
	Opossum (*Didelphis marsupialis*)	8	Secondary forest around rural towns
	Opossum (*Metachirus nudicaudatus*)	3	Secondary forest around rural towns
	Mouse (*Transandinomys bolivari*)	5	Rural towns
	Mocangué (*Proechimys semispinosus*)	11	Secondary forest around rural towns
	Rat (*Rattus* spp.)	2	Rural towns
	Human	31	Rural towns
R. sanguineus s.l.	Dogs	456	Urban and rural towns
	Cats	8	Urban and rural towns
	Ant eaters (*Tamandua mexicana*)	1	Captive animal in Zoo
	Opossum (*Didelphis marsupialis*)	1	Rural towns
	Poncho (*Hydrochoerus isthmius*)	–	Cited in Fairchild et al. (1966)
	Marmoset (*Saguinus geoffroyi*)	1	Captive pet in rural towns
	Human	14	Urban and rural towns

[a] The numbers indicate the number of ticks collected from each vertebrate species.

In rural towns, *A. ovale* was commonly collected close to forested areas, at altitudes of 0–900 m (Fig. 2b). During this study, no *A. ovale* were found questing, but many ticks were found parasitizing dogs. Similarly, *A. oblongoguttatum* were found parasitizing hosts in rural towns close to forests.

As expected, potential distribution models indicate that much of the continental and insular areas of Panama have environmental characteristics suitable for establishment of these tick species. In fact, even at relatively high elevations (800–1200 m) there is a reasonable chance to find suitable areas for *A. ovale* and *R. sanguineus* s.l. (Fig. 2). Average models present 95.76% of sensitivity and HS index up to 76% for *A. mixtum*, 97.41% of sensitivity and HS index up to 79% for *A. ovale*, and 95.1% of sensitivity and HS index up to 80% for *R. sanguineus* s.l. The potential distribution model of *A. mixtum* is affected by environmental conditions from western and eastern highlands, and the same pattern is observed for the potential distribution model of *R. sanguineus* s.l., although to a lesser extent. Alternatively, the potential distribution model of *A. ovale* indicates that Panama comprises environmental conditions suitable for the establishment of the tick throughout the country, with a wider geographical range than the other tick species. According to the PCA, the variable with biggest contribution (highest load) to the climatic distribution of the three ticks in Panama was Bio9. The Jackknife analysis in Maxent for variable contribution shows that this same variable is also the most important for the SDM of *Amblyomma* species, but for *R. sanguineus* s.l. SDM the variable with the greatest gaining was Bio5 (Table 2).

Molecular Assays

Rickettsia DNA was detected in ticks from 21 rural towns (35% of total), and no *Rickettsia* was detected in ticks from

Table 2. Bioclimatic Values for the Habitat of the Three Different Ticks in the Study.

Bioclimatic variable	*Amblyomma mixtum* (46)		*Amblyomma ovale* (36)		*R. sanguineus* s.l. (51)	
	Range	Mean	Range	Mean	Range	Mean
Annual mean temperature (Bio1)	22.5–27.4	26.18	21.9–27.3	26.03	22.5–27.4	26.12
Mean diurnal range (Bio2)	7.2–10.4	8.76	6.0–10.3	8.21	6.0–10.4	8.70
Isothermality (Bio3)[‡]	71–83	75.74	71–88	77.19	71–88	75.96
Temperature seasonality (Bio4)[‡]	386–821	584.41	324–821	574.81	324–821	577.27
Max temperature of the warmest month (Bio5)	28.8–34.4	**32.44**[m]	28.3–34.4	**31.70**[m]	28.8–34.4	**32.27**[m]
Min temperature of the coldest month (Bio6)	16.7–22.5	**20.88**[m]	15.7–22.9	21.08	16.7–22.9	20.82
Temperature annual range (Bio7)	8.9–14.6	**11.56**[m]	7.7–14.1	**10.61**[m]	7.7–14.6	**11.45** [m]
Mean temperature of the wettest quarter (Bio8)	21.8–26.8	25.66	21.2–26.7	25.57	21.8–26.8	25.64
Mean temperature of the driest quarter (Bio9)	22.9–27.7	**26.38**[m]	22.0–27.7	**26.10**[m]	22.9–27.7	**26.26**[m]
Mean temperature of the warmest quarter (Bio10)	23.3–28.3	27.02	22.8–28.3	26.82	23.3–28.3	26.92
Mean temperature of the coldest quarter (Bio11)	21.8–26.8	25.52	21.0–26.7	25.34	21.8–26.7	25.44
Annual precipitation (Bio12)	1111–3732	2214.30	1131–3732	**2281.94**[m]	1111–3372	**2320.86**[m]
Precipitation of the wettest month (Bio13)	210–589	**373.85**[m]	224–614	367.58	210–615	385.14
Precipitation of the driest month (Bio14)	0–103	15.30	0–144	29.17	0–143	23.63
Precipitation seasonality (Bio15)[‡]	36–83	66.91	28–81	**61.19**[m]	29–83	64.39
Precipitation of the wettest quarter (Bio16)	566–1449	963.91	567–1464	938.83	566–1646	991.71
Precipitation of the driest quarter (Bio17)	7–444	**75.24**[m]	13–516	**122.47**[m]	7–448	**100.80**[m]
Precipitation of the warmest quarter (Bio18)	53–750	331.04	53–896	399.61	53–896	363.18
Precipitation of the coldest quarter (Bio19)	400–1379	747.65	400–1287	715.11	400–1646	757.08
Elevation	10–825	106.26	1–825	134.89	1–1200	113.63

Parenthetical values indicate the number of localities used. For each tick species, the variables used in the potential distribution model are denoted with [m].
[‡] Representing values with a scaling factor of 100.

Table 3. Rickettsiae Infesting *A. mixtum* in Human Settlements in Panama (2007–2013).

Humans settlements[a]	Population	No.[b]	*A. mixtum*	*R. rickettsii*	"R. amblyommii"
Lowland urban city (0–250 m)	(≥80,000)	5	0	–	–
Lowland urban city (0–250 m)	(50,000–80,000)	8	0	–	–
Lowland rural towns (0–250 m)	(10,000–50,000)	40	321	3	118
Lowland rural town (250–500 m)	(5000–10,000)	4	21	2	8
Lowland rural town (500–1000 m)	(≤5000)	2	45	0	12
Highland rural town (1000–2000 m)	(≤25,000)	1	0	–	–

[a] Urban areas were considered as free of agricultural activities (e.g., livestock and crops). Rural areas were considered according the primary economic activity (e.g., livestock and crops), or proximity to forested areas.
[b] Corresponding to human settlements analyzed.

urban cities. A consensus sequence of 560 bp was obtained for *omp*A gene, showing a 99% homology to *R. rickettsii* in questing *A. mixtum* (1.29%). Noteworthy, *A. mixtum* infected to *R. rickettsii* were found in locations very close geographically to the recent fatal TBR case in Coclé Province (Tribaldos et al. 2011), and other which has not yet been reported in scientific publication.

Compared with consensus sequences on a BLAST search, we observed 99.7% homology with *C.* "R. amblyommii" for *glt*A and 99.8% homology with *C.* "R. amblyommii" for *omp*A in 148 *A. mixtum* (38%) and in 14 *R. sanguineus* s.l. (3.3%, $n = 408$). Tables 3 and 4 summarize the rickettsiae infecting *A. mixtum* and *R. sanguineus* s.l. in rural and urban towns, respectively. In

Table 4. Rickettsiae Infesting *R. sanguineus* s.l. in Human Settlements in Panama (2007–2013).

Humans settlements[a]	Population	No.[b]	*R. sanguineus*	*R. rickettsii*	"R. amblyommii"
Lowland urban city (0–250 m)	(≥80,000)	5	150	0	0
Lowland urban city (0–250 m)	(50,000–80,000)	8	50	0	0
Lowland rural towns (0–250 m)	(10,000–50,000)	40	112	0	3
Lowland rural town (250–500 m)	(5000–10,000)	4	162	0	4
Lowland rural town (500–1000 m)	(≤5000)	2	34	0	7
Highland rural town (1000–2000 m)	(≤25,000)	1	0	–	–

[a]Urban areas were considered as free of agricultural activities (e.g., livestock and crops). Rural areas were considered according the primary economic activity (e.g., livestock and crops), or proximity to forested areas.
[b] Corresponding to human settlements analyzed.

Table 5. Tick–Rickettsiae Relationship in Rural Areas in Panama (2007–2013).

Tick/host	*N*	*R. rickettsii*			"R. amblyommii"			Total
		L	N	A	L	N	A	
Amblyomma mixtum	**387**							
Horses	228	0	0	0	0	10	108	118
Dogs	24	0	0	0	0	1	7	8
Pigs	18	0	0	0	0	7	0	7
Humans	12	0	0	0	0	0	0	0
Questing ticks	117	0	0	5	0	2	13	20
Amblyomma ovale	**103**							
Dogs	103	0	0	0	0	0	1	1
Rhipicephalus sanguineus Group	**408**							
Dogs	365	0	0	0	0	1	13	14
Cats	23	0	0	0	0	0	0	0
Humans	18	0	0	0	0	0	0	0
Questing ticks	20	0	0	0	0	0	0	0
Total	**928**	0	0	5	0	21	142	168

Bold values indicate the total number of individuals
L larvae ticks, *N* nymph ticks, *A* adults.

addition, of 103 *A. ovale* tested, one was positive for *C.* "R. amblyommii," while 11 *A. oblongoguttatum* were tested without any evidence of infection with rickettsiae. Table 5 summarizes the tick species infected with *Rickettsia*, according the host species and stage.

Discussion

It is known that the distribution and persistence of tick populations is dependent on environmental factors such weather, microclimate, and vegetation, as well as the presence of suitable hosts (Randolph 2004; Szabó et al. 2013). In addition, changes in the landscape and the introduction of domestic mammals can lead to the presence and persistence of some species of ticks, such as *A. mixtum* or *R. sanguineus* s.l., which are considered among the principal vectors of TBR in Latin America (Dantas-Torres 2007; Szabó et al. 2013; Oteo et al. 2014). Approximately 50% of Panamanian native forests have been altered by human activity, especially in the Pacific lowlands where 68% of the Panamanian population lives (Miambiente 2010); thus, under these conditions, natural landscape modifications and decline of biodiversity, *A. mixtum* and *R. sanguineus* s.l. thrive and will likely increase their distribution as anthropogenic transformation continues.

Our results suggest that the rural Panamanian towns provide better environments for ticks and their hosts than the urban cities. For example, we found that *R. sanguineus* s.l. is the only species well adapted to human urban and rural dwellings, while *Amblyomma* species were present only in rural towns. In general terms, rural environments allow more tick–host interactions, which would benefit the *Amblyomma* species, whose cycles involve both small (e.g., rodents or opossum) and large mammals (e.g., horses) (Table 1). Furthermore, since *Amblyomma* species are exophilic, they are more exposed to environmental conditions. Conversely, the presence of dogs is a necessary condition for the maintenance of large populations of *R. sanguineus* s.l. and this species is endophilic, taking advantage of indoor habitats such as wall cracks or furniture to complete its cycle (Guglielmone et al. 2003; Demma et al. 2005; Dantas-Torres 2008). The importance of both species of ticks in public health depends on the differences of life history, behavior, and adaptability to anthropogenic environments, in addition to human conducts that could favor the contact with the ticks.

Rhipicephalus sanguineus s.l. was brought to the Americas from the Old World on infested dogs (Walker et al. 2000; Guglielmone et al. 2003). This group of species has a low affinity for parasitizing humans (Palmas et al. 2001; Szabó et al. 2013); however, it has been associated with TBR outbreaks in the United Sates (Demma et al. 2005) and Mexico (Eremeeva et al. 2011). Furthermore, studies from a TBR-endemic area of Brazil found natural infections of *R. rickettsii* in *R. sanguineus*, which could represent other factors associated with the transmission of TBR in these areas (Cunha et al. 2009; Pacheco et al. 2011). Experimental data suggest that dogs can amplified *R. rickettsii* for immature of *R. sanguineus* s.l., although not maintain the infections in successive generations (Piranda et al. 2011).

In Panama, Calero et al. (1952) introduced the possibility that *R. sanguineus* s.l. was involved in an urban case of TBR, which could be explained by the urban environmental conditions at that time. In our data, we found no *R. rickettsii* infections in *R. sanguineus* s.l., and *C.* ''R. amblyommii'' was only present in these ticks from rural sites. In Brazil, Szabó et al. (2013) presented the possibility that rickettsemic wandering dogs may be a source of infection for different species of ticks, especially *R. sanguineus*, *A. cajennense*, or *A. aureolatum*. Thus, more tick–host interactions and a main mobility of dogs (e.g., homes, pastures, and forest), allowing more conditions for the establishment of rickettsiae in rural areas, and consequently a risk factor for the occurrence of human cases. Furthermore, additional research demonstrating the relevance of Panamanian population of *R. sanguineus* in TBR infections is necessary.

With respect to *A. mixtum* in Panama, Rodaniche (1953) considered *A. cajennense* (*A. mixtum* after Nava et al. 2014 review) as the ''perfect vector'' for *R. rickettsii* for two principal reasons: (1) its affinity for parasitizing domestic animals and humans and (2) high capacity of transmission in experimental conditions. Its wide distribution around rural towns in the Pacific slopes of Panama reflects its ability to take advantage of pastures and cleared areas, parasitizing different species of domestic and synanthropic animals (Fairchild et al. 1966; Guglielmone et al. 2003). In addition, these ticks are more prone to parasitize humans than *R. sanguineus*. According to Fairchild (1943), humans are susceptible to being parasitized by *A. mixtum* adults and nymphs, which we also found in our study.

Our data reflect a much higher prevalence of *C.* ''R. amblyommii'' (35%)-infected *A. mixtum* than *R. rickettsii* (1.29%). However, despite the low detection of *R. rickettsii*, its presence could be indicative of persistence in the environment, and present potential risks of new cases. Other countries present similar results. For example, absence or low prevalence of *R. rickettsii* and high rates of *C.* ''R. amblyommii'' have been reported in ticks from the US, even in ticks from areas with a high incidence of TBR (Smith et al. 2010, Moncayo et al. 2010, Nadolny et al. 2014). In Costa Rica, Hun et al. (2011) reported 67% ($n = 16$) of *A. cajennense* s.l. infected with *C.* ''R. amblyommii'' and any *R. rickettsii*. The low incidence of *R. rickettsii* in ticks has been explained, in part, by its effects on the fitness of certain tick species. Niebylski et al. (1999) reported pathogenicity and lethal effects on immature stages and females of *Dermacentor andersoni* under laboratory conditions. In contrast, experimental models showed a successful trans-ovarial transmission of *C.* ''R. amblyommii'' in *A. americanum* (Burgdorfer et al. 1981).

Candidatus ''R. amblyommii'' has been identified in at least six countries of the Americas and detected or isolated in nine species of ticks, including species with anthropophilic behavior (Labruna et al. 2011; Saraiva et al. 2013; Parola et al. 2013). The effects of *C.* ''R. amblyommii'' on vertebrates are not clearly defined. Laboratory animals inoculated experimentally with *C.* ''R. amblyommii'' seem to not manifest evidence of disease or any alteration of their health (Burgdorfer et al. 1981; Saraiva et al. 2013); however, it can induce an immunologic response in different groups of mammals (Labruna et al. 2007; Melo et al. 2011; Bermúdez et al. 2011; Saraiva et al. 2013). Despite its frequent presence in ticks, there are no confirmed cases of human rickettsiosis caused by C. ''R. amblyommii,'' although some authors report it as causing a tick bite rash (Billeter et al. 2007) and mild fever (Dasch et al. 1993; Apperson et al. 2008; Jiang et al. 2010). Considering the aggressive parasitic behavior of *A. mixtum* toward humans and the relative high prevalence of C. ''R. amblyommii'' in these ticks, this could result in the high seroprevalence of spotted fever rickettsiosis described by Silva-Goytia and Calero (1956) and Bermúdez et al. (2013). However, more studies are necessary to confirm this hypothesis.

Finally, the finding of *A. ovale* and *A. oblongoguttatum* in Panamanian rural sites indicates this species' ability to adapt to parasitism in rural towns and its ability to adapt in new environments and hosts. Both species have been reported to bite humans in Panama (Bermúdez et al. 2012). In the case of *A. ovale*, there is a great capacity to parasitize many groups of vertebrates (Murgas et al. 2013). This idea was strengthened by the findings of Szabó et al. (2012), who reported an alternate nidicolous behavior in *A. ovale* in response to environmental imbalances. Moreover, a Brazilian survey suggested *A. ovale* as the vector of a *Rickettsia* sp. strain Atlantic rainforest of Brazil (Sabatini et al. 2010, Silva et al. 2011).

Conclusions

Although the presence of infected ticks is not the only variable that influences the ecology of the spotted fever group rickettsiosis, it is a critical evidence for predicting areas at risk for future cases. The potential distribution model shows that much of the insular and continental area of Panama has environmental conditions that could favor the establishment of tick populations, especially in rural towns. This includes adequate conditions for the persistence of the main vector (*A. mixtum*), as well potential vectors as *R. sanguineus* s.l., besides offering alternative habitats for *A. ovale* and *A. oblongoguttatum*. To improve the models of distribution, layers with demographic information and use of soil would need to be included.

In urban cities, the current conditions do not demonstrate the presence of rickettsiae in *R. sanguineus* s.l. on dogs. However, the high numbers of dogs infected with *R. sanguineus* s.l. are an important element to consider for health researchers and authorities, especially because of this tick's demonstrated effective role as a *R. rickettsii* vector in other countries and the capacity of dogs as an amplifier reservoir (Piranda et al. 2011; Pacheco et al. 2011). Also, our work suggests that C. ''R. amblyommii'' is widespread in rural towns of Panama. The detection of *R. rickettsii* on *A. mixtum* from TBR-endemic areas merits further studies that include obtaining seroprevalence in humans and other vertebrates.

Acknowledgments

This work was supported by the projects ''Surveillance of Panamanian ectoparasites for rickettsial agents'' (funds CDC, ICGES) and ''Vigilancia epidemiológica de las enfermedades emergentes y re-emergentes del cordón fronterizo Panamá-Colombia'' (funds Ministry of Economic and Finance), and also other field activities from the projects ''Estudio de la diversidad de toxinas producidas por los escorpiones de importancia médica en Panamá, mediante el empleo de técnicas biotecnológicas,'' ''Análisis toxinológico de poblaciones de escorpiones de los géneros *Tityus* y *Centruroides* en áreas endémicas de Costa Rica, Colombia y Panamá, empleando técnicas moleculares e inmunológicas'' (fondos SENACYT), and ''Análisis de la incidencia de leishmaniasis en Capira'' (funds Ministry of Economic and Finance). LEP received funding from the Programa de Doctorados Nacionales del Departamento Administrativo de Ciencia, Tecnología e Innovación (COLCIENCIAS) from Colombia. Special thanks to Nicole Gottdenker, Anayansi Valderrama, and Robyn Nadolny for their comments on the manuscript and assistance with the English revision.

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