

ARTÍCULO ORIGINAL

## Serologic evidence of human *Rickettsia* infection found in three locations in Panamá

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**Introduction:** Since the middle of last century, cases of rickettsiosis have been found in Panamá when outbreaks of murine typhus and spotted fever were reported. Since then, little information exists about its prevalence in this country, since it is most often misdiagnosed as another disease.

**Objectives:** The aim of this paper is to demonstrate the presence of *Rickettsia* infections in humans in three locations in Panamá. These locations are agricultural areas, near forested areas or those who work in zoo.

**Materials and methods:** Three locations were chosen for this study: Tortí, El Valle de Antón and workers in the Summit Municipal Park in Panamá City. All volunteers signed an informed consent and answered a questionnaire. The samples were analyzed for the detection of rickettsial spotted fever and typhus group by the indirect immunofluorescence (using commercial kits) and antigens of *Rickettsia rickettsii* and *R. amblyommii*.

**Results:** Blood samples were taken from 97 volunteers in Tortí (25), El Valle de Antón (37) and Summit Municipal Park (35). Of these, a total of 38 (39%) samples reacted to one of the two methods: eight (32%) in Tortí, 18 (48%) in El Valle and 12 (34%) in Summit Municipal Park.

**Conclusion:** The results show a high prevalence of antibodies to *Rickettsia* belonging to the spotted fever group in each of the three study areas, in addition to presenting evidence of the typhus group *Rickettsia* in El Valle de Antón. These areas could be considered endemic for rickettsiosis as there are conditions for maintaining them.

**Key words:** Rickettsiaceae infections, immunoglobulin G, disease outbreaks, epidemiology, Panamá.

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### Evidencia serológica de infecciones de *Rickettsia* en humanos provenientes de tres localidades de Panamá

**Introducción.** Desde mediados del siglo pasado, se conocen en Panamá casos de rickettsiosis, cuando fueron reportados brotes de tifus en ratones y de fiebres manchadas. A partir de entonces, poca información se tiene sobre su prevalencia en este país, lo cual se debe principalmente a que son confundidos con otras enfermedades.

**Objetivos.** El objetivo de este trabajo fue demostrar la presencia de rickettsiosis en humanos provenientes de tres localidades de Panamá, que corresponden a zonas agropecuarias, cercanas a bosques, o que trabajaban en zoológicos.

**Materiales y métodos.** Se escogieron tres localidades para este estudio: Tortí (provincia de Panamá), El Valle de Antón (provincia de Coclé) y el Parque Municipal Summit en Ciudad de Panamá. Los voluntarios firmaron un consentimiento informado, además de responder un cuestionario. De cada voluntario se extrajo sangre venosa, la que fue analizada por medio de inmunofluorescencia indirecta, utilizando kits comerciales y láminas sensibilizadas con antígenos cultivados de *Rickettsia rickettsii* y *Rickettsia amblyommii*.

**Resultados.** Se tomaron muestras de 97 voluntarios, 25 en Tortí, 37 en El Valle de Antón y 35 en el Parque Municipal Summit. De estos, 38 (39 %) de las muestras fueron positivas en algunas de las dos técnicas practicadas: 8 (32 %) en Tortí, 18 (48 %) en El Valle y 12 (34 %) en el Parque Municipal Summit.

#### Author contributions:

Sergio E. Bermúdez: coordinated the activities of the project, analyzed the results and wrote the manuscript.

Cirilo Lyons: coordinated the laboratory activities.

Gleydis García: collaborated in the analysis and the results

Yamitzel Zaldívar: coordinated the blood draw in volunteers.

Amelia Gabster: collaborated in the interpretation of the data and the English translation.

Griselda Arteaga: collaborated in the interpretation of the data.

**Conclusión.** Se demuestra una alta prevalencia de anticuerpos contra *Rickettsia* del grupo de las fiebres manchadas en las tres áreas de estudio, además de presentarse evidencia de títulos para *Rickettsia* del grupo tifus en El Valle de Antón. Estas zonas podrían considerarse como endémicas por rickettsiosis, ya que existen condiciones que permiten el mantenimiento de las mismas.

**Palabras clave:** infecciones por Rickettsiaceae, inmunoglobulina G, brotes de enfermedades, epidemiología, Panamá.

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Rickettsial diseases are a group of zoonosis caused by Gram negative bacteria of the genus *Rickettsia*. These diseases are among the oldest known vector-borne zoonosis infections, and even today are considered important emerging or re-emerging infectious diseases (1). These bacteria are transmitted by ectoparasites, which spread through the saliva of ticks (spotted fever group *Rickettsia*-SFGR) or by infected flea and lice excretions (typhus group *Rickettsia*-TGR) (2). Because the initial symptoms of rickettsial diseases are not specific, they are often confused with other common diseases like dengue, which results in underreporting and unreliable epidemiologic data (3).

The importance of rickettsial diseases has stimulated research on the different rickettsial species that could be considered pathogenic. For example, until the mid of 20th century, the only pathogenic *Rickettsia* that were considered were *R. rickettsii* (SFGR), *R. prowazekii* and *R. typhi* (TGR); nevertheless, now it is known that 16 of the 25 species are human pathogens (4). Of these, eight species might be involved in pathologies of humans in Latin America (5,6).

In Panamá, rickettsial diseases are known since the first half of the last century, when Calero (7) reported an outbreak of 13 cases of murine typhus in Panamá City. Subsequently, from 1950 to 1953, there were five cases of rickettsiosis by *R. rickettsii* in this country, including two mortal cases (8,9). As in other Latin countries, reports of rickettsiosis disappeared from Panamá for over 51 years, and it was not until the first decade of the 21st century, when there were new reports (10,11).

There is no solid epidemiological data of the extent of human rickettsiosis or of the different species that may be involved in human infections in Panamá.

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Therefore, the aim of this paper is to present preliminary results on the presence of rickettsial infectious in human serum from three locations in Panamá.

## Materials and methods

### Study sites

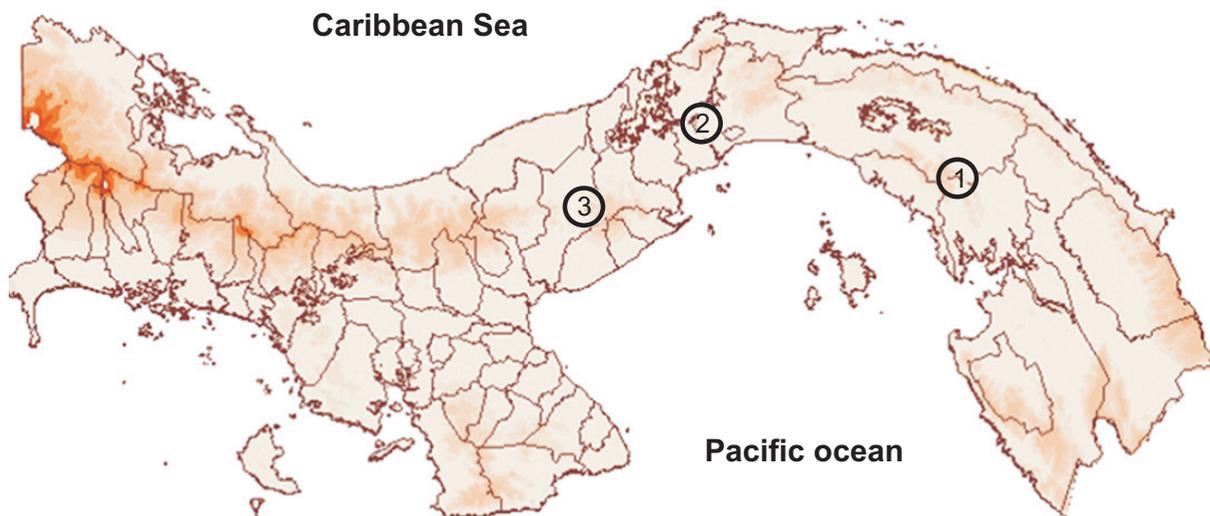
Three sites were chosen for our study, including volunteers from Torti area (provinces of Panamá and Darien), El Valle de Antón (Coclé Province) and workers of the Summit Municipal Park (Panamá Province) (SMP) (figure 1). These locations were chosen because previous reports have shown natural infection of *Rickettsia* within ectoparasites and mammals in these regions.

Torti is a rural community with an economy that is aimed primarily at farming, and it is surrounded by a secondary forest. There is no history of human rickettsial infections in this area. El Valle de Antón is located inside an extinct volcanic crater, at an altitude of 650 meters. This village is surrounded by Natural Reserve Cerro Gaital (1,200 m) and other natural sites that are often visited by locals and tourists. Inhabitants of this village are mainly engaged in agriculture and ecotourism (12). In 2008, a fatal case of spotted fever *R. rickettsii* occurred nearby (11), and as part of the investigation of this case, we obtained serum samples from family members of the deceased and volunteers working at the local zoo, El Níspero.

Summit Municipal Park (SMP) is 15 km from Panamá City and is surrounded by Soberanía National Park. This park consists of a large garden and a zoo with animals representing Panamanian fauna (13,14). We obtained serum samples from garden workers, animal keepers, education personnel, administration and maintenance staff. Unlike the other people from our study, most of the workers of the SMP reside in urban areas (especially in Panamá City).

### Inclusion criteria

As inclusion criteria, the volunteers had to be more than 18 years-old, to live or work at the study sites. Previously, all volunteers had signed



**Figure 1.** Location of study area in Panama: Torti (1), Summit Municipal Park (2), El Valle de Antón (3).

an informed consent, according to the Bio-Ethical Committee standard. At the time of sampling, each volunteer answered a questionnaire about their work (including whether he or she manipulated live animals), the presence of pets at home, history of ectoparasites-bites, and the use of ectoparasites-control measures.

### **Serology**

We extracted approximately 5 ml of venous blood, of which 20  $\mu$ l of serum were used to determine IgG antibodies by indirect immunofluorescence method (IFA), using the Focus Diagnostics  $\text{\textcircled{R}}$  kit (Cypress, USA). For this analysis we followed the protocols recommended by manufacturers. The criteria for a positive result were a seroreaction in dilution of  $\geq 64$ . The slides were read using an ultraviolet microscope (Nikon Eclipse E-400) at 1,000X magnification.

Additionally, we performed IFA using antigens cultivated from Brazilian strains of *R. rickettsii* (Taiacu) and *R. amblyommii* (AC37) from the collection of the Centre for Research in Tropical Diseases, Faculty of Microbiology at the University of Costa Rica. Each serum was diluted in PBS, starting from a 1:64 dilution. Then, ten microliters of diluted sera were added to each well of the antigen slides, and incubated at 37  $^{\circ}$ C for 30 minutes in a humid chamber. The slides were rinsed once, washed twice for 10 minutes per wash in PBS. The slides were incubated with fluorescein isothiocyanate-labeled goat anti-human

IgG, followed the procedures previously described by Labruna, *et al.* (15). Sera reacting at the screening dilution (1:64) were then tested in serial two-fold dilutions to determinate the endpoint titer. To recognize the possible antigens that caused the reaction, we followed the approach proposed by Labruna, *et al.* (15).

### **Results**

We included 97 volunteers in the study: 25 from Torti, 37 from El Valle de Anton, 35 workers from SMP. In total, 38 had positive seroreactions (39%) for one of the two tests performed (commercial antibodies and antigens cultivate). Titers of each locality are showed in tables 2-4. One-hundred percent of the volunteers confirmed having pets, and 41% used control agents against ectoparasites. Findings on awareness of the importance of ectoparasites in public health show that 100% of respondents recognized it as a problem in animal health, and 33% as a human problem. Other responses to questionnaire appear in table 1.

In the village of Torti we analyzed sera of 25 volunteers of which eight (32%) reacted to one of the two tests performed. There were no IgG antibodies responses against TGR (table 2). In the responses to the survey questionnaire, four volunteers said that they were continually exposed to ticks, all had animals at home, and only five applied anti-ectoparasites treatments.

At El Valle de Anton, sera of 37 volunteer were analyzed, of which 18 (48%) reacted to one of the

**Table 1.** Answers to questions by localities

Questions	Tortí	El Valle	SMP
Gender (F/M)	9/16	15/22	13/22
Owner of pets or cattle			
Pets	25	37	35
Cattle	15	2	0
Work with domestic animals			
Horses	20	31	1
Cow	23	27	0
Dogs/Cats	0	2	3
Work with captive animals (Y/N)	0	7	5
Ectoparasites in those animals (Y/N)	11	23	24
Use control for ectoparasites (Y/N)	20	6	10
Ectoparasite on own body (Y/N)	17	15	10
Presence of symptoms after bites (Y/N)			
Fever	0	0	0
Headhache	0	0	0
Spotts on skin	0	0	0
Recognized as ectoparasites as human health problem (Y/N)	5	11	13
Recognized as ectoparasites as animal health problem (Y/N)	20	18	30

**Table 2.** Endpoint of *Rickettsia* antibodies in human sera of Tortí village volunteers (n=25)

No. Volunteer	Commercial kit IgG		Antigens slides		Possible antigen that cause the reaction <sup>1</sup>
	<i>R. rickettsii</i>	<i>R. typhi-prowazekii</i>	<i>R. rickettsii</i>	<i>R. amblyommii</i>	
12	64	-	128	-	<i>R. rickettsii</i>
18	64	-	64	64	SFGR
19	64	-	128	64	SFGR
20	64	-	128	64	SFGR
21	64	-	-	64	SFGR
22	64	-	-	-	SFGR
23	-	-	-	64	SFGR
24	-	-	128	-	SFGR

SFGR: Spotted Fever *Rickettsia* Group

two tests performed. Of these, nine (24%) had IgG antibody responses against SFGR antigens and four samples reacted against TGR (table 3). Importantly, among the nine positives for SFGR, five belonged to the family of the deceased in 2008.

We analyzed the sera of 35 volunteers at the SMP. Twelve (34%) appeared to be positive to one of the two tests performed (table 4). Among the volunteers with positive results, one worked at the greenhouse, three at the nursery, three at the zoo, two worked in education and three in at the department of ground maintenance. Of these, eight said they had pets at home, all had seen ticks at their houses, and only seven used chemicals for tick control.

## Discussion

Our results show a relatively high seroprevalence of human IgG antibodies against *Rickettsia* in

the three study sites. It is important to mention, that the majority of the volunteers (44%) only reacted to endpoint, which could be attributed to past infections. For those samples where only reacted with one of the methods, may be due to cross reactivity with other agents; nevertheless, it is necessary to apply other methods to verify this. Further, the fact that the research was conducted in an area where ectoparasites have been reported infected with *Rickettsia*, reinforces the idea that these bacteria circulating in the environment, and could be into contact with humans through parasitism of infected ectoparasites. Similarly, this study analyzed serum of volunteers at risk of exposure to bites of ectoparasites (farmers and animal keepers at a zoo), which increases the possibility of being infected.

Other research in Latin America, have values of 4% seroreaction: in Jujuy, Argentina (16), 5% in

**Table 3.** Endpoint of *Rickettsia* antibodies in human sera of El Valle village volunteers (n=37).

No. Volunteer	Commercial kit IgG		Antigens slides		Possible antigen that cause the reaction
	<i>R. rickettsii</i>	<i>R. typhi-prowazekii</i>	<i>R. rickettsii</i>	<i>R. amblyommii</i>	
1	64	-	-	-	SFGR
2	64	-	128	-	<i>R. rickettsii</i>
3	64	-	128	256	SFGR
4	-	-	512	-	<i>R. rickettsii</i>
5	-	-	128	-	<i>R. rickettsii</i>
7	64	-	-	128	SFGR
8	-	-	64	64	SFGR
11	-	-	64	-	SFGR
12	64	-	128	-	<i>R. rickettsii</i>
14	-	64	-	-	TGR
15	-	64	-	-	TGR
17	64	-	128	-	<i>R. rickettsii</i>
23	-	64	-	-	TGR
26	64	-	-	-	SFGR
27	64	-	128	-	<i>R. rickettsii</i>
28	64	-	64	-	<i>R. rickettsii</i>
30	64	-	-	-	SFGR
31	-	64	-	-	TGR

SFRG: Spotted Fever *Rickettsia* Group; TGR: Typhus *Rickettsia* Group

**Table 4.** Endpoint of *Rickettsia* antibodies in human sera of Summit Municipal Park volunteers (n=35)

No. Volunteer	Commercial Kit IgG		Antigens slides		Possible antigen that cause the reaction
	<i>R. rickettsii</i>	<i>R. typhi-prowazekii</i>	<i>R. rickettsii</i>	<i>R. amblyommii</i>	
3	64	-	64	-	<i>R. rickettsii</i>
5	64	-	-	128	SFGR
7	64	-	-	-	SFGR
8	-	-	128	-	SFGR
14	64	-	-	-	SFGR
15	-	-	-	128	SFGR
18	64	-	128	128	SFGR
21	-	-	64	-	SFGR
22	-	-	128	128	SFGR
30	64	-	256	-	<i>R. rickettsii</i>
32	64	-	64	-	<i>R. rickettsii</i>
33	64	-	-	-	SFGR

SFRG: Spotted Fever *Rickettsia* Group

Yucatán, México (17), 4.2% in an endemic area for spotted fever, São Paulo, Brazil (18), while Hidalgo, *et al.*, (19) show 40.3% seropositivity in an endemic area for SFGR in Cundinamarca, Colombia. Other studies in São Paulo have a positive rate between 14.3% (20) and 28% (21). Recently, Hidalgo, *et al.*, (22) found a high mortality rate (36%) associated with SFGR an outbreak from Córdoba, Colombia, and explained that the high fatality rate might be the result of treatment failures, as the exact agent of the infectious disease is often not known.

With regard to the high prevalence among Torti volunteers, it is possible that they carry out farming

activities that put them at risk of being parasitized by infected ticks. There are previous records of *R. amblyommii* infection in the ticks *Amblyomma cajennense* s.l., *Rhipicephalus sanguineus* s.l., and *Dermacentor nitens*, in this area (23). This suggests that the ecological conditions that allow for human contact with infected ticks are present.

A similar explanation could apply to the inhabitants of El Valle de Anton, who not only maintain close contact with domestic animals infested with ectoparasites, but also frequent the wilderness areas surrounding the village. This would also explain the positive TGR with the commercial kit.

Rickettsial species within the TGR are known to be present in Panamá since a murine typhus outbreak in the 30's of the last century (7). However, there are no recent reports of *R. typhi* in humans, animals or fleas in Panamá, although this might be the result of a lack of directed studies. It has previously been demonstrated that *R. amblyommii* circulates in ticks from dogs and horses, while *R. felis* circulates in dog fleas and that sera from both horses and dogs were positive to rickettsial infection (12). This fact, combined with the high seroprevalence of *R. rickettsii* and the recent fatal case of rickettsiosis, indicates that SFGR might be endemic to this area. Further evidence for the TGR is needed.

With respect to volunteers working in the SMP, they not only showed exposure to ticks in the park, but also claimed to have pets at home. At least 17 species of ticks have recently been reported for this park, including species that can potentially parasitize humans (13,14).

Most importantly, the majority of the people who filled out questionnaires did not consider ticks and other ectoparasites as vectors of pathogens for human beings, and considered them only as transmitters of diseases in dogs, horses or cattle. Therefore, adequate dissemination of information could raise awareness among people about the consequences of not keeping their animals and living areas tick-free.

Similarly, health authorities should seek more recent information about detection techniques in suspected cases of rickettsiosis. This idea is reinforced by the knowledge that some techniques could yield false positive or nonspecific reactions, making it desirable to include other diagnostic methods (24). Recently, Spolindorio, *et al.*, (6) reported a case of rickettsiosis caused by a possible new agent, using serological and molecular tests, and demonstrated the importance of using multiple methods to detect these agents.

Finally, this study shows that rickettsial pathogens circulate among people who are in close contact with domestic and zoo animals and wildlife in Panamá.

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### Conflict of interests

This manuscript was written with the participation of all authors. We declare that there is no conflict of interest in the results presented in this article.

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