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Seroprevalence of *Rickettsia typhi* and *Rickettsia conorii* infections in the Canary Islands (Spain)

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SUMMARY

Objective: The aim of this work was to evaluate the prevalence of past infection due to *Rickettsia typhi* and *Rickettsia conorii* in the Canary Islands (Spain).
Methods: A representative sample of the population of the seven islands, formed of 662 people aged between 5 and 75 years (368 females, 294 males), was analyzed. Epidemiological data were obtained by direct survey. The detection of serum IgG antibodies against both microorganisms was based on an indirect immunofluorescence test, considered positive if the titers were $\geq$1/80.
Results: Of the analyzed population 3.9% had IgG antibodies against *R. typhi* and 4.4% against *R. conorii*. Out of these positive samples, only three were positive for both species. The seroprevalence was similar in both sexes. Positive results were found in all age groups, but a higher rate was noticed in those aged 46 years and older ($p < 0.05$). *R. typhi* was found to be more prevalent in rural areas of all islands, as well as in farmers.
Conclusions: Our results confirm the presence of antibodies against the causative agents of murine typhus and Mediterranean spotted fever in the Canary Islands. Indirect data suggest that the detection of antibodies to *R. conorii* might be due to a cross-reaction between these species.

1. Introduction

Infections caused by different species of the genus *Rickettsia* are a broad group of zoonoses with a worldwide distribution. However, depending on the species and the vectors involved in their transmission, the incidence of these infections varies significantly in different geographical areas.

Murine or endemic typhus is a zoonosis caused by *Rickettsia typhi*.[1] The classic biological cycle of *R. typhi* infection includes rats (*Rattus rattus* and *Rattus norvegicus*) as a reservoir and the oriental rat flea (*Xenopsylla cheopis*) as a vector. The usual way of transmission is percutaneous inoculation of the microorganisms present in flea feces. However, this classic cycle seems to have been replaced in some regions by the peri-domestic animal cycle (urban and suburban outbreaks) involving cats, dogs, opossums and their fleas (*Ctenocephalides felis*[2]). Inhalation of desiccated flea feces has also been described as an additional mechanism of transmission.[3] The disease usually presents as an acute febrile illness (7–28 days of duration) associated with headache and a skin rash.[4] Its distribution is worldwide, with endemic areas in the southern areas of the USA, South America, Australia, Southeast Asia, and Southern Europe.[5,6] The disease also occurs in Spain, as some reports of sporadic cases[7–12] and a few clinical series[13–16] have shown. In addition, some epidemiological studies in central,[17,18] northeastern,[19] and southern[20–22] Spain have shown a seroprevalence of *R. typhi* ranging between 3.8% and 18%.

Mediterranean spotted fever caused by *Rickettsia conorii* is endemic in some areas of Spain[17,23] and other Mediterranean countries, as well as in different areas of Asia and Africa. The disease is transmitted to humans by the bite of the brown dog tick, *Rhipicephalus sanguineus*, and usually manifests itself as a febrile illness with headaches, arthromyalgia, skin rash, and a characteristic black eschar ('tache noire') at the inoculation site.

Data on the seroprevalence of antibodies against the causative agents of both infections in the Spanish population are scarce, and no such study has ever been conducted in the Canary Islands. The aim of our study was to analyze the seroprevalence and epidemiology of *R. typhi* and *R. conorii* in the Canary Islands.

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2. Subjects and methods

2.1. Characteristics of the geographic area

The Canary Islands are an archipelago of seven islands of volcanic origin in the Atlantic Ocean, located around latitude 27°–29° North and 14°–18° West. There are great differences between the islands with regard to their size and altitude (between 600 and 3700 m), which determine a great climate variability. The presence of trade winds and the actual relief of the islands of Fuerteventura and Lanzarote result in an arid climate, whereas the rest of the islands have different ecosystems.

The area in this study comprised the seven Canary Islands. The estimated population in 1996 was 1 606 549 people (50.4% female, 49.6% male), resulting in a population density of 219 people per square kilometer. The distribution by age was as follows: 20.6% younger than 16 years; 27.7% between 16 and 30 years; 22.5% between 31 and 45 years; 18.7% between 46 and 65 years; and 10.5% older than 65 years. The two main islands, Gran Canaria and Tenerife, account for 85.5% of the population.

2.2. Study method

We analyzed a representative sample of the population by obtaining sera associated with the 1998 National Survey on Nutrition[24] and preserving it at −70 °C until testing was performed. Informed consent was obtained from each subject, so that the serum could be used for our study. The study universe was constituted of the entire population aged between 5 and 75 years. The base population comprised every single person registered in the census. A two-stage sampling by conglomerates was performed, with the municipality (county) being the primary

Table 1
Cases of positive IgG titers (≥1/80) for *Rickettsia typhi* and/or *Rickettsia conorii* obtained from a representative sample (*N* = 662) of the general population on the Canary Islands (Spain)

Case	Age (years)	Sex (M/F)	Island	*R. typhi* (IgG)	*R. conorii* (IgG)
1	58	M	La Gomera	1/2560	≤1/40
2	61	F	La Palma	1/2560	≤1/40
3	46	F	Lanzarote	1/640	≤1/40
4	61	F	Tenerife	1/640	≤1/40
5	58	M	La Gomera	1/640	≤1/40
6	39	F	La Gomera	1/640	≤1/40
7	54	M	Tenerife	1/640	≤1/40
8	68	F	Gran Canaria	1/320	≤1/40
9	21	M	Tenerife	1/320	1/80
10	74	M	La Palma	1/320	≤1/40
11	15	F	El Hierro	1/320	≤1/40
12	70	M	La Palma	1/320	≤1/40
13	64	F	La Gomera	1/320	≤1/40
14	58	M	Fuerteventura	1/160	≤1/40
15	40	F	La Gomera	1/160	≤1/40
16	57	F	La Gomera	1/160	≤1/40
17	15	F	El Hierro	1/160	1/80
18	75	F	El Hierro	1/160	≤1/40
19	14	F	Gran Canaria	1/80	1/80
20	35	F	Gran Canaria	1/80	≤1/40
21	63	M	Tenerife	1/80	≤1/40
22	74	M	La Gomera	1/80	≤1/40
23	55	M	La Gomera	1/80	≤1/40
24	75	M	El Hierro	1/80	≤1/40
25	20	M	La Gomera	1/80	≤1/40
26	58	M	Gran Canaria	1/80	≤1/40
27	65	M	Fuerteventura	≤1/40	1/160
28	59	M	Gran Canaria	≤1/40	1/80
29	69	M	Gran Canaria	≤1/40	1/80
30	68	M	Gran Canaria	≤1/40	1/160
31	47	F	Gran Canaria	≤1/40	1/80
32	55	M	Tenerife	≤1/40	1/80
33	63	M	Tenerife	≤1/40	1/160
34	62	M	La Gomera	≤1/40	1/80
35	8	F	La Palma	≤1/40	1/80
36	54	F	La Palma	≤1/40	1/80
37	69	F	La Palma	≤1/40	1/160
38	8	M	El Hierro	≤1/40	1/80
39	51	F	Tenerife	≤1/40	1/80
40	59	M	Tenerife	≤1/40	1/160
41	69	M	Tenerife	≤1/40	1/80
42	14	F	Tenerife	≤1/40	1/160
43	44	F	Gran Canaria	≤1/40	1/80
44	53	F	Tenerife	≤1/40	1/320
45	64	F	Fuerteventura	≤1/40	1/80
46	63	F	Gran Canaria	≤1/40	1/80
47	61	F	Gran Canaria	≤1/40	1/80
48	44	M	Gran Canaria	≤1/40	1/80
49	63	M	El Hierro	≤1/40	1/80
50	31	M	Gran Canaria	≤1/40	1/80
51	26	M	Gran Canaria	≤1/40	1/80
52	66	M	Gran Canaria	≤1/40	1/80

IgG, immunoglobulin G; M, male; F, female.

unit of the sampling and the individuals registered in it, the secondary unit. A total of 662 sera were tested, 368 from females and 294 from males.

The following epidemiological data were recorded for each person: age, sex, profession or occupation, socioeconomic status (low, medium, high), and place (village, island) and type of residence (rural: <10 000 inhabitants, semi-urban: between 10 000 and 100 000, and urban: >100 000 inhabitants).

2.3. Serological technique

Human serum samples were evaluated by indirect immunofluorescence assay (IFA) with commercially available antigens for *R. typhi* and *R. conorii* (*Rickettsia mooseri*- Spot IF, *Rickettsia conorii*- Spot IF, BioMérieux, Marcy l'Etoile, France). The antigens were obtained from cultures on Vero cells and then fixed on the slides. Titers of immunoglobulin G (IgG) ≥1/80 were considered positive (indicative of past infection). We also evaluated the presence of immunoglobulin M (IgM) antibodies against both species of Rickettsia. According to the manufacturer's specifications, the sensitivity and specificity of these tests are over 90% for both antigens.

2.4. Statistical analysis

Biostatistical analysis was performed using SPSS version 10.0 for Windows statistical package (SPSS, Chicago, IL, USA). The Chi-square test was performed to establish associations between the different variables taken into consideration and the Student's *t*-test for the average comparison of normal values. A *p*-value of <0.05 was considered significant.

3. Results

The seroprevalence (IgG) of *R. typhi* infection was 3.9% (26 positive samples) and the seroprevalence (IgG) of *R. conorii* infection was 4.4% (29 positive samples). The highest titer of antibodies found against *R. conorii* was 1/320 (only one sample), while seven positive samples for *R. typhi* presented higher titers, two of them reaching a value of 1/2560 (Table 1). Only three serum samples had antibodies against both rickettsial species. In these samples the titers against *R. conorii* reached a value of 1/80, while those against *R. typhi* were: 1/80, 1/160, and 1/320 in each case. None of the 662 subjects studied had IgM antibodies against both species of Rickettsia.

Table 2
Epidemiological data for subjects with serological evidence of past infection due to *Rickettsia typhi* and/or *Rickettsia conorii*. Data are expressed as number (*n*) and percentage (%) of positive cases.

	IgG against *R. typhi* ≥1:80		IgG against *R. conorii* ≥1:80	
	n	%	*n*	%
Sex				
Male	14	53.8	16	55.2
Female	12	46.2	13	44.8
Age (years)				
<16	3	11.5	5	17.2
16–30	2	7.7	2	6.9
31–45	3	11.5	3	10.3
46–65	12	46.2	14	48.3
>65	6	23.1	5	17.2
Residence				
Rural	14	53.8	9	31.0
Semi-urban	6	23.1	7	24.1
Urban	6	23.1	13	44.8
Socioeconomic status				
High	6	23.1	9	31.0
Medium	12	46.2	11	37.9
Low	8	30.7	9	31.0
Island				
Gran Canaria	4	15.4	12	41.4
Fuerteventura	1	3.8	2	6.9
Lanzarote	1	3.8	0	0
Tenerife	4	15.4	8	27.6
La Palma	3	11.5	3	10.3
La Gomera	9	34.6	1	3.4
El Hierro	4	15.4	3	10.3

IgG, immunoglobulin G.

Seroprevalence rates for both diseases in relation to sex, age, type of residency, socioeconomic status, and occupation are shown in Table 2. The rates were similar for males and females. Positive titers were found in all age groups, but were significantly higher in people aged 46 years or older ($p < 0.05$). In terms of residence pattern, the seroprevalence of murine typhus was higher in rural

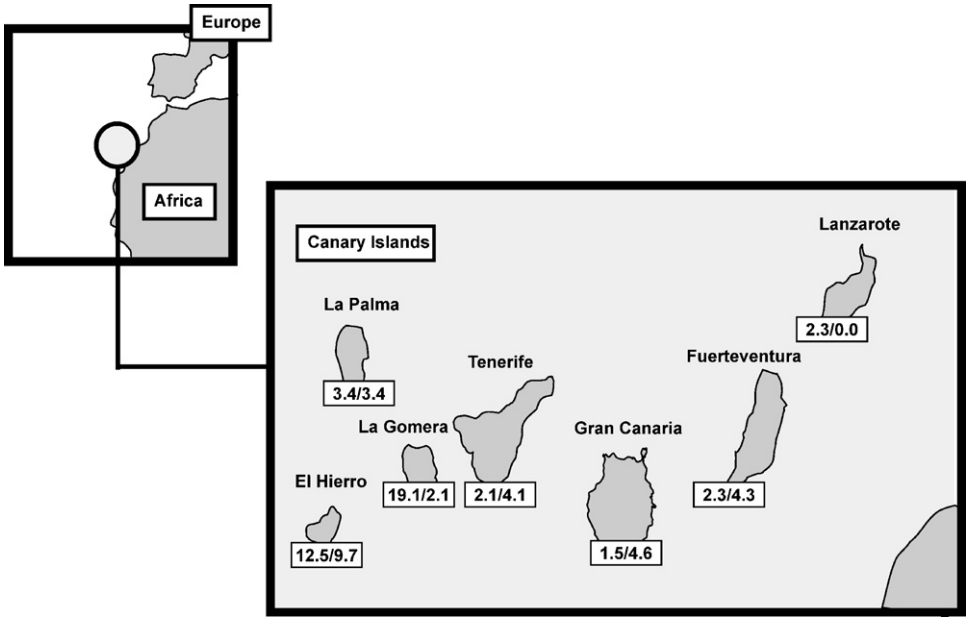

Figure 1. Seropositivity rates for *Rickettsia typhi*/*Rickettsia conorii* on each of the seven Canary Islands, expressed as percentages.

than in urban areas (53.8% vs. 23.1%; $p < 0.05$). On the other hand, positive immunofluorescent antibody test results for *R. conorii* were more prevalent in urban than in rural areas (44.8% vs. 31.0%), although this difference was not statistically significant. In terms of professional occupation, an association with farming was found only for *R. typhi* (11.9%; $p < 0.05$).

The geographical distribution (Figure 1) shows that *R. typhi* is present on all seven Canary Islands, with the highest prevalence rates being on the islands of El Hierro and La Gomera (12.5% and 19.1%; $p < 0.05$). Positive titers against *R. conorii* were not found in individuals from the island of Lanzarote, but were found from the other six islands, with the highest rate being on El Hierro (9.7%).

4. Discussion

Mediterranean spotted fever is a well-known disease in Spain; some endemic areas exist, and cases are clustered in the spring and summer. However, murine typhus, very frequent after the Spanish Civil War in the 1940 s, has became a virtually forgotten disease in the last decades. Information on the prevalence and geographical distribution of these two infections is scarce and extrapolated from clinical series.

As opposed to Mediterranean spotted fever, murine typhus is not considered endemic in any Spanish region, and data on the seroprevalence in the healthy population are even scarcer. In our study, we found that 3.9% of the studied subjects had titers equal to or greater than 1/80 against *R. typhi* and 4.4% subjects were seropositive for *R. conorii*. Although a similar seropositivity could be interpreted as a cross-reaction between *R. typhi* and *R. conorii*,[17,23,25] the analysis of the positive cases (Table 1) makes this interpretation unlikely. In fact, antibodies to both species of rickettsiae were only detected in three subjects, and at low titers. In addition, samples with higher titers against *R. typhi* were negative for *R. conorii*.

The seroprevalence to *R. typhi* in the Canary Islands (3.9%) is notably lower than that recorded by Ruiz-Beltrán et al.[17] in Salamanca (northwest region of the Spanish Iberian peninsula; 12.8%), but higher than that reported by García-Curiel et al. (1984)[21] in Seville (southwest Spain; 0.6–2% for people aged ≥10 years). Interestingly, no clinical cases of murine typhus have been reported in Salamanca and, conversely, the largest series in Spain has been published in Seville, with 104 cases being reported in 17 years, accounting for 6.7% of all patients admitted to the hospital with fever of an intermediate duration in that area.[14] In the previously mentioned study performed in Salamanca,[17] there were no statistically significant differences observed concerning the seropositivity with regard to age, sex, habitat, geographic area, or occupation. Our data present several differences, mainly in terms of geographical distribution, occupation, and age. Thus, the presence of antibodies against *R. typhi* was associated with a rural residence and farming. In addition, the seropositivity was higher as the age of the studied individuals increased. However, the rate in children under 16 years of age was 2.3%. Data reported from several studies suggest that children are equally susceptible to infection, but diagnosis is frequently missed.[26–28]

Until recently, the description of cases of murine typhus from the Canary Islands was anecdotal.[12] However in recent years, two series of cases have been published in Tenerife[15] and Gran Canaria.[16] Moreover, in this last series, the patients showed some clinical differences (i.e., renal involvement) in comparison to those reported in other geographical areas.

With regard to the presence of antibodies against *R. conorii* in the Canary Islands, we found an overall prevalence of 4.4%. This rate is lower than those reported from other Spanish regions in which the disease is endemic.[17,21,29] However, some areas that are not considered endemic such as the province of Soria,[30] show a prevalence rate similar to ours.

An important aspect to note is that while autochthonous cases of murine typhus have been described from the Canary Islands, no case of Mediterranean spotted fever has yet been reported. Although the seroprevalence rate and the IgG titers are low, there is the suspicion of a possible cross-reaction between *R. conorii* and other spotted fever group rickettsiae (mainly *Rickettsia felis* and *Rickettsia massiliae*).[31] In fact for *R. felis*, a flea-transmitted Rickettsia causing a murine typhus-like illness: (1) it can be transmitted by the same vectors,[32] (2) the cross-reactivity with spotted fever group rickettsiae has been demonstrated,[31] and (3) several cases of human infection by *R. felis* have been recorded in the Canary Islands.[33] Moreover, Fernández de Mera et al. described the finding of *R. massiliae* in *Rhipicephalus pusillus* ticks from Gran Canaria,[34] a Rickettsia that has been recognized as a human tick-borne spotted fever group Rickettsia.[35] Therefore, we do think that the detection of antibodies against *R. conorii* represents a cross-reaction against other rickettsiae of the spotted fever group.

Finally, when analyzing the distribution of cases with antibodies to *R. conorii*, the highest prevalence was observed in urban areas, a fact reported previously in France[36] and in our country as well.[37] This change in the epidemiological pattern of the infection consists of a shift from rural towards urban.

In summary, our results confirm the presence of antibodies against the agents of murine typhus and Mediterranean spotted fever in the Canary Islands. Taking into account that no autochthonous cases of Mediterranean spotted fever have been reported from the Canary Islands and the description by our group of other species of Rickettsia (*R. felis*, *R. massiliae*), it is possible that the detection of antibodies to *R. conorii* represents a cross-reaction between these species.

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