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ARTICLE

Seroepidemiological study of *Rickettsia felis*, *Rickettsia typhi*, and *Rickettsia conorii* infection among the population of southern Spain

M. Bernabeu-Wittel · M. D. del Toro · M. M. Nogueras · M. A. Muniain · N. Cardeñosa · F. J. Márquez · F. Segura · J. Pachón

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Abstract *Rickettsia typhi* and *Rickettsia conorii*, the etiologic agents of, respectively, murine typhus and Mediterranean spotted fever, are recognized as frequent causes of fever of intermediate duration in southern Spain; in addition, in recent years *Rickettsia felis* has been detected in potential vectors in this area. Nevertheless, limited data exist regarding the actual prevalence of past infection due to these three pathogens. In the present study, the prevalence of past infection due to *R. felis*, *R. typhi*, and *R. conorii* was determined in a representative population of southern Spain during 2002. In addition, the possible risk factors associated with exposure to these pathogens were investigated. An epidemiological survey was completed by all subjects included in the study. Serum samples were tested by indirect immunofluorescence assay. The prevalence of past infection due to *R. felis*, *R. typhi*, and *R. conorii* among the 504 total subjects was 6.5, 3.8 and 8.7%, respectively. In multivariate analysis, infection due to *R. felis* was independently associated with a high-risk occupation (one that required working outdoors in nature, close contact with domestic animals, or potential contact with rodents) (OR=5.8; 95%CI 2.1–15.6), while infection due to *R. typhi* was associated with older age (factor of 1.04 [95% CI 1.008–1.068]) and frequent insect bites (OR=10.3; 95% CI 2.3–45.5). Two factors were associated with infection due to *R. conorii*: a high-risk occupation (OR=9.3; 95%CI 3.7–23.2), and participation in outdoor activities (OR=7.2; 95%CI 1.4–38.5). The results confirm the widespread prevalence of past infection due to *R. felis*, *R. typhi*, and *R. conorii* in the population of southern Spain.

M. Bernabeu-Wittel (✉) · J. Pachón
Department of Infectious Diseases,
Hospitales Universitarios Virgen del Rocío,
Avenida Manuel Siurot s/n,
41013 Seville, Spain
e-mail: MAXBW@telefonica.net

M. D. del Toro · M. A. Muniain
Department of Infectious Diseases,
Hospital Universitarios Virgen Macarena,
Avenida Dr. Fedriani s/n,
41009 Seville, Spain

M. M. Nogueras · N. Cardeñosa · F. Segura
Infectious Diseases Program, Corporació Sanitaria
Parc Taulí,
c/Parc Taulí s/n,
08208 Sabadell, Barcelona, Spain

F. J. Márquez
Faculty of Biology, University of Jaén,
Paraje de las Lagunillas s/n,
23071 Jaen, Spain

Present address:
M. Bernabeu-Wittel
Department of Internal Medicine,
Hospitales Universitarios Virgen del Rocío,
Avenida Manuel Siurot s/n,
41013 Seville, Spain

Introduction

The presence of rickettsial disease in humans in southern Europe, specifically in Spain, has been known for decades. Of the different types of rickettsial disease present in Spain, Mediterranean spotted fever has been the most frequent, with cases described virtually throughout the entire country [1, 2]. In recent years, murine typhus (MT) has been also recognized as a frequent cause of fever of intermediate duration (FID) in the south of Spain, causing up to 7% of cases [3]. Despite this fact, seroepidemiological population-based studies of *Rickettsia conorii* and *Rickettsia typhi* infection in Spain are scarce and have been conducted in the central and northern provinces of Spain, where

Fig. 1 Geographic location of Seville, in southwestern Spain

prevalences of past infection due to *R. conorii* and *R. typhi* are about 10.5% and 6.8–12.5%, respectively [4–6]. However, the prevalence of past infection in the areas of Spain where MT represents a health problem, i.e. the southern regions, has not been determined.

In addition, *Rickettsia felis*, a newly discovered species of the spotted fever group [7, 8], has been detected in some areas where MT is endemic [9–11]. *R. felis* can be transmitted from host to host (peridomestic mammals) by the cat flea (*Ctnedocephalides felis*). Proven to be pathogenic, it causes a clinical syndrome very similar to that seen in MT [12–14]. The geographical as well as climatic conditions in our area are similar to those in which *R. felis* has been detected. In fact, our team detected a high rate of infections with *R. felis* in cat fleas collected in rural areas of southern Spain [15]. Nevertheless, no human cases of infection have been demonstrated in these zones, hence the impact and prevalence of this rickettsial pathogen are unknown.

The aim of the present study was to analyze the prevalence of past infection with *R. felis*, *R. typhi*, and *R. conorii* in a representative population of southern Spain in order to determine (a) the geographic distribution of these rickettsial pathogens and (b) the possible risk factors associated with exposure to these pathogens.

Materials and methods

Geographical area and study population

The geographical area encompassed the city of Seville and the northern, western, and southern areas of the province of Seville (total population of these areas: 1,194,570). The province of Seville is a 14,000-km^2 area in southwestern Spain with 1,758,720 inhabitants (65% of whom live in urban areas). Its main city is Seville, with 705,000 inhabitants (Fig. 1). The climate is mild Mediterranean, with a mean temperature of 19.6°C (range, 12.9–26.3) and a mean rainfall of 327 l/m^2/year. The topography is divided into cultivated agricultural land (66%), woods (19%), pastures (7.5%), and urban areas (7.5%).

Sample size and stratification

The sample size was established for an estimated seroprevalence of *R. typhi* of 5±2.5% (99% confidence level) in 504 subjects. The Statistical Annuary of Andalusia (2001 census) [16] was used to prestratify subjects according to age (0–14 years [*n*=110], 15–29 years [*n*=135], 30–44 years [*n*=98], 45–64 years [*n*=101], and >64 years [*n*=60]) and type of residential community (>50,000 inhabitants=urban [*n*=310]; 5,000–50,000 inhabitants=suburban [*n*=157]; and <5,000 inhabitants=rural [*n*=37]).

Inclusion criteria and sample collection

With the exception of the youngest age group (0–14 years), subjects were recruited by random telephone solicitation between 1 January 2002 and 30 June 2002. Subjects in the age group 0–14 years were preselected in our two central laboratories, which receive blood samples from the entire region (from primary care clinics, outpatient clinics, and hospitals). From this preselected group, we identified samples that originated in primary care and preanesthesic outpatient clinics (in order to avoid the selection of hospitalized, chronically ill, or immunosuppressed children). We then obtained, by telephone, informed consent from the legal guardians of the children to use the remaining serum for our study purposes. Once informed consent was obtained, a serum sample was collected and an epidemiological survey was distributed to all subjects included in the study (data collected included gender, age, work in a high-risk occupation, type of residential community, participation in outdoor activities, travel within the last 12 months, contact with animals, and insect bites within the last month). High-risk occupations were those that required close contact with animals (farmers, livestock breeders, slaughterhouse workers, veterinarians), outdoor work in natural areas (rangers, foresters), or work in areas likely to harbor rodents (e.g., sewage systems). Subjects unable to answer the epidemiological survey, those hospitalized in the previous 30 days, those with febrile disease in the previous 30 days, and those with any kind of immunosuppression were excluded.

Serological technique

Serum samples were tested by indirect immunofluorescence assay (IFA) using commercially available antigens for *R.*

Table 1 Prevalence of past infection due to *Rickettsia felis*, *Rickettsia typhi*, and *Rickettsia conorii* in the population of the province and city of Seville in 2002, according to age and area of residence

	R. felis	*R. typhi*	*R. conorii*
Age in years			
0–14 (*n*=110)	2 (1.8%)	1 (0.9%)	1 (0.9%)
15–29 (*n*=135)	7 (5.2%)	2 (1.5%)	14 (10.4%)
30–44 (*n*=98)	9 (9.2%)	2 (2%)	12 (12.2%)
45–64 (*n*=101)	13 (12.9%)	5 (5%)	9 (9%)
≥65 (*n*=60)	2 (3.4%)	9 (15%)	8 (13.3%)
Total (*n*=504)	33 (6.5%)	19 (3.8%)	44 (8.7%)
Type of residential community[a]			
Urban (*n*=310)	21 (6.8%)	14 (4.5%)	30 (9.7%)
Suburban (*n*=157)	10 (6.4%)	5 (3.2%)	10 (6.4%)
Rural (*n*=37)	2 (5.4%)	0	4 (10.8%)

[a] Rural: <5,000 inhabitants; suburban: 5,001–50,000 inhabitants; urban: >50,000 inhabitants

typhi (ref no. IF0100; Focus Technologies, Cypress, CA, USA) and *R. conorii* (ref. no. 75901; bioMérieux, Marcy l'Toile, France). *R. felis* antigen was kindly provided by the Unité de Rickettsies, Marseilles, France. It was deposited onto slides, air dried, and fixed with acetone. Twofold dilutions of human sera were applied to the antigens. The slides were incubated in a humidified chamber at 37°C for 30 min. After washes in PBS-Tween and water to remove unbound immunoglobulins, binding sera was detected by using fluorescein isothiocyanate-labelled goat anti-human IgG (bioMérieux). Slides were incubated and washed as described above and were examined with a fluorescence microscope at ×400. Endpoint titers were obtained by serial dilution on positive specimens, with titers 1/64 or higher considered indicative of past infection.

Statistical analysis

Seroprevalence was determined globally, by age, and by type of residential community. Values were described as means and standard deviations. A univariate analysis was performed to determine possible risk factors for past infection. Univariate group comparisons were performed using chi-square and Fisher's exact tests, and correlations using Spearman's rho test. Multivariate analysis using a forward stepwise logistic regression model was performed for factors found to have significant differences in the univariate analysis. Group differences were determined by odds ratios (ORs) and 95% confidence intervals (CIs). A p value of <0.05 was considered significant.

Results

A total of 504 subjects were included in the study. The global prevalence of past infection with *R. felis*, *R. typhi*, and *R. conorii* was 6.5% (33 positive samples), 3.8% (19 positive samples), and 8.7% (44 positive samples), respectively. Geometric mean antibody titers of samples positive for *R. felis*, *R. typhi*, and *R. conorii* were 88 (range, 64–256), 89 (range, 64–256), and 64 (range, 64–256), respectively. Cross-reactions between *R. felis* and *R. typhi* were present in the sera of two subjects (one with an IgG titer of 1/128 and 1/64, respectively, and one with a titer of 1/64 against both rickettsia); between *R. felis* and *R. conorii* in five subjects (2 with an IgG titer of 1/64 against both rickettsia, 1 with a titer of 1/128 against both rickettsia, one with a titer of 1/128 and 1/64, respectively, and one with a titer of 1/64 and 1/256, respectively); and between *R. typhi* and *R. conorii* in two subjects (both with an IgG titer of 1/64 against both rickettsia).

The prevalence of past infection according to age group and type of residential community is detailed in Table 1. The subjects with past infection due to *R. felis* were younger than those with past infection due to *R. typhi* (42±18 vs. 59±2 years; p=0.01) but similar to those with past infection due to *R. conorii* (42±18 vs. 42±21 years). A significant positive correlation was found between age and past infection with any *Rickettsia* species (Spearman's rho=0.11, p=0.01, with respect to *R. felis*; 0.1, p=0.02, with respect to *R. typhi*; and 0.2, p<0.0001, with respect to *R. conorii*). The highest prevalence of past infection due to *R. felis* (9–12%) was detected in subjects aged 30–64 years, while the highest prevalence due to *R. typhi* (15%) was found in subjects aged >65 years. The highest rates of past infection due to *R. conorii* (9–13.3%) were more homogenously distributed in subjects ≥15 years of age. No differences were detected when the prevalence of past infection was analyzed according to the type of residential community.

The geographic distribution of past infections in the province and city is detailed in Fig. 2**a**–**c**. Briefly, in the province of Seville, past infections due to *R. felis* were distributed mainly in the northern and western areas, while those due to *R. typhi* were found mainly in suburban areas

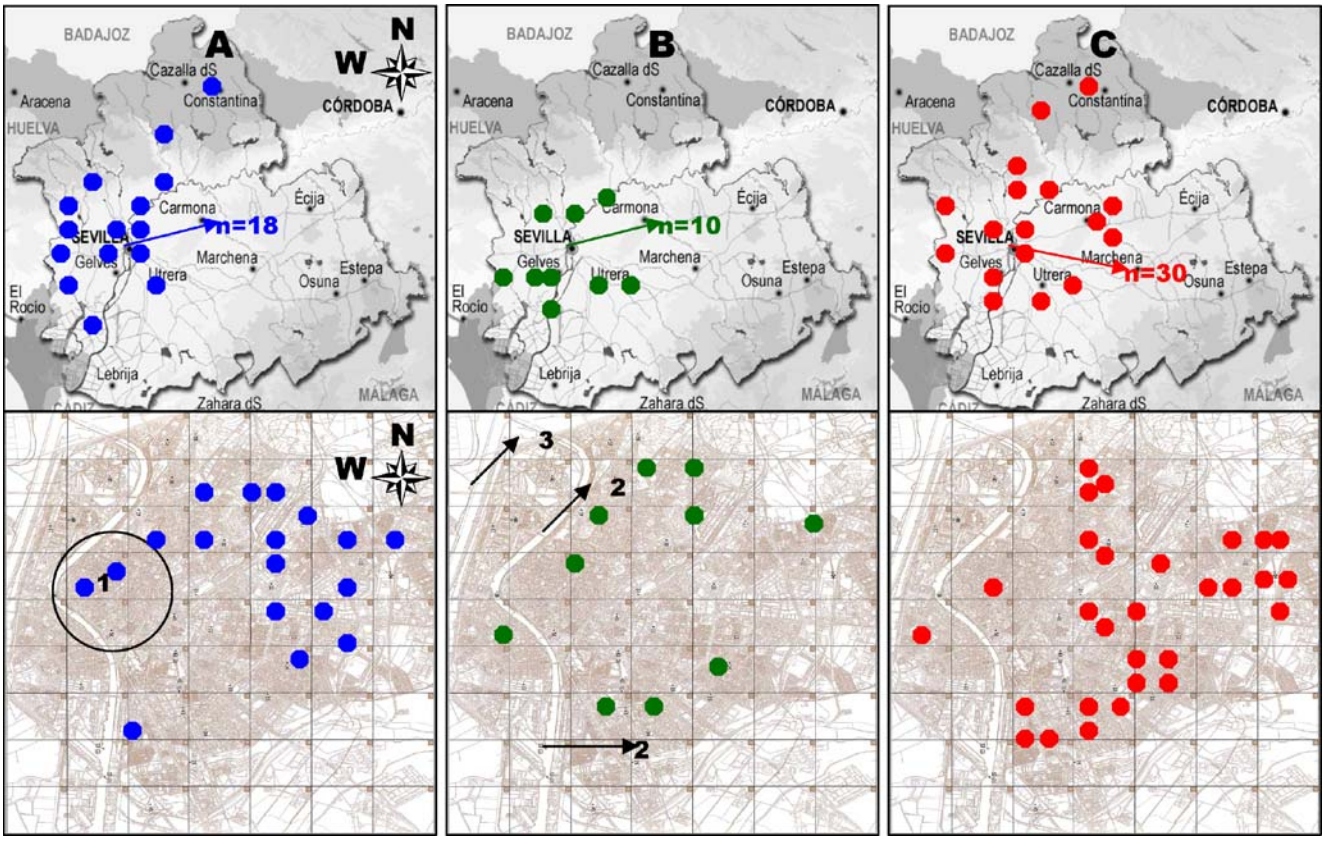

Fig. 2 **a** Geographic distribution of past infections (positive samples) due to *Rickettsia felis* (*black dots*) in the province (*upper image*) (*n* refers to *positive samples* in the city of Seville) and in the city (*lower image*) of Seville, 2002. "1" indicates the historic center of the city. **b** Geographic distribution of past infections (*positive samples*) due to *Rickettsia typhi* (*blue dots*) in the province (*upper image*) (*n* refers to positive samples in the city of Seville) and in the city (*lower image*) of Seville, 2002. "2" indicates the old course of the Guadalquivir River crossing the center of the city. "3" indicates the new course of the Guadalquivir River; this deviation was performed to avoid the frequent floods of the river (annual water flow of 3,362 hm^3/year). **c** Geographic distribution of past infections (*positive samples*) due to *Rickettsia conorii* (*red dots*) in the province (*upper image*) (*n* refers to *positive samples* in the city of Seville) and in the city (*lower image*) of Seville, 2002

near the city of Seville. Past infections due to *R. conorii* were practically homogenously distributed throughout the study area. In the city of Seville, the older areas of the city were almost free of rickettsial past infections; instead, past infections with the three species studied were distributed predominantly in the peripheral areas, and mainly in the northern, eastern, and southern districts.

The characteristics of subjects with past infection due to *R. felis*, *R. typhi*, and *R. conorii* are detailed in Table 2. Subjects infected with *R. felis* as well as those infected with *R. conorii* were more frequently in contact with animals than those infected with *R. typhi* (p=0.013; OR=12, 95%CI 1.4–111 and p=0.009; OR=11, 95%CI 1.3–98, respectively). No other differences in epidemiological characteristics were detected between the three groups.

The details of univariate analysis to detect possible risk factors associated with past infection due to any of the three rickettsial pathogens studied are shown in Table 3. Briefly, there were no differences related to sex, participation in outdoor activities, or recent travel. Although many subjects reported frequent contact with animals, past infection due to *R. felis* was not associated with this variable, while infection due to *R. typhi* and *R. conorii* was more frequent when, respectively, contact with cats and contact with different kinds of animals was reported. Curiously, almost no one reported having seen rodents near their home or in their workplace; however, infection due to *R. typhi* was associated with the awareness of an insect bite in the previous month. Finally, high-risk occupations were associated with past infection due to *R. felis* and *R. conorii*.

In multivariate analysis, the only factor independently associated with past infection due to *R. felis* was a high-risk occupation (p=0.001; OR=5.8, 95%CI 2.1–15.6). Factors associated with *R. typhi* infection were older age (p=0.013; factor of 1.04, 95%CI 1.008–1.068) and the awareness of insect bites within the last month (p=0.02; OR=10.3, 95% CI 2.3–45.5). The factors associated with *R. conorii* infection were a high-risk occupation (p<0.001; OR=9.3, 95%CI 3.7–23.2) and, in subjects who resided in urban areas, participation in outdoor activities (p=0.021; OR=7.2, 95%CI 1.4–38.5).

Table 2 Epidemiological characteristics of subjects with past infection due to *Rickettsia felis*, *Rickettsia typhi*, and *Rickettsia conorii* in Seville, 2002

Characteristic	Subjects with past infection		
	R. felis (*n*=31)	*R. typhi* (*n*=19)	*R. conorii* (*n*=44)
Sex (M/F)	16/15	10/9	17/27
Contact with animals	41%	21.1%	49%
Dogs	19.4%	5.3%	25.7%
Cats	0%	10.5%	0%
Rodents	0%	0%	0%
Other[a]	6.5%	0%	0%
Various types[b]	15.1%	5.3%	23.3%
Parasites in pets/animals	20%	10.5%	9.3%
Fleas	6.5%	5.3%	2.3%
Ticks	3.2%	5.2%	2.3%
Various types[c]	10.3%	0%	4.7%
High-risk occupation	42%	26.3%	44%
Outdoor activity	6.5%	5.3%	7%
Travel within last 12 months	39%	16%	30%
Insect bites within last month	10%	22%	4.7%

[a] Birds, chickens, horses, cattle, etc
[b] Subject reported contact with various species of different animals
[c] Includes fleas and ticks as well as other types of parasites

Table 3 Univariate analysis of risk factors associated with the prevalence of past infection due to *Rickettsia felis*, *Rickettsia typhi*, and *Rickettsia conorii* in the population of Seville, 2002. Significant differences are indicated in bold type

Risk factor	Prevalence of past infection (%)		
	R. felis	*R. typhi*	*R. conorii*
Sex (M/F)	6.5/5.8	4.1/3.5	6.5/10.4
Contact with animals (no/yes)	6.3/6	5.2/2	8/9.8
Dogs	6.3/5.5	5.2/1.5	7.6/10.1
Cats	6/0.9	**5/13 (*p*=0.052)**	7.6/1.3
Rodents	6.3/0.9	5.2/0	7.6/1
Various types[a]	6.3/5	2.7/5.2	**7.5/15 (*p*=0.06)**
Parasites in pets/animals (no/yes)	5.5/13.5	3.8/7	9/7
Fleas	5/14.5	3.8/7.1	8.7/7.1
Ticks	5.6/6.3	3.8/6.3	8/7
High-risk occupation (no/yes)	**5.1/18 (*p*=0.001)**	4.7/6.9	**8.3/26 (*p*<0.001)**
Outdoor activity (no/yes)	6/11	4/0.9	8/17
Travel within last 12 months (no/yes)	5.7/7.1	4.8/1.5	9/7.6
Insect bites within last month (no/yes)	6.7/6.2	**2/9 (*p*=0.045)**	5/9.2

[a] Subject reported contact with various species of different animals

Discussion

Our study confirms the widespread distribution of *R. felis*, *R. typhi*, and *R. conorii* in areas of southern Europe, reflected in the moderate-to-high prevalence of past infection due to these agents in a representative sample of the general population. Of the three rickettsia studied, *R. conorii* was the most prevalent, indicating the endemic and deep distribution of its reservoirs and vectors in our environment. This finding adds to previous data about its known distribution in other areas of Spain [2, 4]. The prevalence of 8.7% was higher than that found in other Mediterranean countries [17, 18] but lower than that found in Central Africa or Asia [19, 20].

MT, endemic in southern Spain, is associated with specific clinical and epidemiological characteristics and continues to be a significant cause of FID in the community [3]; in this sense, the moderate prevalence of past infection due to *R. typhi* detected in this study also shows the persistence of efficient life-cycle elements that enable *R. typhi* to remain viable and potentially transmissible to humans in our urban and suburban areas. Other authors in Spain have found even higher prevalence rates of human infections due to *R. typhi* [5], and the few studies carried out in reservoirs of this pathogen also showed high seroprevalence rates in wild rodents as well as in domestic and peridomestic dogs [21, 22]. This last finding may be of pivotal epidemiological interest because of the newly described life-and-transmission cycle of *R. typhi*, in which other peridomestic mammals (e.g., opossums, and, possibly, cats and dogs) may play the role of reservoirs, with the cat flea acting as vector [9, 23]. This newly described *R. typhi* life cycle could significantly expand the human population at risk, since the number of mammalian pets is in the millions, and the cat flea bites humans more avidly than other species of fleas [9, 23]. In fact, in a previous study by our group, only 19% of patients with MT had seen rodents near their home or workplace [3], and, in the present study, none of the subjects with past infection due to *R. typhi* reported previous contact with rodents. Approximately 15%, however, reported contact with either dogs or cats.

The most striking result of our study is the relatively high prevalence of past infection due to *R. felis* in our area (6.5%). Since its description, *R. felis* has been increasingly detected in cat fleas in areas where MT is endemic [7, 10, 11]; in fact, in the area of the present study, our group recently detected the presence of *R. felis* in *C. felis* collected

from cats and dogs of suburban and rural counties [15]. The human disease caused by *R. felis* has been scarcely studied, probably because its recent discovery, the lack of commercially available and accurate diagnostic tests (most cases described were diagnosed by means of DNA-based techniques, which are routinely available in only certain reference laboratories), and its relatively unspecific clinical picture, which in most cases closely mimics that of MT [7, 12–14]. Hence, there are no extensive studies analyzing the clinical and epidemiological features of *R. felis* disease in humans, and the actual prevalence of past infection due to *R. felis* has not been evaluated; in this sense, ours is the first study that confirms the widespread prevalence of *R. felis* in an area where MT is endemic. Some authors have correlated the prevalence of *R. felis* past infection in potential reservoirs (opossums) with the occurrence of cases of MT in endemic areas [24]; this, together with our data (evidence of past infection due to *R. felis* in the general population), raises the hypothesis of *R. felis* being the cause of an undetermined number of cases of FID, or even the cause of a number of cases diagnosed as MT. In fact, in a previous prospective study evaluating FID, nearly 19% of 926 patients with FID remained without a specific diagnosis after extensive diagnostic testing [3]. Thus, future clinical studies focusing on *R. felis* infection are needed to elucidate this issue.

The use of an appropriate cutoff titer is a critical issue in seroprevalence studies, and this titer should be established (and should vary) according to the incidence of disease and the distribution of the etiologic agent. For this reason, in a previous study, we evaluated the IFA antibody titers against *R. typhi* in 100 sera from healthy controls. We found negative IFA titers in 83 sera, IgG titers of 1/64 in 14 sera, and IgG titers of 1/128 in 3 sera [3]. On the basis of these results, in our area we consider a diagnosis of acute MT to be confirmed when a compatible clinical picture is present together with a single titer of ≥1/512 or when a fourfold increase in titers is detected between acute and convalescent sera, while a past infection is diagnosed when we find single titers below 1/512. With respect to *R. conorii*, there is a general consensus in Spain, where *R. conorii* infection is the most prevalent rickettsiosis, to classify infections as acute when a compatible clinical picture is present together with a single titer of ≥1/512, or when a fourfold increase in titers is detected between acute and convalescent sera (IFA IgG); past infections are diagnosed when single titers below 1/512 are found [1, 2, 4]. Finally, with respect to *R. felis*, there are insufficient data on cutoff titers for acute and past infections (in fact, ours is one of the first studies to address this issue), so we applied the same cutoff points used for *R. typhi* and *R. conorii* infection after evaluating the global epidemiology of rickettsiosis in our area. This strict approach prevented us from diagnosing false-positive infections and, in our opinion, had no impact on the estimation of prevalence of past infections.

The distribution of past infections according to type of residential community and age reflected the local living patterns and sociocultural characteristics of the population studied. A common feature of the three pathogens was their distribution in the peripheral suburbs of the city, probably due to the location of open spaces and green zones in which peridomestic reservoirs are more likely to live. In addition, a relationship between age and progressively higher infection rates due to *R. typhi* and *R. conorii* was found, attributed to the longer exposure time experienced by older people; this feature has also been noted by other authors [17].

Particular features of past infection with *R. felis* were its higher prevalence in the median ages of life (30–64 years), its lower prevalence in the older and younger ages, and its independent association with high-risk occupations. These features probably reflect changes in sociocultural behaviour over the last 20–30 years as well as changes in the ecology of this zoonosis (perhaps related to the high numbers of domestic pets), which may have allowed *R. felis* to thrive in urban, suburban, and rural environments, leading to the subsequent widespread distribution of this rickesttsial pathogen. In fact, studies performed in different countries have found that approximately 20–30% of the cat fleas collected from domestic and peridomestic cats and dogs were infected with *R. felis* [25–27]. As a consequence, many authors now consider *R. felis* a paradigm of an emergent pathogen [9, 23, 28].

In contrast, *R. typhi* infection was characterized by its presence only in urban and suburban areas, its increasing prevalence in older ages, with older age being an independent risk factor, and its independent association with an awareness of insect bites. These data also reveal key local epidemiological features of *R. typhi*. For example, the absence of infections in rural areas probably reflects the fact that reservoirs and vectors have been maintained in urban and suburban zones and have not spread *R. typhi* to rural counties. In addition, the narrow correlation with older age may reflect improvements in social and hygiene conditions over the last 20–30 years (e.g., increase in per-capita income, coupled with public health measures such as the extensive campaigns of rat control carried out by local authorities between 1984 and 1986, which were a pivotal factor in the decrease of MT cases in the following years [3]), which implies a decrease in the population at risk compared to two or three decades ago. Finally, the association with insect bites may be an indirect indicator of the poor social conditions of subjects with past infection due to *R. typhi*, as pointed out by other authors [4–6]. The fact that none of the infected subjects reported contact with rodents requires further evaluation of alternative elements in the *R. typhi* life cycle (peridomestic mammals and cat flea), as commented above; nevertheless, if this alternative cycle existed, the epidemiology of *R. typhi* should resemble that of *R. felis*, and this is not supported by our data.

Finally, the particular features of past infection due to *R. conorii* (practically homogenous distribution according to type of residential community and age, except for the youngest group; correlation with contact with animals; and independent association with high-risk occupations and outdoor activity) clearly indicate how this pathogen affects the human population in southern Spain. The wide and homogenous distribution in practically all ages and residential areas probably reflects the broad and persistent distribution of endemic foci of infected reservoirs and vectors in this area, with the result being that most inhabitants contract *R. conorii* infection early in their lives, making this *Rickettsia* species the most prevalent one in southern Spain. On the other hand, its association with high-risk occupations and outdoor activity are classical predisposing factors also encountered in other studies [4, 17–20].

In conclusion, our data confirm the presence and widespread distribution of *R. felis*, *R. typhi*, and *R. conorii* past infections in the population of southern Europe. Past infection due to *R. felis* and *R. conorii* was associated with high-risk occupations, while that due to *R. typhi* correlated with age and frequent insect bites.

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