SHORT REPORT: SEROLOGICAL EVIDENCE OF INFECTION WITH RICKETTSIA TYPHI AND RICKETTSIA FELIS AMONG THE HUMAN POPULATION OF CATALONIA, IN THE NORTHEAST OF SPAIN

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Abstract. Murine typhus (MT) is a cause of fever of intermediate duration in the south of Spain. Rickettsia typhi has been described as the MT etiological agent. Rickettsia felis produces an infection similar to MT. The aim of the study is to determine their seroprevalence in humans in Catalonia. Antibodies to Rickettsia typhi and Rickettsia felis from 217 serum samples were examined by indirect immunofluorescence assay (IFA). Age, gender, residence area, contact with animals, and occupation were surveyed. Rickettsia typhi and Rickettsia felis seroprevalences were 8.8% and 3.2%, respectively. Rickettsia typhi was present in 7.6% of the samples in urban, 8.5% in semirural, and in 21.4% in rural areas, whereas Rickettsia felis was present in 3.5% in urban, 1.7% in semirural, and 7.1% in rural area. The only statistically significant association observed was that between Rickettsia felis seropositivity and age. Our data seem to indicate the presence of Rickettsia typhi and Rickettsia felis in humans in Catalonia.

INTRODUCTION

Murine typhus (MT) is a rickettsial infectious disease transmitted to humans by fleas. Although it is often acute and mild, MT could sometimes be fatal. MT severity has been associated with old age and delayed diagnosis.¹ This disease is one of the most widely distributed arthropod-borne infections, endemic in many coastal areas and ports throughout the world.² During the past years, sporadic outbreaks have been reported.³,⁴ In addition, MT has been related to the international traveler.⁵ However, MT is often unrecognized because of unspecific clinical symptoms and the lack of specific diagnosed techniques.⁶

Rickettsia typhi is the etiological agent of MT. Until recent years, MT was associated with the presence of rats and their fleas.² Nevertheless, murine typhus could occur in places where these are absent. Thus, the classic rat-flea-rat cycle seems to have been replaced in some regions by the peridomestic animal cycle involving cats, dogs, opossums, and their fleas.⁶

Rickettsia felis was first identified as a human pathogen in 1994. It may produce a clinical syndrome similar to MT.⁷ Their pathogenic role for humans has been demonstrated in cases clinically compatible with MT in different places.⁸,⁹ The first case of Rickettsia felis infection in Europe was reported in 2002.¹⁰ Rickettsia felis has already been detected in fleas and mammals from different countries.¹¹–¹⁵

MT has been long known in Spain. During the past years, murine clinical cases have increased in Spain. In Seville, MT was an important cause of fever of intermediate duration,¹⁶ and some cases have been reported in the Canary Islands.¹⁷ However, only three studies were published about the human seroprevalence of Rickettsia typhi in Spain.¹⁸–²⁰ On the other hand, Rickettsia felis has been identified in Ctenocephalides felis from Cadiz in 2002.²¹ Our previous data indicates the presence of human antibodies reactive with Rickettsia felis in Seville.²²

Because Rickettsia typhi seems to be widely distributed throughout Spain and Rickettsia felis may be involved in cases clinically compatible with MT, the aim of the current study is to determine the seroprevalence of Rickettsia typhi and Rickettsia felis in the population of the northeast of Spain. In addition, their distribution according to several demographic variables will be evaluated. The results may be indicative of possible risk factors associated with exposure as well as possible transmission cycles.

MATERIALS AND METHODS

Geographical area. The study was undertaken in Vallés Occidental (Catalonia), a predominantly urban area near the coast in the northeast of Spain. A total of 11 municipalities (356,266 inhabitants) participated in the study.

Samples. Serum samples from 217 patients who had attended at Sabadell’s Hospital were collected for the survey. The collection of samples took place during a 5-month period, from September to January. The sample includes adults undergoing minor surgery and children cared for in the Pediatrics Emergency Service.

Taking into account the actual population of Vallés Occidental, the study population was stratified by age (0–14 years, 15–29 years, 30–44 years, 45–64 years, ≥ 65 years) and by residential area: rural, semirural, and urban. Like other seroprevalence studies published, the residential area was determined considering the number of inhabitants who live in the municipalities. In fact, municipalities with < 5,000 inhabitants were included in rural area group, municipalities with 5,000 to 50,000 inhabitants were considered semirural areas, and municipalities with > 50,000 inhabitants were regarded as urban areas. This objective criterion allows comparisons between different seroprevalence studies.

Informed consent was obtained from all adult participants and from parents or legal guardians of minors. Each patient

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filled out a questionnaire in which the following variables were registered: age, gender, place of residence, contact with wild animals, contact with farm animals, contact with domestic animals, and occupation. Those inhabitants unable to answer the epidemiologic survey were excluded.

Blood samples were collected into tubes containing heparin. The sample of heparinized blood was sedimented and the supernatant was collected and stored at −80°C. A serological survey was carried out according to the ethical guidelines of the ethics committee of Sabadell Hospital.

Of the 217 subjects, 118 were men and 99 women. The mean age was 34.36 years (0–91 years). Cases were reported by 11 towns, and 144 (66.4%), 59 (27.2%), and 14 (6.5%) subjects lived in urban, semirural, and rural areas, respectively. In the group of 161 adults, there were 11 (6.8%) students, 27 (16.8%) retired, 37 (23%) housewives, 53 (32.9%) workers, and 26 (16.1%) unemployed. In seven people the occupation was unknown.

**Sero logical technique.** Human serum samples were evaluated by indirect immunofluorescence assay (IFA). We used a commercial antigen (MRL Diagnostics, Cypress, CA) to determine antibodies to *Rickettsia typhi*. *Rickettsia felis* antigen was kindly obtained from the Unité de Rickettsies, Marseille, France. Antigens were applied to 10-well microscope slides (BioMérieux, Marcy l’Etoile, France) with a pen nib. After air drying, they were fixed with acetone for 10 minutes at room temperature. Twofold dilutions of human serum specimens in phosphate-buffered saline (PBS)–3% nonfat dry milk were applied to the antigens. The slides were incubated in a humidified chamber at 37°C for 30 minutes. After two 10-minute washes in PBS-Tween and one 5-minute wash in purified water to remove unbound immunoglobulins, slides were air dried. Binding sera were detected by using fluorescein isothiocyanate–labeled goat anti-human IgG (BioMérieux) diluted 1:100 in PBS containing Evans blue. The slides were incubated and washed as described above. Dried slides were mounted with buffered glycerol (pH 8.0) and examined with a fluorescence microscope at 400×. The highest dilution, at which distinct and specific fluorescence was seen, was scored as the end-point titer for the serum sample. Titers ≥ 1/40 were considered positive.

**Statistical analysis.** The software application SPSS was used. Seroprevalence was determined globally and by residence areas. A univariate analysis was performed to determine possible risk factors. Univariate group comparisons were performed using χ² and Fisher exact test. Group differences were determined by odds ratio (OR) and confidence intervals of 95%. Quantitative variables were compared by means of Student t test. Multivariate analysis was performed using a forward stepwise logistic regression model, for factors with significantly differences among groups, in univariate analysis. A P < 0.05 was considered significant.

**RESULTS**

Considering titers ≥ 1/40 as positive, the seroprevalence of *Rickettsia typhi* infection in humans was 8.8% and the seroprevalence of *Rickettsia felis* infection was 3.2%. The relationships between the rickettsial antibody positive rate and the surveyed items are shown in Table 1.

Nineteen samples had antibodies against *Rickettsia typhi*. Eight of them had an IgG titer of 1/40, seven a titer of 1/80, and four a titer of 1/160. Seven serum samples were found with antibodies reactive with *Rickettsia felis*; four, two, and one at IgG titers of 1/40, 1/80, and 1/160, respectively.

The *Rickettsia typhi* seroprevalence were 7.6% in urban areas, 8.5% in semirural areas, and 21.4% in rural areas. Mean ages of subjects with presence or not of antibodies to *Rickettsia typhi* were 41.92 (1–78 years) and 33.63 years (0–91 years), respectively. There were no significant differences in the rates of antibodies to *Rickettsia typhi* related to any of the items of surveyed information.

The *Rickettsia felis* seroprevalence were 3.5% in urban areas, 1.7% in semirural areas, and 7.1% in rural areas. The only statistically significant association observed was that between *Rickettsia felis* seropositivity and age. The mean age of seropositive subjects was 61 years (42–82 years) and the mean age of seronegative subjects was 33.47 years (0–91 years) ($P = 0.001$). When age was stratified, there was a statistical difference in oldest category ($P = 0.039$, OR = 6.102 (1.3–29.1)). The study of risk factors in different living area showed that age was only significant in urban area ($P = 0.003$).

Only one serum sample presented antibodies against both rickettsial species at the same titers (1/40). The subject was a 49-year-old housewife who lived in an urban area.

**DISCUSSION**

This study represents the first evidence, by immunofluorescence assay, of antibodies against *Rickettsia typhi* and *Rickettsia felis* in the northeast of Spain. *Rickettsia typhi* is described as the murine typhus (MT) etiological agent. On the
other hand, human infections with *Rickettsia felis* appear to be widespread and may produce a clinical syndrome similar to MT. 7

MT occurs worldwide, particularly in warm and humid climates. In Seville, it is an important cause of fever of intermediate duration. 16 Also, there are some cases reported from Tenerife (Canary Islands). 17 Although MT has been long known in our country, there are few epidemiologic studies. *Rickettsia typhi* human seroprevalence has been described in the south (Seville) 18 and in central Spain (Salamanca), 19 Madrid 20. In addition to the latter studies, the presence of human antibodies to *Rickettsia typhi* in the northeast of Spain suggests that *Rickettsia typhi* could be widely distributed in our country.

Like other countries, 23 geographical seroprevalence is not homogeneous in Spain. Seropositives rates differ from one another due to the different epidemiologic and climatic environments. Highest seroprevalence have been found in the central and the northeast regions, being more urbanized. In fact, *Rickettsia typhi* seroprevalence in our population is 8.8%. Positive sera titers ranged from 1/40 to 1/160 and 5.5% samples had titers ≥ 1/80. This data (8.8%) is coherent with the data obtained in central Spain: Madrid (6.8%) and Salamanca (12.8%). On the contrary, our result is higher than those obtained from Seville (2%).

Approximating others study, 19.24 a significant correlation between *Rickettsia typhi* seropositivity and any of the items of surveyed information has not been obtained.

So far, murine typhus has been described as a disease of mostly urban or port areas, where humans and rats share the same habitat. 2 According to our data, seroprevalence in urban area is not the highest seroprevalence rate. Similar results have been observed in Madrid, 20 suggesting a widespread presence of *Rickettsia typhi* in rural areas. Human behavior modification as well as environmental changes may be allowing the expansion of *Rickettsia typhi* into rural and semirural areas through the peridomestic cycle involving cats, dogs, peridomestic rodents, and their fleas. 6

Moreover, studies carried out in Spain showed elevated *Rickettsia typhi* seropositive rates in both dogs and rodents. 25,26 In both studies, there were no differences between seropositive animals from rural or urban areas. There was no significant difference with regard to contact with animals in our survey, probably owing to the small number of people who reported contact with animals.

Considering the presence of antibodies in the human population, our data are a preliminary point of view about *Rickettsia typhi* infection in our region. Further studies are necessary to identify reservoirs as well as *Rickettsia typhi* spread. *Rickettsia felis* was identified in Cadiz fleas in 2002 for the first time in Eurasia. 21 Considering *Rickettsia felis* may produce a clinical syndrome similar to murine typhus, 7–9 it could also be implicated in cases clinically compatible with murine typhus in Spain. In fact, in 1999, a Seville study 16 showed that approximately 19% of patients with fever of intermediate duration remained without a specific diagnosis after wide diagnostic tests. *Rickettsia felis* seroprevalence in our region is 3.2%, which is quite lower than the 6.5% found in our previous study in Seville. 22 Catalonia titers ranged from 1/40 to 1/160, and 1.38% of samples had titers ≥ 1/80.

Although there were no significant differences in rates of antibodies to *Rickettsia felis* related to contact with animals, seroprevalence of individuals that reported contact with domestic animals tends to be higher. This observation agrees with those reporting the presence of *Rickettsia felis* in domestic and peridomestic animals and their fleas. 10,11,13,21 Nevertheless, the small number of samples with antibodies reactive with *Rickettsia felis* does not allow to establish a statistical relationship between seropositive rates and contact with animals nor other items surveyed.

The only statistically significant association observed was that between *Rickettsia felis* seropositivity and age. *Rickettsia felis* seropositivity was significantly more prevalent in subjects older than 65 years than in younger people. The significant increase in antibody prevalence to *Rickettsia felis* in older people could be due to the opportunity for infections to increase with time.

Our data agrees that *Rickettsia felis* has low cross-reaction with *Rickettsia typhi*. In fact, only one sample is seropositive to both *Rickettsia typhi* and *Rickettsia felis* at same titer. In our previous study (Bernabeu and others, unpublished data), the same results were observed.

The most frequent rickettsiosis in Europe, Mediterranean spotted fever, is endemic in Catalonia, and *Rickettsia conorii* and Bar29 are present in this zone. 27 Our results seem to indicate the presence of antibodies against *Rickettsia typhi* and *Rickettsia felis*, in addition to the presence of those against *Rickettsia conorii* and Bar29. *Rickettsia felis*, *Rickettsia conorii*, and Bar29 are classified in the spotted fever group, and some cross-reaction could be possible among them. In our previous study (Bernabeu and others, unpublished data), in which antibodies reactive with *Rickettsia felis*, *Rickettsia typhi*, *Rickettsia conorii*, and Bar29 were surveyed, 66.66% (22 of 33) of sera with antibodies against *Rickettsia felis* did not present cross-reaction with either *Rickettsia conorii* or Bar29. According to these results, although cross-reactions could be possible, presence of antibodies to *Rickettsia felis* in the population of the northeast of Spain might be suspected. However, additional studies are required to evaluate etiology of reaction as well as to determine the actual *Rickettsia felis* seropositive rate in the northeast of Spain. In fact, serology for *Rickettsia conorii* and Bar29 plus Western blot and cross-adsorption analysis will be considered in further studies.

On the other hand, *Rickettsia typhi* is classified into the typhus group. Besides, our previous study (Bernabeu and others, unpublished data) shows very low cross-reactions between *Rickettsia typhi* and *Rickettsia conorii* (1 of 19) and no cross-reactions with Bar29. In the same way, the Salamanca survey 19 shows Rickettsia typhi seropositivity cannot be interpreted totally as a cross-reactivity with *Rickettsia conorii*. Therefore, our results would agree with the actual *Rickettsia typhi* seroprevalence in Catalonia.

In conclusion, we present first serological evidence of *Rickettsia typhi* infection in the human population of the northeast of Spain. *Rickettsia typhi* seroprevalence is similar to other urban regions of Spain. Moreover, our data seem to show *Rickettsia felis* might also be present in this zone. Nevertheless, two facts must be considered: first, *Rickettsia felis* seroprevalence obtained is low; second, some cross-reaction could be possible among *Rickettsia felis*, *Rickettsia conorii*, and Bar29. Therefore, further studies should be necessary to confirm *Rickettsia felis* infection in Catalonia. As cross-reaction between *Rickettsia typhi* and *Rickettsia felis* is low, it is required to include both antigens in serological testing.
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