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ORIGINAL ARTICLE

Seroprevalence and risk factors associated with rickettsiosis *(Rickettsia rickettsii)* in humans in Baja California, Mexico

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Abstract

Introduction: *In Mexico, rickettsiosis, caused by R. rickettsii (obligate intracellular bacterium), is transmitted by Rhipicephalus sanguineus and is able to infect vertebrates, including human beings. Symptoms are high fever, headache, myalgia, nausea, vomiting, abdominal pain and cough. Mortality can be as high as 30% in untreated patients.* **Aims:** *To demonstrate the existence of rickettsiosis in Ensenada, Baja California (Mexico), since there are no cases reported in human beings.* **Materials and methods:** *This prospective, descriptive, cross-sectional study was conducted between October 2009 and August 2011, and included 384 samples of patients older than 1 year of age from Ensenada (Baja California). Antibodies against R. rickettsii were measured with the R. ricketsii ELISA® Helica kit (Biosystems Inc.), for use in dogs and adapted for humans using a anti-human immunoglobilin G conjugate (anti-IgG). To determine sensitivity and specifity in humans, 32 samples were subjected to immunofluorescence assays (IFA). Specific primers were used for molecular diagnosis of R. rickettsii in dogs and ticks.* **Results:** *Adjusted seroprevalence of rickettsiosis by R. rickettsii in humans was 3.9% (95% confidence interval [CI]: 0.8-5.3) and seropositivity showed no association with sex, age, occupation and mobility of dogs between home and the street.* **Conclusions:** *From the κ result of substantial agreement between the enzyme-linked immunosorbant assay (ELISA) and IFA, it is inferred that the seroprevalence results of this work are reliable.* (Gac Med Mex. 2015;151:38-42)

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Introduction

Rickettsioses, diseases caused by bacteria of the *Rickettsia* genus, are a serious public health problem world wide, because they can affect broad sectors of the population, they are difficult to diagnose and outcomes are fatal when there is no early therapeutic intervention[1,2,8]. There are 24 identified rickettsia species, out of which 16 cause rickettsial diseases. Most of these bacteria are associated with ticks, which are both their vectors and reservoirs, but some are transmitted by lice, fleas or mites; therefore, geographic distribution of these infections depends on the geographic distribution of their vectors[3,6,5,9]. Rickettsias are divided in two groups: the spotted fever group and the typhus group, according to several characteristics. In the spotted fever group, ticks are mainly associated as vectors *(R. rickettsii, R. japonica, R. conorii)* and transmit the disease transovarially (to their entire progeny) and transstadially (at any stage), but also

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fleas *(R. felis)* and mites *(R. akari)* do[5,17,21]. The typhus group is associated with lice *(R. prowasekii)* and fleas *(R. typhi),* which have mammals as reservoirs[22,24,25]. It is hard to establish which of these bacterias is the most pathogenic for humans, but *R. rickettsii* is the one that causes more deaths per year[1,27,29]. Rocky Mountains spotted fever is caused by *R. rickettsii,* an obligate intracellular bacterium[11,14]. These species are associated with bacteria-associated arthropods and are capable of infecting vertebrates, including humans, generally as accidental hosts. These organisms are short, cocco-basillary in shape, Gram-negative, 0.8-2.0 µm long and with 0.3-0.5 µm in diameter[2]. In the U.S.A., the most important vectors for this disease are *Dermacentor andersoni* in the Rocky Mountains and *D. variabilis* in the East, the Atlantic Coast and the West Coast. In Mexico, the most important vectors are *R. sanguineus* and *Amblyomma americanum,* same as in Central and South America[2].

Based on the above, the following objective was proposed: to estimate the seroprevalence of rickettsiosis *(R. ricketsii)* in human beings and to measure its association with risk factors in Ensenada (Baja California, Mexico).

Material and methods

Study design

This prospective, descriptive, cross-sectional study included 384 samples of patients older than 1 year of age attending to one of the three private diagnostic laboratories of the Ensenada (Baja California) urban area that took part in the study. Analyses of the samples were carried out in the Veterinary Public Health Laboratory at the Institute of Veterinary Research and Sciences of the Universidad Autónoma de Baja California.

Ethical considerations and inclusion criteria

This study complied with the research and ethics guidelines established in the Declaration of Helsinki (1964), revised in Tokio (1975), Venezia (1983) and Hong Kong (1989). Patients older than 1 year, from both sexes, that attended to the clinical diagnostic laboratory for any reason participated in the study. The patients were required to have an informed consent letter from a parent, or legal guardian when the case required so.

Sample size calculation

The sample was collected randomly, with no replacements. The p-value was included within 1.96 $(p\,[1-p]/n)^{1/2}$ for π; thus, the sample size (n) was obtained by applying the formula:

$$n = \pi\,(1 - \pi)\,\left[\frac{z}{d}\right]^{2}\ {}^{13}$$

Where: n = sample size (384); π = 50% maximum variance estimator; z = 1.96; and d = 5%.

Sample taking

The procedure for extraction, processing, identification and storage of blood samples was carried out as follows: at least 2 ml of blood were collected in 3 ml empty test tubes without ethylenediaminetetraacetic acid (EDTA), intended for serum testing. The sample was taken by radioulnar venipuncture, after antisepsis of the area with isopropyl alcohol. The collected blood samples were identified with numbers corresponding to the questionnaire applied to each suject; those contained in tubes without EDTA were centrifuged at 3,500 rpm for 10 min in order to obtain the serum for the ELISA and IFA testing. The serum obtained from each sample was kept in 1.5 ml-capacity containers, identified and stored at –20 °C until the moment of serologic testing.

Serologic testing

The samples were collected by personnel of the corresponding clinical laboratory. Antibodies against *R. rickettsii* were measured with the *R. rickettsii* ELISA® kit (Helica Biosystems, Inc.), which detects and semi-quantifies IgG in dogs with sensitivity and specificity values of 99.5 and 96%, respectively. This kit was adapted to be used in humans by using an anti-human IgG conjugate at a 1:40,000 dilution. In order to determine sensitivity and specificity in humans, 32 samples were subjceted to IFA, since this technique is the gold standard with regard to serologic diagnosis, and the κ statistic was used to measure agreement between IFA and ELISA. Interpretation of the κ result was based on established criteria[14].

The employed dilution of blood serum was 1:100 with phosphate buffer solution (PBS). The cutoff value was obtained with the average of 20 sera of clinically healthy subjects from the region without a previous

history of clinical signs consistent with rickettsiosis and tick bites plus two standard deviations[15]; according to this, the cutoff value was 0.569 optical density. Two sera were used as positive controls: one that was declared to be positive by the Parasitology Laboratory of the Department of Preventive Veterinary Medicine and Animal Health of the University of São Paulo and the other was donated by ISESALUD and declared positive by the Institute of Epidemiological Diagnosis & Reference (INDRE – *Instituto de Diagnóstico y Referencia Epidemioógicos).*

Sera that tested positive to ELISA were tested with IFA; additionally 20 of the seronegative ones were included. The IFA plates were produced in the Parasitology Laboratory of the Department of Preventive Veterinary Medicine and Animal Health of the University of São Paulo.

Risk factors and clinical signs

To assess risk factors in this study, a questionnaire was applied to the subjects at the moment the blood sample was taken.

Statistical analysis

A database was designed with the Excel program (Microsoft) for the capture and management of information generated in the project. The database was organized in two tables with the following information: serologic analyses results in humans and epidemiological questionnaire results.

The seroprevalence value was generated as follows:

$$\text{Prevalence} = \frac{\text{number of positive results}}{\text{total number in the group}} \times 100$$

Adjusted prevalence was generated by applying the previously described equation[17].

The hypotheses to be proved were the following:

1. The generated seroprevalence value was equal to or different from 0.
2. The generated seroprevalence value was equal in males than in females, as well as between age and occupation groups.
3. There was association between risk factors and rickettsiosis seropositive and seronegative results.
4. The rates of sensitivity and specificity of the ELISA kit modified for humans were adequate to use it as screening test.
5. The level of agreement between the serologic assays of the study (IFA and ELISA) was good.

ELISA	Positive	Negative	Total
Positive	(a)	(b)	(a + b)
Negative	(c)	(d)	(c + d)
Total	(a + c)	(b + d)	(a + b + c + d)

Figure 1. *Gold Standard (IFA).*

Hypotheses 1 and 2 were assessed with the Z-test for analysis of proportions (one proportion and comparison between two proportions). Hypothesis 3 was tested with the chi-square test. Sensitivity and specificity values to test hypothesis 4 were assessed according to a contingency table (Fig. 1). Hypothesis 5 was assessed with the κ agreement test.

$$\text{Sp} = \frac{\text{a (positives)}}{\text{a (positives)} + \text{c (false positives)}}$$

$$\text{Sp} = \frac{\text{d (negatives)}}{\text{d (negatives)} + \text{b (false negatives)}}$$

Serologic analysis

For the determination of serologic diagnosis, antibodies against *R. rickettsii* were measured with the *R. rickettsii* Elisa® commercial kit (Biosystems, Inc.), which detects and semi-quantifies IgG in dogs with a sensitivity and specificity of 99.5% and 96%, respectively. Optical density was determined at 450 nm. Samples were analyzed in an ELISA reader using a 450 nm filter. The adjusted seroprevalence was calculated with the obtained results.

Results

- The seroprevalence value generated in this study was higher than 0 ($p < 0.05$).
  R. rickettsii-associated rickettsiosis adjusted seroprevalence was 3.9% (95% CI: 0.8-5.3). This result was obtained by using the commercial kit, standardized for use in humans. Of all patients

included in the study, 12.7% (49/384) were considered suspicious of the disease for having at least 4 of the following clinical manifestations: fever, erythema, epistaxis, headache, vomiting, edema and/or having been bitten by a tick.

– The seroprevalence generated value was not different between males and females ($p < 0.05$), as well as between age and occupation groups.

  In this study, adjusted rickettsiosis seroprevalence did not show a similar distribution ($p > 0.05$) between sex classes and age and occupation groups.

– No association was observed ($p < 0.05$) between the risk factors to be assessed and rickettsiosis-seropositive results.

– With regard to the assessment of clinical signs association with rickettsiosis-seropositivity, no association was found ($p > 0.05$) with claudication, nervous problems, blood transfusions or being bitten by a tick, but a group of clinical manifestations (epistaxis, vomiting, arthralgia, myalgias) showed a significant association ($p < 0.05$) with seropositive patients.

– With regard to the test for agreement between the ELISA (modified to be used in humans) and the IFA results, it is worth mentioning that the sensitivity and specificity values generated by the *R. rickettsii* ELISA® commercial kit (Biosystems, Inc.), modified to be used in humans, were 84 and 94%, respectively. When the ELISA results were confronted with those of IFA through statistic k, a value of 0.80 was generated, different from 0 ($p < 0.05$), the magnitude of which indicates it is superior, high in substantial agreement[9,14].

Discussion

The high specificity (94%) of the *R. rickettsii* ELISA® commercial kit (Biosystems, Inc.), modified to be used in humans, may be due to the quality of the antigen fixed in the plates of the kit, since sensitivity and specificity of ELISA depend on the quality of the antigen fixed in the plate. There are some 30 specific lipopolysaccharides of the outer layer of the bacterium *(R. rickettsii)* that have been used as antigen for ELISA plates, which has increased its specificity and, consequently, its economic value; sensitivity is moderate (84%), and this may be due to the high value of the cutoff point.

The 0.80 result for the κ statistic between ELISA and the confirmatory test (IFA) translates into a high reliability of the ELISA commercial kit modified to be used in humans for diagnosis of the disease. The high level of agreement between both tests may be due to the fact that both detect IgG, even though one measures fluorescence and the other, colorimetric changes[12].

Conclusion

The results of this study demonstrate that there is serologic evidence of rickettsiosis in humans and dogs in the city of Ensenada; therefore, its testing should be included in differential diagnoses performed at the health sector, since this is a short evolution disease with 30% mortality in untreated patients[12,19].

Serologic diagnosis of this disease can be made by means of ELISA, which is a reliable, quick and easy to perform technique, due to the agreement values it generates with regard to IFA[9,10,13].

Clinical manifestations of the disease are unspecific and difficult to assess in seroprevalence studies, since they generally occur within the first two weeks post-infection and antibodies are detectable in blood for 66 weeks[16].

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