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ORIGINAL

DETECTION OF ANTIBODIES TO *ANAPLASMA*, *BARTONELLA* AND *COXIELLA* IN RURAL INHABITANTS OF THE CARIBBEAN AREA OF COLOMBIA

DETECCIÓN DE ANTICUERPOS CONTRA *ANAPLASMA*, *BARTONELLA* AND *COXIELLA* EN HABITANTES RURALES DE UN ÁREA DEL CARIBE COLOMBIANO

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ABSTRACT

Objetive. To estimate the seroprevalence of antibodies to *Anaplasma phagocytophilum (formerly Ehrlichia)*, *Bartonella* spp. and *C. burnetii* in Cordoba and Sucre departments, an important cattle raising and farming region of Colombia. **Materials and methods.** We analysed a representative cross-section of the population by collecting sera in 2003. All of the livestock farming individuals living in towns within Cordoba and Sucre departments served as the base population from which samples were obtained, and all rural workers between 16 and 65 years of age were eligible to enrol. All sera were examined by IFA for the detection of Ig*G* antibodies to *Bartonella spp, Anaplasma phagocytophilum* and *Coxiella burnetii*. **Results.** The overall seroprevalence of antibodies to one or more of the studied agents was 56.8%. Of 81 serum specimens tested antibody to *C. burnetii* 23.6%, were seropositive, 37.7% had antibody reactive with *Bartonella* and 20% of individuals tested were seropositive to *Anaplasma phagocytophilum*. **Conclusions.** Our data indicate that the prevalence of antibodies to *Bartonella, A. phagocytophilum and C. burnettii* is high in our region. Our results suggest that infectious zoonotic diseases are very common among residents of the Caribbean area. This study demostratres for first time the presence of these microorganisms in Colombia.

Key words: Arthropod-borne, Colombia, *Anaplasma,* Bartonella, Coxiella, seroprevalence.

RESUMEN

Objetivo. Establecer la seroprevalencia de *Bartonella spp, Anaplasma phagocytophilum* (antes *Erlichia*) y *Coexiella burnetii.* **Materiales y métodos**. Se analizaron sueros representativos de un sector de la población en el año 2003, recolectados de personas que trabajan en actividades del campo en los departamentos de Córdoba y Sucre que sirvieron como población base de las muestras que se obtuvieron. Los trabajadores rurales elegidos a participar tenían entra 16 – 65 años de edad. Los sueros fueron examinados por IFA para detección de anticuerpos contra IgG para *Bartonella*

spp, Erlichia Anaplasma phagocytophilum y *Coexiella burnetii.* **Resultados**. La seroprevalencia de anticuerpos de todos los microorganismos estudiados fue de 56.8%. De 81 muestras de suero analizadas el 26.6% fueron seropositivas contra *C. burnetii*, el 37.7% tuvieron anticuerpos contra *Bartonella* y el 20% de los individuos evaluados fueron seropositivos para *Anaplasma phagocytophilum*. **Conclusiones**. Nuestros datos indican que la prevalencia de anticuerpos contra *Bartonella*, *A. phagocytophilum* y *C. burnetii* son altos en nuestra región. Los resultados indican que estas enfermedades zoonoticas son muy comunes en las personas que residen en el área del caribe colombiano. Este estudio demuestra por primera vez la presencia de estos microorganismos en Colombia.

Palabras clave: Artropodos, Colombia, *Anaplasma,* Bartonella, Coxiella, seroprevalencia.

INTRODUCTION

Human granulocytic anaplasmosis (HGA), bartonellosis and Q fever are emerging zoonoses described in many areas of the world (1-3). Due to changes in livestock production practices, international trade in animals and their products, and increasing anthropogenic disturbance of natural habitats, zoonoses are becoming increasingly recognized as an important source of human morbidity and mortality. Individuals living in rural areas, particularly in developing countries, are at high risk for contracting zoonoses, since they often work closely with domestic livestock or come into contact with wildlife. Knowledge of the incidence and prevalence of such diseases in rural areas and the way they spread geographically through time is important for their control. No survey of HGA, bartonellosis and Q fever has been conducted in Colombia. We undertook a one-year seroepidemiological study to look for evidence of zoonotic infections in rural villages in Cordoba and Sucre departments, Colombia, where inhabitants work almost exclusively in livestock production.

HGA is an emerging tick-borne disease first described in 1994 in the midwestern United States, (2-4). The etiologic agent is an ehrlichial species closely related to *Anaplasma phagocytophilum*. It is often referred to as the HGA agent and was recently named *Anaplasma phagocytophilum* (2). The causative agent of HGA is a gram-negative obligate intracellular bacterium that invades granulocytic leukocytes (5).

A wide variety of infections with various *bartonella* species is recognized in humans and animals, (6). Three species are well-known human pathogens: *Bartonella bacilliformis, B. quintana*, and *B. henselae*, *B. quintana* and *B. henselae* are species of wide-reaching geographic distribution. *B. quintana* was first described as the agent of trench fever in 1918 and is now known to be responsible for louse-borne bacteremia and endocarditis in homeless people and bacillary angiomatosis in AIDS patients, (6). Humans are the only known reservoir of *B. quintana*, and transmission among individuals occurs via the body louse (*Pediculus humanus*). *B. henselae*, a species first recognized in 1990, is the main etiologic agent of cat scratch disease and is also responsible for bacillary angiomatosis and peliosis hepatitis in immunocompromised (mostly AIDS) patients, (6, 7), as well as bacteremia and endocarditis. *B. henselae* comprises two different genotypes, *B. henselae* Houston and *B. henselae* Marseille, (6). Cats are the main reservoir of *B. henselae*, and persons become infected following cat scratches or bites. The cat flea (*Ctenocephalides felis*) has been proposed to be a vector for human transmission, (6, 8).

Q fever, which often manifests as a systemic illness, occurs worldwide and is caused by *Coxiella burnetii*, an obligate intracellular bacterium (9). While this bacterium must divide intracellularly, it has the ability to live on and spread in cell-free media, (3, 9). *C. burnetii* may remain viable outside the host for long periods; high resistance to UV radiation, heat, dehydration, pressure and osmotic and oxidative stress has been confirmed, (3, 9).

In Colombia, arthropod-borne zoonoses are not reportable diseases. However, the first seroepidemiologic study for tick-borne disease in humans in Colombia was conducted in

Cienaga de Oro (Cordoba department) in 2001, (10), In this study, 49% of the population had IgG antibodies against *Rickettsia* spp., as measured by immunofluorescence assay (IFA). These results encouraged us to undertake further investigation into the prevalence of antibodies to other zoonotic bacteria, including *Bartonella*, *Coxiella* and *Anaplasma*.

The aim of our study was to estimate the seroprevalence of antibodies to *Anaplasma*, *Bartonella* spp. and *C. burnetii* in Cordoba and Sucre Departments, an important cattle raising and farming region of Colombia.

MATERIALS AND METHODS

Description of the geographic area. The region included in this study represented the most important areas of cattle ranching and farming in Colombia. Cordoba department is located on the Caribbean coast (Figure 1). The annual average temperature is 32°C, average humidity reaches 80% annually, and all of the study area is located in humid tropical forest habitat. Inhabitants of the villages included in the study work almost exclusively in livestock production (rearing cattle, swine and sheep). The major cattle ranching areas studied are at an elevation of 4-15-m.

Population studied. The approximate population of Cordoba department in 1993 was 1,460,000 (51% female, 49% male). Rural workers in the department were estimated to number 677,000. The distribution by age was as follows: 30% under 16 years, 30% 16-30 years, 35% 31-47 years, 5% older than 47 years, 90% of total rural workers are men. Approximately 85.5% of the study population lived in the Sinú River basin.

Study method and serum collection. We analysed a representative cross-section of the population by collecting sera in 2003 and preserving the samples at -70°C until testing by IFA. All of the livestock farming individuals living in towns within Cordoba and Sucre departments served as the base population from which samples were obtained, and all rural workers between 16 and 65 years of age were eligible to enroll. A two-step sampling technique was carried out in non-randomized conglomerates. The towns were considered the principal sampling unit, and the people selected were the secondary unit. The inhabitants of the rural communities were informed about zoonotic diseases and the reason for the study before each blood collection, and they were cooperative and enthusiastic about participation in the project. The committee of investigation of the University of Cordoba, faculty of Veterinary Medicine approved the project.

Epidemiological and clinical data. A questionnaire was designed to collect all pertinent epidemiologic and clinical information from study participants. We recorded the following data about each subject: place of residence, age, sex, time spent in farming

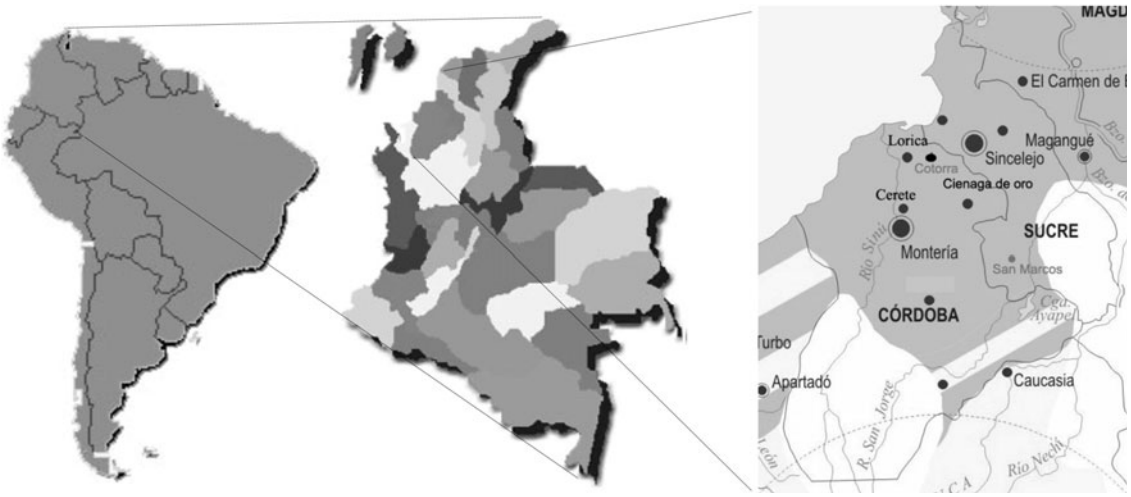

Figure 1. Diagram of study area depicting the villages where sampling was conducted in the departments of Cordoba and Sucre, Colombia.

labours (in years), the presence of ticks in his/her workplace, and whether he/she had experienced any of the following symptoms: fever, rash, and myalgia. All of the people studied belong to groups of low socioeconomic status; thus the living conditions in those rural areas are primitive (no water and sanitary resources, no electricity in some cases). Approximately 60% of study participants were illiterate.

Serological assays. All sera were examined by IFA for the detection of IgG antibodies to *B. quintana* and *B. henselae*, *A. phagocytophilum* and *C. burnetii*. All assays were performed double-blind, with coded specimens lacking identification markers and clinical or other information. We used kits from Focus Technologies (Cypress, Ca, USA), following the manufacturer's instructions.

Sera collected in 2003 were examined for the detection of:

1. IgG against *B. quintana, B. henselae* and *A. phagocytophilum* by indirect immunofluorescence assay (Focus Technologies, Cypress, Ca, USA), in 1:64 for both (Single IgG serum endpoint titers > 1:64 are suggestive of infection at an undetermined time and are suggestive of either past infection or early response to a recent infection).

2. IgG against *C. burnetii* by indirect immunofluorescence assay (Focus Technologies, Cypress, Ca, USA), in 1:16 for phase I and phase II (Single IgG serum endpoint titers > 1:16, strongly suggest *C. burnetii* infection).

Statistical analyses. We used Epi Info (CDC, Atlanta, Georgia, USA, version 2000) to perform statistical analyses The statistics used were the Student's *t*-test for independent samples (differences among means and proportions) and the Chi-square independence test. A value for $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 is a summary of our findings of antibodies to *Anaplasma, Bartonella and Coxiella* of human serum samples. The overall seroprevalence of antibodies to one or more of the studied agents was 56.8%. The prevalences among villages varied from 33.3% in Monteria to 81.2% in Lorica (Table 1). No statistically significant difference in prevalence was observed between Monteria and Cotorra villages ($p > 0,05$) Significant differences were observed when prevalences were compared among Lorica, Monteria, San Marcos and Cienaga de Oro ($p < 0,05$).

Of the 81 sera tested, 37.7% had antibody reactive with *Bartonella* by IFA. Equal proportions (34%) had antibody to *Bartonella henselae* and *Bartonella quintana*. The prevalences of *Bartonella* antibody among villages varied from 7.1% in Monteria to 56.2% in San Marcos and Lorica (Table 1) ($p < 0,05$). Statistically significant differences were observed when prevalences for *Bartonella* were compared among Lorica, Monteria, San Marcos and Cienaga de Oro ($p < 0,05$).

Table 1. Prevalence (% positive) of antibody to four zoonotic agents in serum samples from humans living in five villages in two departments the Caribbean area of Colombia.

Villages	*B. henselae*	*B. quintana*	*Bartonella spp*	HGA	*C. burnettii*	Seroreactivity patients
San Marcos (n=16)	9/16 (56%)	9/16 (56%)	9/16 (56%)	2/16 (13%)	1/16 (6%)	**68,8%**
Cotorra (n=16)	3/16 (19%)	4/16 (25%)	4/16 (25%)	2/16 (13%)	3/16 (19%)	**37,5%**
Lorica (n=16)	6/16 (38%)	7/16 (44%)	9/16 (56%)	2/16 (13%)	8/13 (62%)	**81,3%**
Montería (n=15)	1/14 (7%)	1/14 (7%)	1/14 (7%)	6/13 (23%)	5/13 (38%)	**33.3%**
Cienaga de Oro (n=18)	7/15 (47%)	5/15 (33%)	7/15 (47%)	6/13 (46%)	0/15 (0%)	**61,1%**
Total positive cases	**26/77 (34%)**	**26/77 (34%)**	**30/77 (39%)**	**15/75 (20%)**	**17/72 (24%)**	**56,8%**

Bartonella spp indicates samples that were positive to either or both Bartonella species in two Colombian Caribbean departments % positive cases.

We found antibody against the HGA agent in 20% of individuals tested (Table 1). The prevalences among villages varied from 12.5% in Lorica, Cotorra and San Marcos to 42.9% in Cienaga de Oro (Table 1). No statistically significant differences in prevalences were observed among Lorica, Cotorra and San Marcos villages ($p > 0,05$). Significant differences were observed when prevalences were compared among Cienaga de Oro, Monteria, Lorica, Cotorra and San Marcos ($p < 0,05$).

Of 81 serum specimens tested antibody to *C. burnetii* by IFA, 23.6%, were seropositive (Table 1). The prevalences among villages varied from 0% in Cienaga de Oro to 61.5% in Lorica (Table 1). Statistically significant differences were observed when prevalences were compared among Cienaga de Oro, Monteria, Lorica, Cotorra and San Marcos ($p < 0,05$).

Eight sera showed cross reactivity among the antigens studied (Table 2). Five sera had cross-reactivity with *Bartonella* and *Anaplasma*; two sera had antibodies against *Anaplasma* and *Coxiella* and one had seroreactivity against *Bartonella* and *Coxiella*.

Table 2. Cross-reactivity among sera samples analyzed by IFA

# Patient	Village	*Bartonella spp*	*A. phagocytophilum*	*C. burnettii*
13	San marcos	(+)	(+)	(-)
252	Lorica	(+)	(+)	(-)
72	Montería	(+)	(+)	(-)
44	Cienaga de Oro	(+)	(+)	(-)
119	Cienaga de Oro	(+)	(+)	(-)
180	Montería	(-)	(+)	(+)
174	Montería	(-)	(+)	(+)
23	Cotorra	(+)	(-)	(+)

DISCUSSION

To the best of our knowledge, this is the first demonstration of infection by any of these bacterial zoonotic agents in humans in Colombia.

We found that 37.6% of the population tested had IgG antibodies to *B. henselae* or *B. quintana*. A total of 12.4% had IgG antibodies to both *Bartonella* species, 33.7% only to *B. henselae*, and 33.7% only to *B. quintana*. Although we conclude that this seropositivity most likely reflects past infection with these *Bartonella* species, we cannot reject non-specific serologic cross-reactivity with other antigens. It is well known that antibody to *Bartonella* spp. cross-reacts with other antigens such as *Rickettsia, Treponema, Mycoplasma* and *Chlamydia,* (6, 11-14). However, all 81 farm workers in this survey were examined by IFA and found to be seronegative for these microorganisms. In spite, that studied towns are small, with little variation in environmental, socio-economic and geo-climatic conditions, it is contradictory that our observation had significant differences ($p < 0,05$) occurred in the seroprevalence of antibodies to the *Bartonella* in the five villages studied (Table 1). This cross-reactivity, which has also been observed in other patient groups, (15), indicates that this serologic analysis genus specific, but not species specific.

In a trial carried out in Switzerland, 20 of 20 (100%) children with cat scratch disease (CSD) had high IFA titers of antibody to *B. henselae,* (11); (16) and 60% of controls living in diverse urban and rural counties were seropositive. In contrast, only 3% (11 of 332) of the controls had high titers above cutoff level proposed for this assay (11); (16) in another work over 3,000 serum samples submitted for *Bartonella* serology to the Centers for Disease Control and Prevention were tested by IFA. Of those patients,

only 86 (2.9%) had antibody to either *B. henselae* or *B. quintana,* (17).

Regarding HGA, our data suggest that HGA cases may occur in Colombia. Since such cases have been not been published to date, they are likely underdiagnosed. Further investigation is needed to demonstrate the presence of the HGA agent in ticks in Colombia. The prevalence of antibody to *A. phagocytophilum* was identical among Lorica, Cotorra and San Marcos. Nevertheless, differences were observed among Cienaga de Oro, Monteria, Lorica, Cotorra and San Marcos ($p < 0, 05$). We have no explanations to this fact, because the five villages studied are small, with modest dissimilarity in environmental and climatic conditions.

Several serosurveys of the prevalence of antibodies to the HGA agent have been conducted across Europe and Asia (15, 18-25). The seroprevalence of HGA observed in our study group (20%) is similar to that found in surveys carried out in Switzerland (17.1%) (25) Slovenia (15.4%) (26) and southern Germany (14%) (27). Antibody prevalences in our study were 2.3 - 2.7 times higher than those found in Sweden (11.4%) (28), Italy (8.6%) (29) and Bulgaria (7.4%) (30). Our prevalence was much higher than those observed in Bulgaria (2.9%) and Germany (1.9%) (27, 30). This finding, at least in part, could be attributed to the fact that the prevalence in these countries was based on blood donors, unlike our survey.

We did not find any association between the results of the HGA-IFA test and either a history of tick bite or occupation among the residents of the Caribbean area of Colombia. This observation is in accordance with the results of (31), who were unable to distinguish any increased risk for prior exposure to HGA on the basis of history of exposure to ticks or behavioural and employment features among the inhabitants of northwestern Virginia, USA. (32); who studied English rural employees, and (28); who studied people of the Koster Islands (Sweden), found no relationship between self-reported tick bite and positivity for HGA, human monocytic ehrlichiosis or Lyme borreliosis. These data reinforce doubts concerning the usefulness of a history of tick bite among inhabitants of areas where Lyme borreliosis and HGA are endemic, particularly for the differential diagnosis of these zoonoses by clinicians. We did not test for antibodies against *Borrelia* spp, because in previous studies we were unable to detect seroreactivity to the Lyme disease agent, (10).

This study provides evidence that *Anaplasma phagocytophilum* is present in the Caribbean area of Colombia. The percentage of seropositive individuals decreased upstream along the Sinú valley (Table 1). Cienaga de Oro, was the most inland town in Sinú valley where seroreactivity to *Anaplasma phagocytophilum* were found.

We detected *Coxiella burnetii* infection in four of the five villages surveyed (Table 1). Of 81 serum specimens tested by IFA, 23.6% had antibody to *C. burnetii* (Table 1). There were statistically significant differences in antibody prevalence among the five villages. In contrast to the high prevalence of antibody to *A. phagocytophilum* (Table 1), we found no evidence of infection with *C. burnetii* among residents of Cienaga de Oro.

Studies conducted in Spain with similar methodology using IFA found that prevalence of antibody to *C. burnetii* varied according to geographic area, from 21.5% in Canary Islands, 5.1% in Huelva (southwest of Spain) to 40.6% in Leon (north central), with intermediate prevalences of 12.7% in Madrid and 20.8% in Soria (central and north central areas, respectively), (9). In the Basque country (northern Spain), (9), using IFA, a seroprevalence was 38.5%. The average seroprevalence in Spain was 23.8%, prevalence similar to that found in our study (23.6%) (9).

Among the serum samples that showed cross reactivity, 5 (6%) reacted with both *Bartonella and A. phagocytophilum*, 2 (2.4%) reacted with *A. phagocytophilum and Coxiella* and only 1 (1.23%) reacted with *Bartonella* and Coxiella (Table 2). This fact, which has been observed elsewhere, may result from co-infection or cross-reactivity to two or more antigens, (6, 7, 11, 13, 14).

Although IFA has both advantages and disadvantages, it is the most widely used method for the identification *Anaplasma phagocytophilum, Bartonella quintana*, *B. henselae* and *Coxiella burnetii* antibodies. We found the assay to be

relatively simple and dependable, and very useful for studies such as ours.

Finally, it is reasonable to believe that as reliable, validated, and safe methods for detection of antibody to *Bartonella, Anaplasma phagocytophilum and Coxiella burnettii* become routine in many clinical laboratories, the recognition of zoonotic diseases in Colombia will continue to expand. Our data indicate that the prevalence of antibodies to *Bartonella, Anaplasma phagocytophilum and Coxiella burnettii* is high in our region and that physicians should evaluate serologic results in combination with clinical symptoms. Our results suggest that infectious zoonotic diseases are very common among residents of the Caribbean area of Colombia and the local health personnel should include them in differential diagnosis.

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