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Molecular Study of Rickettsiae in Serum Cattle

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Abstract

A molecular survey of 230 serum samples from cattle was studied by PCR-amplification of the citrate synthase gene gltA, the gene coding for protein 190 kDa—*omp*A—and the gene *omp*B. The study was carried out in the Junta of Castilla y León (northern Spain). The results suggest that the molecular study of the serum cattle would not make a good method in epidemiological studies on rickettsiae in this region. But it is necessary to continue and expand the work with more sensitive molecular methods.

Keywords

Rickettsiae, Epidemiology, Molecular Assay

1. Introduction

Rickettsioses are caused by obligate intracelular Gram-negative bacteria of the genus *Rickettsia* that comprises the spotted fever group (SFG) rickettsiae and the typhus group (TG) rickettsiae [1]. Domestic animals are the hosts of several arthropod species, reservoir of some tick-pathogens and arthropods can transmit rickettsiae to vertebrates during blood meals.

As happening with other studies made in horses [2], we initially supposed that extensive cattle that forge in the marsh are more susceptible to arthropod and tick-borne pathogens than farm animals. People who works with these cattle or live in tick infested areas might be a risk of infection with one or more zoonotic rickettsiae. But not only, because the economic impact of these infections is also an economic problem in livestock areas.

In this study we start from the premise that ticks are abundant on the grass and animals in rural area, and cattle have been recognized as hosts of *Rickettsia* spp. So in the regions studied, provinces of Castilla-León (Northeastern Spain) present a spotted fever group rickettsiosis, caused by *Rickettsia conorii*, recog-

nized in human population since 1975 and cause of disease since 1981 [3]. Isolated cases are originating from the rural milieu. The knowledge of prevalence of tick-borne pathogens in domestic animals is important to implement measures to control transmission to human and animals.

It is true that the most of the works in this sense have been carried on by serological methods which have frequent cross-reactions with non-rickettsial antigens [3] and usually cannot identify the causative agent [4]. There are other methods, as molecular ones, more sensitive and specific capable to detect the rickettsia species, in which because rickettsiae is intracelular, the blood or the buffy coat is considered preferable samples for PCR test [5].

PCR methodology is employed for research and clinical purposes and is a highly useful tool for the direct diagnosis of rickettsiosis. In fact it has been successfully used to detect new and old rickettsiae pathogens in new locations in order to suggest new regions of risk of the rickettsiosis [6]. PCR simply takes a short time, and detects low concentrations of specific target DNA sequence, as other authors have proposed that, sera could be used as a valuable sample to detect *Rickettsia* spp. by molecular techniques, in order to give an alternative method when other samples as blood or tissue biopsy is not available [7].

With all these premises we asked to us the following question, is the serum sample in extensive asymptomatic cattle a good candidate for the detection of rickettsiae to carry on an epidemiological study in endemic areas?

A positive response could provide us of a useful and fast method when the antibodies are not detectable to implement for veterinary use. That was the reason because we started to research in this sense.

2. Materials and Methods

2.1. Animal Samples

The serum samples used in this work—from 230 cattle—form part of our group's frozen (−20˚C) serum collection. The cattle from which these samples came were cows from extensive farming systems of the Junta of Castilla y Leon: Avila province-50, Burgos province-30, León province-10, Palencia province-10, Salamanca province-25, Segovia province 65, Soria province-10, Valladolid-10, Zamora province-20 (**Figure 1**).

The animals ranged from two year to 23 years in age (68 were less than 9 years old, 88 were between 9 and 16 years old, and 44 were over 16 years old). The collection of specimens lasted one year (2013).

All the samples come from cows raised in the countryside (extensive), and therefore in contact with arthropods such as ticks, which are the main vectors of diseases caused by rickettsia; in this environment, it is absolutely impossible that these animals do not have contact with ticks, although we do not have specific information on the status of parasitation of the analyzed samples.

This work was approved by the Research Bioethics and Animal Welfare Committee of the Universidad de Alcalá (Protocol number CEI 2011034).

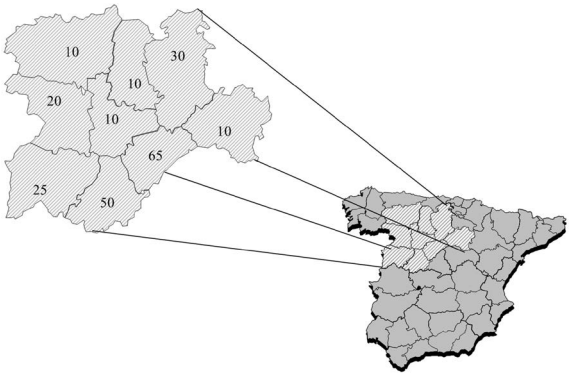

Figure 1. A sketch map of Spain showing the provinces in which cattle serum was collected.

2.2. DNA Extraction and PCR

Total DNA was extracted from animal serum using the COBAS AmpliPrep® system (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's recommendations. DNA of *Rickettsia* genera was detected by PCR-amplification of: 1) the citrate synthase gene gltA, using primers RpCs 1258 and RpCs 877, which amplify a 381 bp fragment; 2) the gene coding for protein 190 kDa—*omp*A—using the primers Rr 190.70p and Rr 190.602n, which amplify a 532 bp fragment; and 3) the gene *omp*B, using the primers 120-M59' and 120-807, which amplify a 833 bp fragment. The first two amplifications were performed following the protocol of Regnery *et al.* [8]; the third was performed following that of Roux and Raoult [9]. To prevent DNA contamination and the carry-over of amplified products, sterile tools were used at all times in each step of the analysis (DNA extraction, preparation of the reaction mixture, and amplification and analysis of the PCR products). Each step was performed in separate work areas. A negative control (Milli-Q water) was included in all amplifications and two positive controls (DNA from *R. conorii*—gift from Dra. Bacellar, Portugal) were included in all PCR runs.

3. Results and Discussion

No rickettsial DNA was detected by PCR in any animal sample.

PCR assay is a sensitive and specific method for the detection of rickettsial agents in sera because serological evidence of infection occurs no earlier than the second week of illness and antibodies are not detectable [10]. However, the present study is the first stage of a project and these are the first results of a series of ongoing studies, they should be considered preliminary. We have used only conventional PCR, and the detection rate may be low. Some previous reports detected rickettsial gene by nested PCR. Other assays will be used to validate these results in following investigations with nested PCR.

Due to ticks are abundant on the grass and the animals in this rural area, we expected a positive result consequence of bites of ticks carrying *Rickettsia* spp., but results were not as we suspected because the experiences realized after a

conventional PCR detected no *Rickettsia* spp. DNA in any of the animal sample tested. Cattle were not rickettsemic by polimerase chain reaction. Sequence analyses of amplicons of gltA, *omp*A and *omp*B gene reveals no positive results in any of the serum samples analyzed, despite the study were carried on in the Castilla-León provinces located in the northeastern Spain areas composed of both urban and rural areas with numerous rural municipalities dedicated fundamentally to agriculture and cattle racing.

But this is not a strange thing. In fact, some authors founded the absence of detectable levels of bacterial DNA in samples from some animals [11]. So, despite *Rickettsia conorii* is believed to be enzootic in Egypt, were not detected by PCR in cattle [12]. In Kenya [13], despite the cattle serum tested for IgG antibodies to SFG rickettsiae were high were not found evidence of the pathogen in blood specimens of cattle. The same that were observed by Boretti *et al.* [14], and Ortuño *et al.* [15], in which were not found bacterial DNA in tissue or blood samples even though the donor animal had high antibody titers.

In Burgos province, one of the Castilla-León provinces (northeastern Spain), studies carried on by our research group observed a high seroprevalence in cattle but no rickettsiae species in DNA in any of the animal sample tested [16].

As we know vertebrates have an important role in the maintenance of *Rickettsia* spp. in the nature. In many cases the natural epidemiological cycle is well known but not in other times. The specific relationship between the arthropod and the rickettsia species is one factor. Other is the biology of the rickettsiae, with an effective transovarial and transstadial transmission. The other is the reservoir. An example is related with the studies of Ruiz-Beltrán *et al.* [17], that proposed wild rabbits as a reservoir in the nature of *R. conorii*, though in other previous studies were impossible to isolate rickettsiae [18] probably due to the low pathogenicity of the rickettsiae parasitizing the biosystem.

However ticks and a high number of animals in a zone are not enough to causes infection, and the risk for a person is related to the contact and living zones that facilitates contact with the bacteria.

But perhaps the simple question is that cattle are not a good reservoir in maintenance of rickettsiosis, though they are exposed to ticks bite. Perhaps cattle could play a role in the natural history of *Rickettsia* spp. infection expanding population of tick-borne pathogens rather than a reservoir for *Rickettsia* spp. as other authors have proposed [19].

4. Conclusion

In summary, cattle appear not to be an ecological niche of rickettsiae in the host-parasite system despite cattle forage in the marsh, are being exposed to tick bites and stay at endemic zone. But either way, negative results obtained in this paper are of interest because recognition of routes of transmission and study of domestic reservoirs that are near to people are of great importance to know and prevent future infection in humans. But, further research should attempt to de-

termine the role of cattle in the epidemiology and ecology of these infections, with other kinds of samples, other methods more sensitive and specific as nested PCR, and involve other areas of the country.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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