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Short communication

First molecular evidence of *Coxiella burnetii* infecting ticks in Cuba

Angel A. Noda[a,*], Islay Rodríguez[a], Jorge Miranda[b], Verónica Contreras[b], Salim Mattar[b]

[a] *Instituto de Medicina Tropical "Pedro Kourí", Habana, Cuba*
[b] *Instituto de Investigaciones Biológicas del Trópico, Universidad de Córdoba, Montería, Colombia*

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ABSTRACT

Coxiella burnetii is the causative agent of Q fever. In order to explore the occurrence of *C. burnetii* in ticks, samples were collected from horses, dogs and humans living in a Cuban occidental community. The species most commonly recovered were *Amblyomma mixtum* (67%), *Rhipicephalus sanguineus* s.l. (27%) and *Dermacentor nitens* (6%). Specific IS1111 PCR and amplicon sequencing allowed the identification of *C. burnetii* DNA in *A. mixtum* collected from a domestic horse. These findings, for first time in Cuba, indicate the need for an in-depth assessment of the *C. burnetii* occurrence in hosts and humans at risk of infection.

1. Introduction

Q fever is a widespread zoonosis caused by an obligate intracellular bacterium named *Coxiella burnetii*. Domestic ruminants are identified as the main sources of human infections, considered to be acquired through inhalation of aerosols contaminated with parturient products or from the urine or feces of infected animals (Cardinale et al., 2014). Ticks as an important reservoir in nature, may play a critical role in the transmission of *C. burnetii* among wild vertebrates (Maurin and Raoult, 1999).

Isolation of *C. burnetii* is not usually performed for epidemiological purposes because it is difficult, time consuming and requires biosafety level 3 laboratories. Molecular tests based on polymerase chain reaction (PCR) present nowadays a very useful method for the detection of *C. burnetii* DNA. The PCR assay with primers targeting IS1111, the repetitive, transposon-like element, has been found to be very specific and sensitive for the detection of *C. burnetii* in different clinical samples (Vaidya et al., 2008).

Within Latin America, there has been only a single report of *C. burnetii* infecting ticks, namely *Amblyomma tigrinum* and *Amblyomma parvum* in Argentina (Pacheco et al., 2013). In Cuba, there have been no records of *C. burnetii* infection in either invertebrate or vertebrate hosts, including humans. The current paradigm that *C. burnetii* has a cosmopolitan distribution (Anderson et al., 2013; Capin et al., 2013), coupled with the scarcity of studies in Latin America (Nava et al., 2014) motivated the present study, which aimed to explore the occurrence of *C. burnetii* infecting ticks in Cuba.

2. Materials and methods

During August and September 2014, 468 ixodids (partially engorged) were collected from horses, dogs and humans in a population settlement in Sierra del Rosario, located in Candelaria, Artemisa province. Ticks were placed in a sterile tube containing absolute ethanol.

Ticks were individually identified by taxonomic keys (Barros-Battesti et al., 2006; Cooley, 1946); for ticks of the *Amblyomma cajennense* complex, we followed morphological descriptions and redescriptions reported by Nava et al. (2014). Specimens were grouped into 102 pools according to host, species, sex and life stages: 68 pools containing 1–4 *A. cajennense* sensu lato (*A. cajennense* s.l.) individuals (adults or nymphs), 26 pools containing up to 5 *Rhipicephalus sanguineus* s.l. individuals and 8 pools including 2–4 *Dermacentor nitens* ticks. DNA was isolated by using a QIAamp DNA Mini-Kit (Qiagen, Chatsworth, California, USA) after cutting the tick into pieces with surgical blades, and eluted in a final volume of 100 μL. Purified DNA was stored at −20 °C until used as a template for PCR.

The efficiency of tick DNA extraction was tested by amplifying the tick mitochondrial 16S rRNA gene using tick specific primers: TQ16S + 1F 5'-CTGCTC AATGATTTTTTAAATTGCTGT GG-3' and TQ16S-2R 5'-ACGCTGTTATCCCTAGAG-3' (Halos et al., 2004).

In addition, a PCR assay targeting a 435-bp fragment of IS1111, a transposon-like repetitive region, was used to detect

* Corresponding author.
E-mail address: angelalberto@ipk.sld.cu (A.A. Noda).

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C. burnetii in collected ticks by using CoxP4 (5′-GGCTGCG-TGGTGATGG) and CoxM9 (5′-GTCCCGGTTCAACAATTCG-3′) as specific primers (Panning et al., 2008). DNA from *C. burnetii* isolate 120 LHR-BR was used as a PCR-positive control. In addition, *C. burnetii* positive ticks were subjected to PCR for *Rickettsia* spp. (Labruna et al., 2004), *Borrelia* spp. (Noda et al., 2013), *Anaplasma* spp. (Liz et al., 2002), *Babesia* spp. (Tavassoli et al., 2013) and *Escherichia coli* (Herzer et al., 1990) in order to confirm detection specificity and/or elucidate possible coinfections.

Amplicons from positive ticks were gel-purified with the QIAEX II (Qiagen, Chatsworth, California, USA) commercial kit following the manufacturer's instructions. Sequencing of the gel-purified amplicons was performed using the GenomeLabTM Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter®; Washington, D.C., USA). Generated sequences were submitted for basic local alignment search tool (BLAST) analysis to determine closest similarities to available corresponding DNA sequences in GenBank.

3. Results

Tick species most commonly recovered were *Amblyomma mixtum* (67%; nymphs 18%, adult males 33% and adult females 16%), followed by *R. sanguineus* s.l. (27%; nymphs 6%, adult males 16% and adult females 5%) and *D. nitens* (6%; nymphs 1%, adult males 3% and adult females 2%). *A. mixtum* was found on horses, dogs and humans, whereas *D. nitens* and *R. sanguineus* s.l. were collected only from horses and dogs, respectively.

Concerning *Coxiella* detection in the tested arthropods, the absence of PCR inhibition was verified by the detection of tick mitochondrial DNA. *C. burnetii* specific DNA was identified by IS1111 PCR coupled with sequencing (KP763486) from two *A. mixtum* tick pools corresponding to 4 adult males and 2 females collected from a domestic horse. Nucleotide sequence analysis by BLAST was consistent with isolates of *C. burnetii* with more than 99% identity for IS1111 gene (e.g. JF970261 from Brazil, LK937696 from Netherlands, KF146935 from Iran) and 97% nucleotide matching was identified relative to DNA used as positive control, confirming the absence of PCR contamination. Importantly, both tick pools were sequenced and identical DNA sequences were achieved.

Finally, DNA from the additional microorganisms tested in ticks infected by *C. burnetii* was absent, confirming the specificity of the PCR assay.

4. Discussion

C. burnetii has been isolated from approximately 40 species of ticks, which act as reservoirs for the bacterium in nature; however, the role of ticks as vectors of *C. burnetii* for humans remains to be confirmed (Szymańska-Czerwińska et al., 2013).

Our collections show a high abundance of *A. mixtum*, a tick species that belongs to the *A. cajennense* species complex (Beati et al., 2013; Nava et al., 2014). Consistent with this notion, the only species of *A. cajennense* s.l. detected in Cuba to date is *A. mixtum* (Nava et al., 2014).

In nature, several species of wild and domestic mammals have been recorded as hosts for the immature and adult stages of *A. cajennense* s.l. (Nava et al., 2014). Moreover, this taxon is relevant from a medical point of view, because all stages of *A. cajennense* s.l. are usually recorded biting humans (Guglielmone et al., 2006).

In addition, *R. sanguineus* s.l. is a three-host tick species associated mostly with dogs, although humans can be accidentally bitten by this tick (Barros-Battesti et al., 2006). *D. nitens* has a one-host life-cycle and is associated mainly with horses in tropical areas of America (Barros-Battesti et al., 2006).

C. burnetii DNA detected in tested ticks has important repercussions for the knowledge of this non-studied infectious agent in Cuba and allows a better understanding of the behavior of *C. burnetii* in Latin America. According to available information, occurrence of *C. burnetii* in ticks from Latin American region has only been reported in two *A. parvum* males and one *A. tigrinum* female from 105 ticks collected in Argentina (Pacheco et al., 2013); however, Brazil reported a case of Q fever in a man who presented with fever of 40 days duration associated with thrombocytosis (Lemos et al., 2011). Also in Brazil, *C. burnetii* DNA was amplified from six milk and two blood samples from goats and from dogs, respectively (Monteiro et al., 2014). Our results strongly suggest that the circulation of the Q fever agent in Latin America is not restricted to Argentina and Brazil as confirmed to date.

In spite of the low positivity, our study represents the first records of *C. burnetii* infecting ticks in Cuba. Hence, given the implication and novelty of these findings, in order to have a better insight into the epidemiology of *C. burnetii* infections in Cuba and its role in domestic animals diseases and human, further studies to include more ticks from different parts of Cuba are warranted.

5. Conflict of interest

Authors declare to have no conflict of interest.

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