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Short Communication

Molecular identification of the agent of Q fever – *Coxiella burnetii* – in domestic animals in State of Rio de Janeiro, Brazil

***Maria Angélica Monteiro de Mello Mares-Guia*[1], *Tatiana Rozental*[1], *Alexandro Guterres*[1], *Raphael Gomes*[1], *Daniele Nunes de Almeida*[1], *Namir Santos Moreira*[1], *Jairo Dias Barreira*[2], *Alexsandra Rodrigues Favacho*[1], *Andrea Lopes Santana*[3] *and Elba Regina Sampaio de Lemos*[1]**

[1]. Laboratório de Hantaviroses e Rickettsioses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ. [2]. Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, RJ. [3]. Secretaria de Saúde do Município de Itaboraí, Itaborarí, RJ.

ABSTRACT

Introduction: Over the last recent years, the number of Q fever cases have has increased throughout the world. An epidemiological investigation was performed in the area in which the first molecular documentation of Q fever in Brazil was previously reported. **Methods**: Indirect immunofluorescence assay (IFA) and PCR of *Coxiella burnetii* targeting the *htp*AB gene were performed in samples from 14 dogs (blood); 1 cat (blood); 10 goats (blood, milk, vaginal swab and anal swab); 3 sheep (blood); and 2 horses (blood). **Results**: Two dogs, two sheep and five goats were seroreactive. DNA was amplified from 6 milk and 2 blood samples from goats and from dogs, respectively. The sequence of the amplicons exhibited 99% sequence similarity with the homologous sequence of the *htp*AB gene of *C. burnetii* RSA 331 (GenBank - CP000890). **Conclusions**: The results confirm *C. burnetii* infection in animals in Rio de Janeiro and reinforce the need for the surveillance of Q fever in Brazil.

Keywords: Q fever. *Coxiella burnetii.* Domestic animals. Molecular diagnosis. Brazil.

Q fever is a zoonosis caused by *Coxiella burnetii*, a small obligate intracellular gram-negative bacterium of the Legionellales order that has tropism for monocyte and macrophage cells[1]. This zoonosis is transmitted to humans through the inhalation of aerosols from contaminated soil and animal excrement, primarily parturient fluids. Ingestion, especially drinking raw milk, is also likely a minor factor in the transmission of *C. burnetii*. Although *C. burnetii* has been isolated from arthropods, primarily ticks, the arthropod-borne transmission of Q fever in humans is unlikely to be significant[2]. In humans, the disease has a broad spectrum of clinical manifestations, ranging from a limited febrile illness, pneumonia and hepatitis to life-threatening forms such as endocarditis and meningoencephalitis[2].

The definitive diagnosis of Q fever is based on serological, molecular and isolation methods. Serological testing is the most commonly used method, specifically using the indirect immunofluorescence assay (IFA). Acute and chronic infections are characterized by different serological profiles; antigen phase II and phase I tests are used for the diagnosis of acute and chronic Q fever, respectively[2].

Q fever is widespread throughout in the world and cases occur on all continents, with the exception of New Zealand[2]. Although cattle, sheep and goats are considered to be the primary reservoirs of *C. burnetii,* several species of wild and domesticated vertebrates, including mammals, reptiles and birds, have been associated with this zoonotic disease agent[2]. Since the 1990s, several outbreaks of Q fever have been described worldwide, especially in Europe, where more than 3,500 reported cases occurred in the Netherlands[3]. In Brazil, although serological tests have been available for Q fever for more than five decades, the human disease is not a nationally notifiable disease and its occurrence and geographic distribution in the Brazilian territory are completely unknown, with limited information in the Southeast region[4-6].

This paper reports an investigation into the source of *C. burnetii* infections in a rural area in the Itaboraí municipality, State of Rio de Janeiro, where the first human case of Q fever in Brazil was confirmed by molecular analysis. In 2008, the patient, after contact with the birth and abortion products of dogs and goats, presented fever with a duration of >40 days that was associated with abdominal pain, headache, nausea, fatigue, malaise and depression. Although the results of the physical examination were unremarkable, except for abdominal pain on palpation, the laboratory investigation revealed a high erythrocyte sedimentation rate, leukocytosis

Address to: Drª Maria Angélica Monteiro de Mello Mares-Guia. Lab. de Hantaviroses e Rickettisoes/IOC/FIOCRUZ. Av. Brasil 4365, Pavilhão Hélio e Peggy Pereira, Sala B115, Manguinhos, 21040-900 Rio de Janeiro, RJ, Brasil.
Phone: 55 21 2562-1712; **Fax**: 55 21 2562-1897
e-mail: amguia@ioc.fiocruz.br
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and thrombocytosis, which were associated with significantly higher titers of antibodies to the phase II *C. burnetii* antigen (titers of 1024). Samples from the patient were PCR positive for *C. burnetii* DNA[4].

Ethical approval: Not required. Although Q fever is not a notifiable disease in Brazil, after confirmation that a patient was infected by *C. burnetii*, all the procedures involving animals were performed in the context of the epidemiological surveillance of rickettsiosis in Brazil. The National Reference Laboratory for Rickettsiosis is part of the Laboratory for Hantavirosis and Rickettsiosis.

The study was conducted in December 2008 and March 2009 in the City of Itaboraí (22º44'51" S and 42º51'21" W), Rio de Janeiro State of Brazil. Biological samples from domestic animals were collected in two areas. In area 1, at the patient's property, blood samples were collected from 14 dogs and 3 sheep in 2008. In area 2, at the property to which the goats were subsequently transferred, blood samples from 1 dog, 1 cat, 2 horses and 10 goats as well as milk and vaginal and anal swab samples from goats and fecal samples from the environment were collected in 2009. The serum samples were evaluated for anti-*C. burnetii* antibodies, phases I and II, using a commercial IFA for the detection of IgG (SCIMEDX™, Denville, New Jersey, USA) with a cut-off titer of 64. Blood, milk, vaginal and anal swabs and fecal specimens were evaluated by PCR assay as previously described, using primers targeting the repetitive transposon-like region of *C. burnetii* - Trans1 (5'TATGTATCCACCGTA GCCAGTC-3') and Trans2 (5'-CCCAACAACACCTCCTTATTC-3') - and producing an expected amplification product of 687 bp[8,9]. DNA was extracted from blood samples from all animals by a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's instructions. DNA from anal/vaginal swabs, after extensive washing in PBS and from milk was extracted using ATL (QIAmp Blood DNA kit, Qiagen™) and proteinase K. Each sample (200 µL) was mixed directly with the ATL and proteinase K and incubated at 56°C overnight, followed by an incubation step for 10min at 70ºC[7]. Fecal samples (2g) were mixed with 10ml of PBS and 1ml of chloroform in a 15ml falcon tube with autoclaved beads at the bottom. This mixture was vortexed for 30 min and then centrifuged at 3,390g for 25min. The supernatant (200µL) was treated with proteinase K and ATL buffer for 30min at 70°C, followed by the treatment with AL buffer for 10min at 70°C. The extraction process was continued with a QIAmp DNA blood kit (Qiagen™) following the manufacturer's instructions. Negative controls were included in each extraction to check for possible DNA contamination.

The PCR reaction was performed on 4µL of DNA from each prepared sample in a total volume of 25µl. The final reaction mixture contained 6pmol of each primer (IDT/Prodimol, Belo Horizonte, MG, Brazil), 200µM of dNTPs (20mM of each deoxynucleoside triphosphate), 1.5mM $MgCl_2$, 0.1U of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and nuclease-free water (Promega, Madison, WI, USA). The amplification consisted of an initial denaturation at 95°C for 5min, followed by 40 consecutive cycles of denaturation at 95°C for 30s, annealing at 60°C for 30s and extension at 72°C for 1min and a final extension at 72°C for 7min.

The PCR products were run on an agarose gel to detect amplification of the target product and then purified using Kit BigDye Terminator® X-Purification (Applied Biosystems, Foster City, CA, USA). The sequencing reactions were performed using a BigDye® Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3100 Nucleic Acid Sequence Analyzer (Applied Biosystems). Partial sequences were compared with the *htp*AB gene from *C. burnetii* sequences available in the GenBank database using the BLAST® program[10].

In area 1, two (15.3%) of the thirteen dogs and two (66.6%) of the three sheep had anti-*C. burnetii* IgG phase II antibodies with a titer of 64. In area 2, only the serum samples from five goats (50%) were reactive (with titers of 64 and 128).

Products of the expected size for the repetitive transposon-like region of *C. burnetii* (687bp) were obtained from blood samples collected from two seronegative dogs in area 1 and from six milk samples and one anal swab from goats in area 2. Two of these six goats were PCR positive but seronegative. The molecular analyses of the fecal samples were negative for *C. burnetii*. **Table 1** presents the serological and molecular data from the samples.

The eight nucleotide sequence analyses of the amplicons obtained from the milk and blood samples were compared with other sequences of *C. burnetii* available in the GenBank database and exhibited between 98% and 100% identity with the sequence of the *htp*AB gene of *C. burnetii* RSA 331 (GenBank accession number CP000890). All sequences obtained in this study were submitted to the NCBI GenBank database, and the accession numbers are shown in **Table 1**.

This report is the first describing a partial sequence of the *C. burnetii htp*AB gene in domestic animals in Brazil. The DNA sequence of the repetitive transposon-like region generated in this study confirms that *C. burnetii* is circulating in goats and dogs in the rural area of Itaboraí, State of Rio de Janeiro.

The identification of two PCR-positive dogs and two IFA-positive sheep in area 1, after the occurrence of the first confirmed human case of Q fever and the identification of anti-*C. burnetii* antibodies in the family members of the patients and dogs from the patient's property in 2008, demonstrated the persistence of this bacterium in the environment and indicated the risk of further cases of Q fever in this region[4].

The study performed in area 2, where the goats were transferred months after the occurrence of the Q fever case, identified the infectious agent in the milk and anal swabs from the goats. Because of its stability in the environment and the potential for aerosol dispersion, these results indicated the possibility of an increase in goat-associated *C. burnetii* infections in humans and animals in the City of Itaboraí and its surroundings. Furthermore, as demonstrated by the large outbreaks of Q fever in humans that have been recently described in Europe, where sheep and goats are considered to be the major sources of human exposure to *C. burnetii*[3], the

TABLE 1 - Serological and molecular analysis of *Coxiella burnetii* in samples from domesticated animals in State of Rio de Janeiro, Brazil.

Area	Animal	IFA (titers)	PCR	GenBank accession number (%)[a]
Area 1	dog 1	unreactive	negative	-
	dog 2	unreactive	negative	-
	dog 3	unreactive	negative	-
	dog 4	unreactive	negative	-
	dog 5	unreactive	negative	-
	dog 6	unreactive	negative	-
	dog 7	unreactive	negative	-
	dog 8	reactive (64)	negative	-
	dog 9	unreactive	negative	-
	dog 10	unreactive	positive[b]	JN966900 (98% identity)
	dog 11	unreactive	negative	
	dog 12	unreactive	positive[b]	JN966901 (99% identity)
	dog 13	reactive (64)	negative	-
	sheep 1	reactive (64)	negative	-
	sheep 2	unreactive	negative	-
	sheep 3	reactive (64)	negative	-
Area 2	dog1	unreactive	negative	-
	cat	unreactive	negative	-
	horse 1	unreactive	negative	-
	horse 2	unreactive	negative	-
	goat 1	reactive (64)	positive[c]	JN966899 (100% identity)
	goat 2	unreactive	positive[d]	JF968205 (100% identity)
	goat 3	reactive (64)	positive[c]	JF972642 (100% identity)
	goat 4	reactive (128)	negative	-
	goat 5	reactive (64)	positive[c]	JF972643 (100% identity)
	goat 6	unreactive	positive[c]	JF972644 (100% identity)
	goat 7	reactive (64)	positive[c]	JN972645 (100% identity)
	goat 8	unreactive	negative	-
	goat 9	unreactive	negative	-
	goat 10	unreactive	negative	-

[a](%) maximum similarity with the sequence of the *htp*AB gene of *Coxiella burnetii* RSA 331; [b]serum samples; [c]milk sample; [d]milk sample that was sequenced and anal swab. PCR: polymerase chain reaction; IFA: immunofluorescence assay.

possibility exists that goats were, in fact, the source of the *C. burnetii* infection in the Itaboraí municipality.

Finally, because Q fever is not a notifiable disease and many human cases could have been misdiagnosed as some other infection, the public health impact of *C. burnetii* infection will be not understood until suitable epidemiologic surveillance is effectively employed in Brazil.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Voth DE, Heinzen RA. Lounging in a lysosome: the intracelular lifestyle of *Coxiella burnetii*. Celular Microbiology 2007; 9:829-840.
2. Maurin M, Raoult D. Q Fever. Clin Microbiol Rev 1999; 12:518-553.
3. Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, et al. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. Emerg Infect Dis 2011; 17:668-675.
4. Lemos ER, Rozental T, Mares-Guia MA, Almeida DN, Moreira N, Silva RG, et al. Q fever as a cause of fever of unknown origin and thrombocytosis: first molecular evidence of *Coxiella burnetii* in Brazil. Vector Borne Zoonotic Dis 2011; 11:85-87.
5. Siciliano RF, Ribeiro HB, Furtado RHM, Castelli JB, Sampaio RO, Santos FCP, et al. Endocardite por *Coxiella burnetii* (febre Q). Doença rara ou pouco diagnosticada? Relato de caso. Rev Soc Bras Med Trop 2008; 41:409-412.
6. Costa PSG, Brigatte ME, Greco DB. Antibodies to *Rickettsia rickettsii*, *Rickettsia typhi*, *Coxiella burnetii*, *Bartonella henselae*, *Bartonella quintana*, and *Ehrlichia chaffeensis* among healthy population in Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 2005; 100:853-859.
7. Rodolakis A, Berri M, Héchard C, Caudron C, Souriau A, Bodier CC, et al. Comparison of *Coxiella burnetii* Shedding in Milk of Dairy Bovine, Caprine, and Ovine Herds. J Dairy Sci 2007; 90:5352-5360.
8. Rozental T, Mascarenhas LF, Rozenbaum R, Gomes R, Mattos GS, Magno CC, et al. *Coxiella burnetii*, the agent of Q fever in Brazil: its hidden role in seronegative arthritis and the importance of molecular diagnosis based on the repetitive element IS*1111* associated with the transposase gene. Mem Inst Oswaldo Cruz 2012; 107:695-697.
9. Willems H, Thiele D, Frolich-Ritter R, Krauss H. Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). Zentralblatt fur Veterinarmedizin B 1994; 41:580-587.
10. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990; 215:403-410.