Q Fever: characteristics and reports of an important neglected zoonosis in Brazil

Febre Q: características e relatos de uma importante zoonose negligenciada no Brasil

DOI:10.34119/bjhrv5n2-125

Recebimento dos originais: 27/01/2022
Aceitação para publicação: 25/02/2022

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**ABSTRACT**
The Q fever is a zoonotic disease neglected in many countries all over the world. This zoonosis is caused by the bacteria *Coxiella burnetii*, a pathogen that presents stability and environmental resistance with high capacity to cause human infection, which could be fatal. This literature review study aims to describe the general aspects of Q fever, presenting the main cases occurred in Brazil and discussing ways to avoid this zoonosis negligence and underreporting in Brazil. The Q fever is still a disease unknown by the larger part of healthcare professionals in Brazil. In addition, the disease in humans presents a clinical picture similar to that of other acute feverish diseases. Therefore, cases of Q fever cannot be diagnosed and their treatment can be
erroneous, which can increase the chances of chronic Q fever occurrence. The inclusion of Q fever as a disease of mandatory notification in humans and the utilization of the "One Health" approach are essential for the confrontation of the disease. Moreover, measures for the control, investigation, and prevention of Q fever will contribute to avoid the occurrence of outbreaks and possible worsening resulting from this zoonosis.

**Keywords:** Q Fever, *coxiella burnetii*, zoonosis, public health, neglected disease.

**RESUMO**

A febre Q é uma doença zoonótica negligenciada em muitos países do mundo. Essa zoonose é causada pela bactéria *Coxiella burnetii*, um patógeno que apresenta estabilidade e resistência ambiental com alta capacidade de causar infecção em humanos, podendo ser fatal. Este estudo de revisão da literatura tem como objetivo descrever os aspectos gerais da febre Q, apresentar os principais casos ocorridos no Brasil e discutir formas para que essa zoonose deixe de ser negligenciada e subnotificada no Brasil. A febre Q ainda é uma doença desconhecida por grande parte dos profissionais de saúde no Brasil. Aliado a isso, a doença em humanos apresenta um quadro clínico similar ao de outras doenças febris agudas. Dessa forma, casos de febre Q podem não estar sendo diagnosticados e sendo tratados de forma equivocada, o que pode aumentar as chances de ocorrência da febre Q crônica. A inclusão da febre Q como uma doença de notificação compulsória em humanos e a utilização da abordagem "One Health" são fundamentais para o enfrentamento da doença. Ademais, medidas de controle, de investigação e de prevenção da febre Q contribuirão para evitar a ocorrência de surtos e possíveis agravamentos decorrentes dessa zoonose.

**Palavras-chave:** Febre Q, *coxiella burnetii*, zoonose, saúde pública, doença negligenciada.

**1 INTRODUCTION**

Currently, the global public health has been impacted by the occurrence of several neglected tropical diseases. Such a fact has been approached by the World Health Organization (WHO); which emphasizes the necessity to be approaching the "One Health" concept for the combat to these diseases.¹ In this context, it is important to highlight that the Q fever is still a neglected disease in many countries all over the world, including Brazil.²,³,⁴,⁵

In the year of 1937, in Queensland, Australia, Derrick has investigated an undiagnosed feverish disease that affected 20 out of 800 workers of a meat factory in Brisbane, passing to call it "query fever". The word "query" was used because of the unexplainable nature of the disease; for this reason, the name “Q fever” has appeared”, in which the "Q" indicates "query". Still in 1937, Burnet and Freeman have isolated the etiologic agent of this disease from the urine and blood of patients in Australia, calling it Rickettsia (*R. burnetii*). In 1938, Davis and Cox isolated the pathogen of ticks in Montana, USA, being it called *R. diaporica*. Furtherly, the causing agent of Q fever was renamed *Coxiella burnetii* to honor both investigation groups that started the studies about this disease.⁶
The Q fever is a zoonosis whose clinical picture in humans can vary since the absence of clinical manifestation up to severe and fatal pictures. The disease can be classified as acute or chronic, presenting a broad, variable, and unspecified clinical aspect, in which the patient could present since flu-like symptoms up to a prolonged fever, pneumonia, hepatitis, among others.\textsuperscript{7} We should reinforce here that \textit{C. burnetii} is one of the most infectious agents to human beings and that a single bacterium is able to cause infection\textsuperscript{8} and, as it will be verified forward, it is classified as biological risk. The incubation period of such pathogen, depending on the size of the inoculum, could last from two to three weeks.\textsuperscript{9}

In the absence of specific treatment with antibiotic therapy, this infection could evolve to the appearance of endocarditis, osteomyelitis or vascular infections, especially in individuals with valvular cardiac alterations and immunosuppressed. In Brazil, despite the serological evidence of its occurrence since 1953, only from the year of 2008, cases of Q fever passed to be irrefutably confirmed, based on molecular analysis in patients of the state of Rio de Janeiro.\textsuperscript{10,11,12,13}

Even being considered as a zoonosis with global distribution, there are few reports of Q fever in Brazil, which could be due to the fact to be a few diagnosed and possibly under-reported disease\textsuperscript{5} in the country\textsuperscript{7,14}. Besides, few studies about this zoonosis have been developed in Brazil, a fact that characterizes an absence of information about it, whose clinical picture is similar to that of other diseases, especially the influenza and dengue fever. Between the more common clinical manifestations, stand out body aches, fever, nausea, vomit, weariness, and cephalgia. So, the small number of reports of this zoonosis, considering the unfamiliarity about it between the healthcare professionals, reinforces the hypothesis that the number of cases should be higher than the cases diagnosed in Brazil\textsuperscript{5} and that part of the resources utilized and allocated for the treatment of dengue and influenza could be saved.

It is important to report that Q fever appears in the attachment of the Normative Instruction nº 50, dated September 24\textsuperscript{th}, 2013, elaborated by the Ministry of Agriculture, Livestock and Supplies, and it is present in the list of mandatory notification infirmities, however in animals.\textsuperscript{15} Nonetheless, differently of other counties such as Australia, Netherlands, and United States, in Brazil the Q fever is not considered yet a disease of mandatory notification in humans.\textsuperscript{16}

Therefore, this present review has as purposes to describe the general aspects of Q fever, present the main publications of cases occurred in Brazil, besides to discuss manners to avoid this zoonosis negligence and underreporting in Brazil.
2 METHODS

Work developed from a literature review in the LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde), SciELO (Scientific Eletronic Library Online), PUBMED (National Library of Medicine and The National Institute of Health) and Google Scholar databases.

In this research, the following descriptors have been used in isolated form or in combination: “C. burnetii”, “Q fever”, “diagnosis”, “epidemiology”, “Brazil”, “febre Q”, “diagnóstico”, “epidemiologia” and “Brasil”.

Publications presenting contents that contributed in a relevant way for the accomplishment of the objectives of this present work were selected, being 41 articles in Portuguese or English, independent from the year of publication.

The boundaries of the regional divisions of Brazil (States and Regions) and South America countries were obtained on the website of the Brazilian Institute of Geography and Statistics (IBGE) (https://www.ibge.gov.br/). Data processing, visualization, and map create were performed via ArcGIS software (http://www.arcgis.com/).

3 RESULTS & DISCUSSION

Etiologic Agent

The etiologic agent of Q fever, the bacteria C. burnetii, is a small coccobacillus with measures of 0.2 to 0.4 μm of width and 0.4 to 1 μm of length. A cellular wall very similar to that of Gram-negative bacteria is presented, however, for many times it cannot be stained with the Gram stain technique, being utilized then the method of Gimenez in clinical samples or laboratory cultures. It is an intracellular pathogen, which replicates into eucaryotic cells, and presents an estimated time of duplication between 20 and 45 hours in in vitro cells culture.9

Initially C. burnetii was classified in the Rickettsiales order, in the family Rickettsiaceae and in the group Rickettsiae, together with genders Rickettsia and Rochalimaea. Based on the sequence analysis of gene 16S rRNA, the bacteria was reclassified from the order Rickettsiales to Legionellales, pertaining to gammaproteobacteria subdivision, in which are included Legionellae spp., Francisella tularensis and Rickettsiella spp..17 In the work of Seshadri et al.18, it was published the complete sequence of C. burnetii genome of the Nine Mile RSA493 strain, an isolated from tick identified in the year of 1935, using the aleatory “Shotgun” method and embracing 1,995,275 pairs of bases.

Two distinct forms of C. burnetii can be observed, the variant of large cells (LCV) and the variant of small cells (SCV), corresponding to a biphasic cycle of development. The bacteria
LCV is a form of exponential replication, with size larger than 0.5 µm, a disperse chromatin and a cellular envelope that is similar to that of classic Gram-negative bacteria. Conversely, the bacteria SCV is a non-replicant stationary form; they are small sticks (0.2 to 0.5 µm of length) characterized by condensed chromatin, a thick cellular envelope and an unusual internal membrane system. The SCVs are stable in the environment and highly resistant to osmotic, mechanic, chemical, thermal, and desiccant stresses. The Centers of Control and Prevention of Diseases of the United States of America (USA) have classified C. burnetii as a category B agent of biological threat due mainly to its high virulence, possibility of aerosolization and its environmental stability.9

An important characteristic of C. burnetii is its antigenic variation, named phase variation, and related mainly to alteration in the lipopolysaccharide (LPS). In phase I, bacteria isolated from arthropods, from infected animals or humans express a virulent form with LPS of smooth variation, highly contagious. In phase II, the bacteria can express a form that could be avirulent or virulent. The phase II, avirulent with LPS of rough variation, is obtained in laboratories after serial passing in embryonated eggs or in cellular cultures. The virulent bacteria of phase II present an LPS molecule rough and truncated, and could differ in the composition of surface proteins, superficial charge and cellular density. The phase variation, probably, is not is not a single step process, as types of LPS of intermediate or semi-rough phase were described.5,19,20 This antigenic variation is important and through it, it is possible to distinguish by laboratory the acute infections, in which predominate the production of anti-C antibodies. Phase II burnetii, of chronic infections, in which predominate the production of anti-C antibodies. Phase I burnetii.16 The phase II antigens are more immunogenic than the phase I antigens.5

Transmission & risk factors

Farm animals such as cows, sheep, and goats are the main reservoir for C. burnetii in the rural area, while, in the urban area, the main reservoirs are the pet animals such as dogs, cats and rabbits. The referred pathogen is present in large amount in the amniotic liquid and in the placenta of such animals and during a childbirth it is estimated that a gram of placenta of infected animals could contain 10⁹ bacteria of C. burnetii. Besides, it is estimated that one milliliter of contaminated milk could contain 10³ bacteria of C. burnetii. The inhalation of contaminated aerosols is the main path of transmission from animals to humans.21 It is important reinforce that the bacterial aerosols can be dispersed by the wind for, at least, 30 km of distance, which contributes for the occurrence of Q fever cases far from the primary
contamination areas. So, cases of the disease can be diagnosed even in persons without recent contact with animals.\textsuperscript{9}

The desiccation of some substances such as urine, stool, milk and birth products, such as the placenta and the amniotic liquid of infected animals could deposit many infectious bacteria in the environment where these animals are maintained; these remnants could remain viable in the environment for years and be widely dispersed by currents of air.\textsuperscript{22} The consumption of raw milk, its derivatives and the tobacco use are also related as risk factors for the development of the disease.\textsuperscript{21} In this context, it is necessary to reinforce the artisanal cheeses, as some works have already identified the presence of genetic material of \textit{C. burnetii} in this food.\textsuperscript{23,24} In addition, in the study of Barandika et al.\textsuperscript{24}, it has been verified the viability of \textit{C. burnetii} in maturated artisanal cheeses for a period up to 8 months, which reinforces the transmission possibility through its consumption as well the stability and resistance of the pathogen.

Between the main risk factors for human infection by \textit{C. burnetii} it is possible to mention the home location close to rural environments, as the bacterial aerosols could be dispersed by the wind and achieve large distances. As other additional risk factors, it is observed that trips to areas where the pathogen is endemic and occupations with direct or indirect contact with parturient ruminants, that is, people working in farms, abattoirs, veterinary physicians and investigators. Although less frequent, it is also possible to mention the gender, as there are reports that men have larger probability to be positive for antibodies against \textit{C. burnetii} or to be diagnosed with Q fever.\textsuperscript{9,25}

According to results presented by Meurer et al.\textsuperscript{26}, it is possible to deduce that living in the rural area increases the chances of exposition to Q fever causing pathogen. In addition, according to Borawski et al.\textsuperscript{27}, Q fever should be considered in the differential diagnosis in cases of fever involving agriculturists and veterinarians who have contact with bovine cattle.

\textbf{Diagnosis}

The clinical diagnosis of Q fever is not easy to be done, as it resembles many diseases, infectious or non-infectious. In the face of it, serological tests have been utilized frequently for the confirmation of Q fever diagnosis, as such tests are easy to be established, being developed mainly in paired blood samples (phase of symptoms and convalescence). The serologic diagnosis is confirmed when an increase of four times occurs in the antibody title, between the paired samples. Besides, the serology enables the differentiation between the chronic and acute
infections of Q fever.\textsuperscript{5,19,28,29} It should be emphasized that the seroconversion, generally, occurs ten to 15 days after the acute disease starting.\textsuperscript{30}

The detection of specific antibodies against the variants of both phases of \textit{C. burnetii} is considered the golden-standard of Q fever diagnosis, being the indirect immunofluorescence (IFI) the reference technique. The ELISA method presents high importance in the serosurveillance investigations or in outbursts; however, its reactive results should be confirmed by the immunofluorescence test. The acute disease can be distinguished from the chronic disease due to the level of titles of antibodies (IgG and IgM, phase I and II). It is convenient to observe that after an acute disease, the respective as classes of Ig could partially decrease during months and be detectable up to several years after the infection, which could complicate the evaluation and the time of an infection, mainly to determine if there is a course of chronic disease.\textsuperscript{31}

Additionally, the cut-offs for a single positive serological title could vary between the countries; however, generally, phase II IgG title $\geq 200$ and phase II IgM title $\geq 50$ are considered significant for the diagnosis of acute Q fever infection, and a phase I title of IgG $\geq 800$ is highly predictive and sensible for the chronic Q fever diagnosis.\textsuperscript{9,32}

Between the molecular methods, the PCR method for detection of \textit{C. burnetii} DNA has become an important tool for the acute Q fever diagnosis, enabling its diagnosis soon after the disease starting, in the three first weeks after symptoms initiation, many times before the occurrence of seroconversion. As the serological response develops itself, the PCR becomes negative in patients who don’t develop the chronic Q fever. As a manner of routine follow-up to evaluate the possible evolution of the disease for the chronic phase, it is indicated that patients do, at least, three consecutive serological tests in the first year after the diagnosis of acute Q fever.\textsuperscript{30}

Many PCR based assays were elaborated for the detection of \textit{C. burnetii} in clinical samples, and the first systems had as targets sequences of different types of plasmids, the RNA 16S-23S, the gene of superoxide dismutase, the gene \textit{coml} or the repetitive elements IS1111 in human or animal samples. The PCR in real time or quantitative PCR (qPCR) is a technique less lengthy than PCR and has the advantage to quantify the number of bacteria in clinical samples, becoming a method very utilized for the diagnosis of Q fever,\textsuperscript{9} besides to be considered indispensable for the early diagnosis of acute Q fever from serum samples.\textsuperscript{33}

The culture could be developed by reference laboratories from clinical samples such as blood, cardiac valves or other specimens of biopsy of surgical tissue; however, this requires a laboratory of biosafety level 3 (BSL-3). The vial technique is still the most utilized method for
the isolation of *C. burnetii*. The pathological analysis of samples of infected tissues, after the immune-histochemical staining, is an interesting tool for the diagnosis when such samples are available.9

The immune-histochemical analysis is very important to examine samples of cardiac valves excised from patients with endocarditis and negative in culture and chronic Q fever suspicion. Such a method is used to detect the presence of *C. burnetii* antigens in tissues soaked with paraffin and fixed in formol, even after the reception of antibiotic therapy. In addition, it could provide a retrospective diagnosis of endocarditis by Q fever, previously not recognized, in patients who relapse after surgery of valvar replacement.29

According to Kantsø et al.34, an adequate diagnosis of Q fever should combine the patient’s clinical data with a follow-up serology and the PCR method.

At Table 1, It is presented an abstract about the diagnosis criteria for Q fever in face of the qPCR and IFI methods.

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<th>Table 1. Criteria of diagnosis for Q fever.</th>
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<td><strong>Diagnosis Method</strong></td>
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<td>qPCR (Single Sample: a sample in the phase of symptoms)</td>
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<td>IFI (Single Sample: a sample in the phase of symptoms)</td>
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<td>IFI (Paired Samples: a sample in the phase of symptoms and another in the phase of convalescence)</td>
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**Treatment & prevention**

Currently, there is not a single one management strategy for the treatment of *C. burnetii* infection due, mainly, to its clinical polymorphism, and studies have revealed that, in every situation, a specific treatment and follow-up should be developed. In the primary infection, after the diagnosis, it is convenient to do a screening of possible risk factors for further complications and start a prophylactic treatment with doxycycline (200 mg/day) and hydroxychloroquine (600 mg/day) to avoid the infection progression. The early diagnosis of endocarditis and vascular infections contributes for the initiation of an adequate antibiotic therapy and the rapid discussion about the necessity, or not, of a surgical treatment. Specific therapeutic measures should be taken in case of infection in pregnant women and in children, due to the fact that these are considered special situations.9
The prevention of Q fever in humans is executed following rules of personal hygiene during the contact with animals and depends on the maintenance of special precautions. If the disease is recognized in animals, special medical care, such as the monitoring of presence of antibodies against *C. burnetii*, should be taken with the people close to these animals. In cases of confirmed contact with the infected animals, it is advisable to use antibiotics prophylactically. Another important measure in the epidemic areas is the suspension of blood donation.  

The “One Health” interventions have the potential to overcome political, social, and economic barriers that restrict the healthcare provision, providing a better control of endemic and neglected zoonoses such as Q fever. Besides, this approach, when strengthening the healthcare systems by means of a better intersectoral collaboration, achieves to increase the capacity of responses to emergent menaces of zoonotic diseases.  

Attempts to produce a safe and effective vaccine occur since 1937, when the etiologic agent of Q fever was discovered. According to Angelakis and Raoult, three types of vaccine were proposed: a living and attenuated vaccine (produced and tested in Russia, but furtherly abandoned due to safety worries); a vaccine extracted with residue of chloroform-methanol (tested in animals, but not in humans); and a vaccine of whole cells inactivated with formalin (Q-Vax), considered acceptable for the human being.  

The Q-Vax vaccine, produced and licensed in Australia, is available since 1989. Its efficacy has been tested in a randomized study controlled with 200 workers of abattoirs. No one between these workers vaccinated against Q fever has developed the disease. Additionally, in Brazil, it was not found any report about the use of vaccine in humans against Q fever.  

**Q fever in Brazil**  

In Brazil, the Q fever is not widely known. São Paulo, Rio de Janeiro, Minas Gerais, Bahia, and Pernambuco are states that presented information about serological evidences in humans and/or animals (Figure 1).
A serological survey in workers of a cold store in the municipality of São Paulo, in the year of 1953, has described Q fever for the first time in Brazil. In this survey, 2 samples, from a total of 643 analyzed ones, were positive with significant value.\textsuperscript{10} Publication in the year of 2006, has reported that a patient of male gender, aged 45 years, with endocarditis, history of rheumatic vascular heart disease and valvar replacement, admitted at the Heart Institute of the Faculty of Medicine of the University of São Paulo, has received the serological diagnosis of infection by \textit{C. burnetii}.\textsuperscript{40}

Also in the year of 2006, clinical and serological evidences of symptomatic infection by \textit{C. burnetii} were identified in 16 patients (2.2\%) of the 726 evaluated ones between 2001 January and 2004 June, in the municipality of Juiz de Fora, located in the state of Minas Gerais.\textsuperscript{41}

A severe case of endocarditis by autochthonous \textit{C. burnetii} with fatal endpoint, despite the adequate antibiotic therapy and valvar surgical treatment, has been reported in the year of 2007 in the state of Bahia; the patient was a 41 years aged man, natural and native of the municipality of São Felipe, Bahia.\textsuperscript{7}
In a study developed by Lamas et al.\textsuperscript{42} in 2009 in the city of Rio de Janeiro, it was verified that the seroprevalence for \textit{C. burnetii} in HIV-positive individuals was of 3.2%, that is, four (women) out of 125 patients studied (64 women and 61 men).

The first molecular evidence of \textit{C. burnetii} in Brazil was reported by Lemos et al.\textsuperscript{11} in 2011, in a patient of male gender, living in the municipality of Itaboraí, in the state of Rio de Janeiro, which presented fever with 40 days of duration associated to thrombocytosis. The infection by \textit{C. burnetii} was confirmed by serological and molecular (PCR) analysis. Blood samples have been also collected from the blood of patient’s family and dogs. The patient’s wife and 13 examined dogs have shown serum reactivity.

In the year of 2013, Lamas et al.\textsuperscript{43} utilized the PCR method to detect \textit{C. burnetii} in cardiac valves of patients operated by endocarditis with negative hemoculture. Such samples have been obtained from a reference hospital in the state of Rio de Janeiro, between the years of 1998 and 2009. Among the 51 tested valves, one was positive for \textit{C. burnetii}.

A study developed by Mares-Guia et al.\textsuperscript{44} in 2016, in the municipality of Itaboraí, placed in the state of Rio de Janeiro, has investigated, using the PCR technique, the presence or not of DNA fragments of \textit{C. burnetii} in blood samples from 272 patients with dengue suspicion. From this total, nine (3.3%) were diagnosed with active Q fever. Additionally, it is important to reinforce that in the samples of 26 patients (10%), anti-\textit{C. burnetii} antibodies have been detected.

Lemos et al.\textsuperscript{45} have investigated in 2018 if cases of Q fever were occurring in 47 cadets registered in a training program of the Military Academy of Firefighters Dom Pedro II, in Rio de Janeiro. The Q fever was described in five cadets, being this one the first report of an outbreak of this disease between military people in Brazil. Also in 2018, Souza et al.\textsuperscript{39} have investigated the presence of anti-\textit{C. burnetii} antibodies in sheep and goat in the municipality of Petrolina, located in the state of Pernambuco. Anti-\textit{C. burnetii} antibodies have been detected in 2.2% (9/403) of sheep and 2.1% (9/412) of goat.

In 2020 Mioni et al.\textsuperscript{46} have estimated the prevalence of \textit{C. burnetii} in bovines forwarded to abattoirs in the state of São Paulo. The authors have found 23.8% (360/1515) of seroprevalence. In the same year, Rozental et al.\textsuperscript{23} investigated the presence of DNA of \textit{C. burnetii} in artisanal cheeses produced in the microregion of Serro, state of Minas Gerais, with detection in five (9.43%) out of 53 samples analyzed.

In 2021, data from Meurer et al.\textsuperscript{26} have shown 4.8% of prevalence of anti-\textit{C. burnetii} antibodies in serum samples from 437 patients with dengue suspicion in the state of Minas Gerais.
4 CONCLUSION

Considering the previously exposed, differently from the occurrences in many countries, this zoonosis requires more studies and investigation in Brazil. This is a disease with high infectivity, its clinical picture is similar to that of other acute febrile diseases and it could become chronic with risk of death. The disease pathogen presents environmental resistance and stability, its main reservoirs are livestock animals (bovines, sheep and goats) and its transmission occurs mainly by the inhalation of contaminated aerosols, which can be dispersed by the wind for, at least, 30 km of distance. In face of the exposed, the Q fever cannot be neglected in Brazil, as recent publications have shown the presence of the disease and the circulation of its pathogen in the country.

The inclusion of Q fever as a disease of mandatory notification in humans in Brazil will be an important step for the end of its negligence and underreporting. Additionally, it is of large relevance, in the context of public health, to consider Q fever as an option during investigations of acute febrile diseases and cases of endocarditis with negative hemoculture, avoiding so, possible mistakes in the diagnosis and, consequently, unnecessary expenses of public resources addressed to health.

The execution of epidemiological studies for the investigation of seroprevalence of Q fever in humans and animals in Brazil will provide important information about this zoonosis, as for the sector of human health as for the sector of animal health, in order to take, together, within an “One Health” perspective, decisions that decrease the losses caused by this disease.

Funding
This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and by the Programa de Pesquisa para o SUS (PPSUS) [grant number CDS APQ 04335/17].

ACKNOWLEDGMENTS
The authors would like to thank FAPEMIG, the PPSUS and the Programa de Pós-Graduação em Saúde at the Universidade Federal de Juiz de Fora by the full support in carrying out this study.
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