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Seroprevalence estimate and risk factors for *Coxiella burnetii* infections among humans in a highly urbanised Brazilian state

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Background: Q fever is among the top 13 global priority zoonoses, however, it is still neglected and under-reported in most of the world, including Brazil. Thus, we evaluated the seroprevalence of and the risk factors for *Coxiella burnetii* infections in humans from Minas Gerais, a highly urbanised Brazilian state.

Methods: *Coxiella burnetii* was searched for patient samples (n=437), which were suspected of then later confirmed as negative for dengue fever, by the indirect immunofluorescence technique and real-time PCR. Risk factors for infections and spatial clusters for both *C. burnetii*-seropositive individuals and livestock concentration were evaluated.

Results: We found that 21 samples (4.8%; 95% CI 3.0 to 7.2%) were reactive for at least one class of anti-*C. burnetii* antibodies (titer of ≥64), with rural residence (p=0.036) being a risk factor. Also, two spatial clusters of seropositivity were found within a significant area by Scan, and a probable relationship between the Scan result and the livestock concentration by area was found.

Conclusions: Seropositive individuals were associated with rural residence, with a likely relationship with the livestock concentration. Thus, this study establishes baseline figures for *C. burnetii* seroprevalence in humans in a state of Brazil, allowing the monitoring of trends and setting of control targets, as well as more representative longitudinal and risk analysis studies.

Keywords: *Coxiella burnetii*, public health, Q fever, seroprevalence, zoonosis

Introduction

Neglected tropical diseases have a major impact on global public health and are being addressed and discussed by the WHO through an ambitious roadmap focused on the control and elimination of these diseases by the year 2030.[1] In this context, Q fever due to *Coxiella burnetii* has a relevant impact on public health in several countries.[2-4]

Coxiella burnetii presents environmental resistance and stability, and is one of the most infectious microorganisms to humans. This pathogen has a worldwide distribution and its most common reservoirs are cattle, sheep and goats. It is found in urine, faeces, milk and the birth fluids of infected animals.[5-7] Human infections mainly occur after inhalation of contaminated aerosols, and ingestion of unpasteurised milk or cheese is not negligible as an additional risk.[8] Such aerosols could be dispersed by the wind, thus contributing to the occurrence of Q fever cases far from the primary contamination areas. *Coxiella burnetii* is also an important tick-borne pathogen, but its role in human infection is still controversial.[5,7]

Large outbreaks of Q fever have been reported from numerous geographical locations, including the USA, Australia and Europe.

The largest series of outbreaks occurred in the Netherlands in 2007–2010, with >4000 Q fever cases documented, although it was estimated that there may have been as many as 40 000 cases. Although the outbreak was controlled, the postepidemic has raised concerns regarding the possibility of future chronic cases.[9] Since then, serological surveys[2,4,10] and molecular studies[3,11,12] on Q fever have been carried out in increasing numbers in several countries, which demonstrates the global concern regarding this zoonosis.

This disease is among the top 13 global priority zoonoses in terms of its impact on human health, amenability to livestock interventions, severity of disease and emergence. Q fever is a true zoonotic disease considered as emerging or re-emerging in many countries. However, as it has only been investigated in a few countries, it is difficult to identify hotspots.[13] In this context, Q fever prevention strategies should incorporate human, animal and environmental domains with a One Health approach. This would engage cross-sectoral collaboration among multiple stakeholders to control Q fever holistically with more efficiency and cost-effectiveness.[14]

In Brazil, the first human serological evidence of *C. burnetii* was reported in 1953,[15] and over the past decade, case reports, seroprevalence studies and, more recently, outbreaks and molecular detection of the pathogen involved, have been published regarding Q fever.[11,16-19] However, these studies do not yet provide a global scenario of Q fever in Brazil, which to date has neither Q fever nor coxiellosis national control programmes. Even so, case reporting has been mandatory for animals since 2013 and for humans since 2014, but cases are overlooked and underreported.[11,20]

Q fever is a less well-known, difficult to diagnose, notifiable zoonosis, causing difficulties with recognition or suspicion of the disease by health professionals. In people, around 50% of infections may be asymptomatic; other patients have influenza-like symptoms, such as are common in influenza, malaria and dengue fever; a minority have atypical pneumonia or hepatitis; in around 5% of patients, chronic infection becomes established.[13]

From 1995 to 2015, dengue fever accounted for >18 million cases across the American continent. Brazil contributed to 55% of that total, and 48% of the 8788 consequent deaths in the Americas over the same period.[21] Accordingly, early differential diagnosis of rickettsial diseases and Q fever from dengue fever may permit early antibiotic treatment.[22]

Unlike what occurs in several countries of the world, there are few studies in Brazil that use molecular diagnosis to confirm *C. burnetii* in human specimens.[11,16,23] With this methodology, Mares-Guia et al.[11] found higher rates of *C. burnetii* DNA in febrile patients suspected of dengue fever confirmed negative than in those who were positive. Brazil has a large number of febrile diseases, such as dengue fever, which make diagnosing Q fever more difficult; it still has the second largest livestock population and one of the largest beef cattle herds for export in the world[24] and, finally, *C. burnetii* has been detected in humans and livestock.[11,16] Considering this scenario, more robust seroepidemiological surveys for a more complete assessment of *C. burnetii* are justified in this country.

Thus, this cross-sectional study aimed to estimate the prevalence of *C. burnetii*, by qPCR and the indirect immunofluorescence technique (IFI), among febrile patients suspected of dengue fever whose samples were confirmed negative in Minas Gerais, Brazil. In addition, it aimed to evaluate the factors associated with *C. burnetii*-seropositive individuals. Minas Gerais is the second most populous Brazilian state, with about 21.2 million inhabitants,[25] and it is one of the largest livestock and beef exporters in Brazil.[26]

Material and Methods

Design, study location and sampling

This was a randomised, cross-sectional survey to evaluate the prevalence of and associated factors for *C. burnetii*-seropositive individuals in Minas Gerais, a highly urbanised state of Brazil.

The search for antibodies encompassed single serum samples from febrile patients suspected of having dengue fever from January 2017 to August 2018; having presented negative results, the samples were sent to the Ezequiel Dias Foundation, the central state public health laboratory. One of the inclusion criteria was that the samples had the ages of 1 and 10 d from the start of febrile symptoms. This criterion was based on previous publications to strengthen causal links between febrile symptoms and Q fever, if *C. burnetii* DNA was found.

The sample size to estimate the seroprevalence was based on a finite population of 31 200 samples confirmed negative for dengue fever, a margin of error of 4.7%, confidence level of 95% and an expected proportion of 50% *C. burnetii*-positive exposure. As a rule, we used 50% of expected positive samples, as we had no prior knowledge of what percentage of anti-*C burnetii* antibodies we would find among the febrile patient samples analysed. Thus, the calculated sample size was 429, according to Fleiss et al.[27] Additionally, the sample size for the unpaired case-control study (risk factors analysis) was based on a 95% bilateral (1-alpha) confidence level, 80% power (% probability of detection), control-to-case ratio of 4, hypothetical ratio of 40% exposed controls prevalence, according to the higher ratio described by Grace et al.,[13] and less extreme ORs of 2.00 to be detected, based on Lacheheb and Raoult's findings.[10] The sample size with continuity correction was 445, according to Fleiss et al.[27] For logistical reasons, we used a final sample of 437, an average taken from the two previous values, which was randomly drawn from among the available ones, encompassing 126 municipalities of the state of Minas Gerais, Brazil. The sample size was calculated by Epi Info version 7 for Windows software (Center for Disease Control and Prevention, Atlanta, GA, USA).

Data collection

Study subjects' information such as age, gender, skin colour, residential zoning and global positioning system were obtained from the Brazilian National Health Information System.

The Digital Mesoregions, Federation Units (states) and Municipalities Mesh of the Brazilian Political-Administrative Division were obtained from The Brazilian Institute of Geography and Statistics (IBGE; https://www.ibge.gov.br).

Real-time PCR

TaqMan real-time PCR (qPCR) was used for the molecular detection of *C. burnetii*. The extraction of the *C. burnetii* DNA from the

Table 1. Sequences of primers and probes used in the qPCR

Gene	Primer forward sequence	Primer reverse sequence	Probe sequence
IS*1111*[a]	5′-AAAACGGATAAAAAGAGTCTGTGGTT-3′	5′-CCACACAAGCGCGATTCAT-3′	5′ FAM-AAAGCACTCATTGAGCGCCGCG-BHQ 3′
RNaseP	5′-AGATTTGGACCTGCGAGCG-3′	5′-GAGCGGCTGTCTCCACAAGT-3′	5′ FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1 3′

[a]The use of the IS*1111* gene insertion sequence is considered the ideal target for the detection of *C. burnetii* by PCR; the repetitive element of this target has a multiple gene with 7–110 copies per isolate of *C. burnetii*,[6] thus a greater sensitivity of the assay is shown.

patient's serum samples and the positive control was performed following the instructions recommended by the manufacturer, using the commercial kit QIAamp DNA stool MiniKit (QIAGEN, Hilden, Germany).

Thus, the diagnosis was determined by the presence or absence of a specific amplification curve for the IS*1111* element of *C. burnetii*. Briefly, qPCR reactions had a final volume of 25 μL, containing 12.5 μL of 2x buffer containing CXR (GoTaq Probe qPCR Master Mix kit, Promega, Madison, Wisconsin, EUA), 900 nM of forward and reverse primers, 200 nM of probe[12] and 10 μL of DNA extracted from each clinical sample. To detect internal control, the human RNaseP gene forward and reverse primers and probe were used. Sequences of the forward and reverse primers and probe are shown in Table 1. The DNA was initially denatured at 95°C for 2 min and amplified at 95°C for 15 s and 60°C for 1 min, for 45 cycles, in the StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, Massachusetts, EUA) real-time cyclers.

Immunofluorescence

Anti-*C. burnetii* antibodies were searched for in the serum of individuals using the IFI. IgM and IgG anti-*C. burnetii* antibodies were investigated utilising commercial blades (SCIMEDX Corporation, Denville, NJ, USA) containing phase I and II antigens, according to the manufacturer's recommendations.

The initial screening of serum samples, positive and negative controls was conducted using dilutions of 1:16 in phosphate buffered saline (PBS) and a pH of 7.3–7.5 (to avoid the prozone phenomenon). Then 25 μL of diluted serum was spotted in duplicate onto a glass slide coated with *C. burnetii* phase I or phase II antigen (SCIMEDX). After incubation at 37°C for 30 min, the slides were washed with PBS (2x, during 5 min) and dried in the air at room temperature before adding the 25 μL of combined conjugate containing fluorescein-labelled goat antihuman IgG and IgM (H + L). The incubation and washing steps were repeated and the slides were dried, covered with a coverslip containing 50–80% glycerol in PBS (pH: 7.5–8.9), kept under the shelter of light and humidity until the time of reading and read under a fluorescence microscope (400×; Olympus BX51 Fluorescence Microscope).

The serum was considered positive if fluorescence was observed at a dilution of 1/16 or greater. All antibodies used in the reaction were manufactured by KPL/SeraCare (Gaithersburg, MD, USA).

As we used a single sample in the phase of symptoms, for a sample to be considered as active Q fever disease according to the IFI, the following situations were utilised as cut-off points: an IgG titer of phase II $\geq$200 and IgM titer of phase II $\geq$50 and/or IgG titer of phase I$\geq$800.[5,28] In addition, for the calculation of anti-*C. burnetii* antibodies seroprevalence from a single sample, the ones which were reactive and suggestive of previous exposure to *C. burnetii* were those with an antibodies titer of$\geq$64,[17] for at least one class of the antibodies investigated.

Explanatory variables

Rural residence, concentration of livestock animals by area and the number of inhabitants in each municipality were analysed as the main explanatory variables.

The numbers of bovine animals, goats and sheep, considered as the main animals related to the transmission of *C. burnetii* to humans according to the literature,[5,7] were obtained from the 2018 Municipal Livestock Research IBGE data. Additionally, livestock concentration indicators were created by dividing the numbers of bovine animals, goats and livestock animals by the respective territorial area (km^2) and 1000 inhabitants of each municipality based on the 2019 IBGE data.[25]

Statistical analysis

Coxiella burnetii seroprevalence with 95% CI, and its significantly associated factors ($p \leq 0.05$), was assessed using χ^2 and Fisher's exact tests. The OR with 95% CI was used as a measure of association. SPSS version 21 software (IBM Corporation, Armonk, NY, USA) was used in these analyses.

Spatial analysis

Spatial analyses were performed as follows. A map of spatial distribution of *C. burnetii*-seropositive and negative individuals in the municipalities of Minas Gerais was drawn. Also, spatial distribution (choropleth) maps of the livestock concentration by both area and inhabitants were drawn using the quantile classification. Kernel density estimation (KDE) was performed to identify clusters of *C. burnetii*-positive sample occurrences (quadratic function, density calculation and adaptive radius) and a spatial scanning (Scan) map was drawn to identify spatial clusters with statistical significance (purely spatial analysis scanning using the Bernoulli model). Data processing, interpretation, visualisation and analysis were performed using ArcGIS (http://www.arcgis.com/), SatScan (https://www.satscan.org/) and TerraView (http://www.dpi.inpe.br/terralib5/wiki/doku.php) software.

Table 2. Reactive samples for *C. burnetii* by the IFI

Municipality	Days of febrile symptoms	IgM phase II	IgG phase II	IgM phase I	IgG phase I
Arcos	9	-	1:128	-	-
Arcos[a]	6	-	-	1:16	-
Belo Horizonte[a]	6	-	-	1:16	1:16
Belo Vale	6	-	1:64	-	-
Brumadinho	7	-	1:64	-	-
Campos Gerais	5	-	1:64	-	-
Cristina	8	-	-	1:128	1:128
Divinópolis	5	1:128	1:128	1:128	1:128
Divinópolis[a]	9	-	1:16	-	-
Igarapé[a]	8	-	-	1:32	1:32
Lima Duarte	6	-	1:64	-	-
Mariana	9	-	-	1:128	1:128
Oliveira	10	1:64	1:64	-	-
Pedro Leopoldo	7	-	-	1:128	-
Pedro Leopoldo	7	-	-	-	1:64
Rio Piracicaba	7	1:64	-	-	-
Sabará	7	1:64	-	-	-
Santa Bárbara	6	-	1:128	-	-
Santana do Deserto	8	-	-	1:64	1:128
São José da Barra	9	-	1:128	-	-
Sarzedo	10	-	1:128	-	-
Sarzedo	6	1:64	-	-	-
Três Corações	3	-	-	1:128	1:64
Varginha	6	-	1:64	-	1:64
Varginha	9	-	1:64	-	-
Mean±SD		76.8±28.6	84.9±37.7	85.3±52.5	83.6±45.1

[a]Samples with antibodies titre <64 not considered suggestive of previous exposure to *C. burnetii*.

Results

qPCR

Among 437 clinical samples tested, 403 showed non-detectable *C. burnetii* DNA and a detectable curve of amplification for specific endogenous control (human RP gene). The remaining 34 analysed samples did not present a specific amplification curve, in the qPCR, for both investigated targets and therefore were considered inappropriate for molecular diagnosis due to the absence of genetic material. As evidence of the quality of this qPCR, we reinforced some aspects. First, the limit of detection was 20 fg of *C. burnetii* DNA. Second, the negative controls presented undetectable results, evidencing absence of contamination. Third, all positive controls presented detectable results, showing that this technique was able to identify the targeted DNA sequence.

Immunofluorescence

Among the 437 samples analysed by the IFI, 25 were reactive for at least one class of anti-*C. burnetii*. Of these, 21 samples (4.8%; 95% CI 3.0 to 7.2%) presented antibodies with a titer of ≥64, thus were considered as having been previously exposed to *C. burnetii*. Table 2 shows the municipality residence of those individuals who had samples reactive to *C. burnetii*, the days of symptoms up to the date of blood collection, as well as the classes and titers of the reactive antibodies.

It is possible to emphasise three situations related to samples with an antibodies titer of≥64. First, six patients (28.57%) had reactive samples only for phase I classes of anti-*C. burnetii* antibodies, indicating possible development of chronic Q fever in the past and that phase II anti-*C. burnetii* antibodies probably decreased over time to the point of not being detected anymore, which suggests an older exposure. Second, 13 patients (61.91%) had reactive samples only for the class of phase II anti-*C. burnetii* antibodies, which suggests that they could have developed acute Q fever in the past, without becoming chronic. Third, two patients (9.52%) had reactive samples for the phase I and phase II classes of anti-*C. burnetii* antibodies, which indicates that they could have developed chronic Q fever more recently, different to those patients described in the first situation.

Descriptive epidemiology and associated factors for previous exposure

Table 3 describes the individuals whose samples were analysed, as well as evaluating the associated factors for previous exposure

Table 3. Characteristics of seroreactive patients (titer of ≥64) and their associated factors

Variable	Sample (n=437)	Number of reactive patients (%)	p	OR (95% CI)
Residence zone			0.036[a]	
Rural	40	5 (12.50)		3.31 (1.14 to 9.58)
Urban/periurban	387	16 (4.13)		1.00
Missing	10	0 (0.00)		
Age, y			>0.05	
0–9	46	2 (4.35)		-
10–19	66	2 (3.03)		
20–29	97	2 (2.06)		
30–39	77	4 (5.19)		
40–49	63	5 (7.94)		
50–59	52	4 (7.69)		
60–69	27	2 (7.41)		
70–79	7	0 (0.00)		
80–89	0	0 (0.00)		
90–99	1	0 (0.00)		
Missing	1	0 (0.00)		
Gender			>0.05	
Female	228	12 (5.26)		-
Male	208	9 (4.33)		
missing	1	0 (0.00)		
Skin colour			>0.05	
Yellow	14	0 (0.00)		-
White	140	7 (5.00)		
Brown	115	5 (4.35)		
Black	21	1 (4.76)		
Missing	147	8 (5.44)		

[a]p by Fisher exact probability test.

to *C. burnetii* (titer of≥64). Rural residence was considered to be a risk factor for previous exposure to *C. burnetii* (p=0.036).

Figure 1 shows the geographical distribution of the IFI results. The spatial analysis (Figure 2) shows the presence of two *C. burnetii*-seropositive clusters of high and medium densities, respectively, in the Metropolitana de Belo Horizonte and Sul/Sudoeste de Minas regions of the study state. These two clusters are within a statistically significant spatial cluster by Scan analysis. Figure 2C shows that there is also probably a relationship between the Scan result and the animal concentration by area.

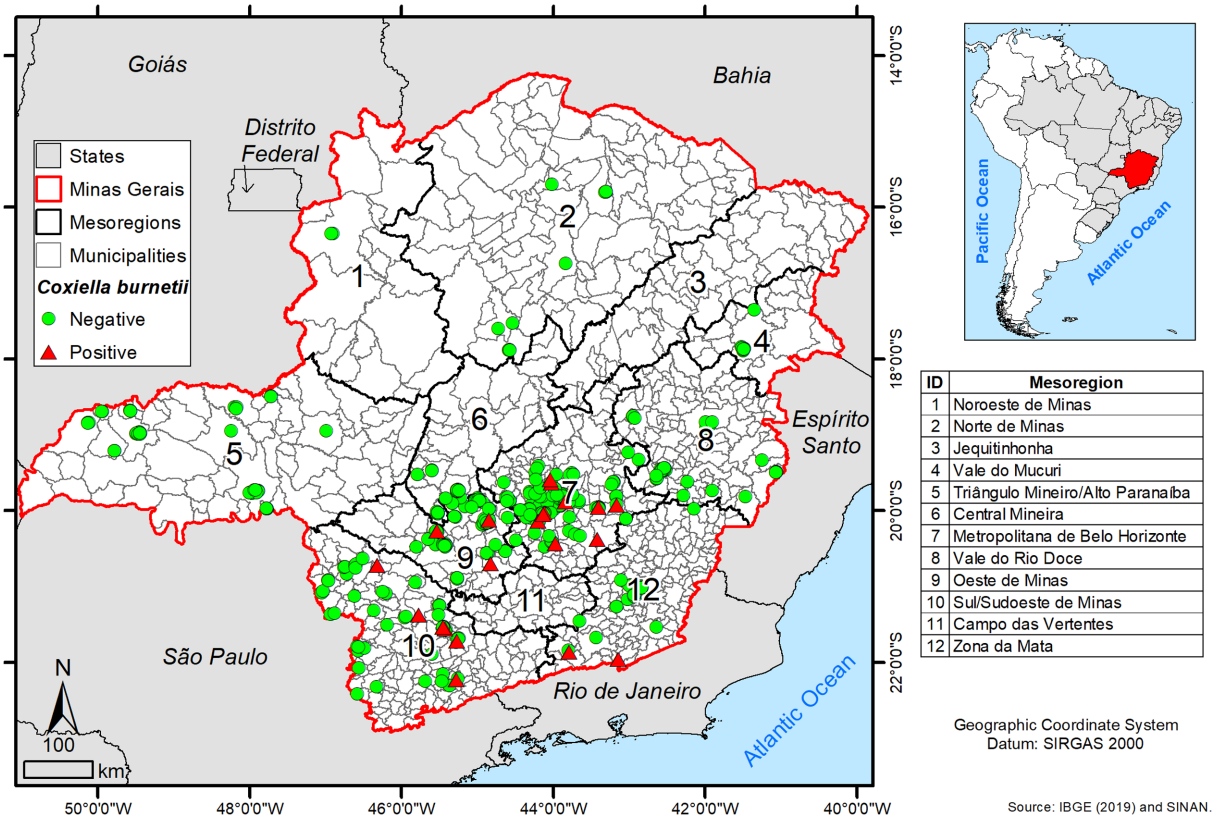
Table 4 shows the bovine, goat and sheep herds (units) by municipalities with at least one reactive sample. The concentration of livestock animals by both territorial area and 1000 inhabitants of these municipalities are also shown in this table. Bovine herds made up >93% of them.

Discussion

Our results showed that 21 samples (4.8%; 95% CI 3.0 to 7.2%) were considered to be previously exposed to *C. burnetii* among the 437 analysed, and that rural residence was a risk factor for exposure. Also, two *C. burnetii*-seropositive spatial clusters were detected.

These results on human exposure to *C. burnetii* are important because this pathogen causes Q fever, a neglected disease that is considered one of the 13 priority global zoonoses. It is estimated to cause 3 500 000 cases with 3000 deaths worldwide annually.[13] However, in Brazil it is almost neglected and there are still no health programmes aimed at humans or animals. Accordingly, an earlier, less representative study in territorial terms included only two municipalities of the same state but reported acute cases of *C. burnetii* among febrile patients,[18] which illustrates the need for continuous and more robust monitoring in this country and, thus, the importance of the current study.

Regarding seroprevalence, Neare et al.[2] in Estonia, Gidding et al.[4] in Australia, Costa[18] among HIV-negative patients from the Juiz de Fora municipality in Brazil, Costa[18] among HIV-negative patients from the Piau municipality in Brazil and Lamas et al.[17] among HIV-positive patients from the Rio de Janeiro municipality in Brazil, reported results of 3.9, 5.6, 3.6, 3.9 and 3.2%, respectively, which were comparable with the current study. Another Brazilian study, using similar methods and interpretation to our study, found a higher seroprevalence (10%) among patients suspected of having dengue,[11] perhaps because that study



ID	Mesoregion
1	Noroeste de Minas
2	Norte de Minas
3	Jequitinhonha
4	Vale do Mucuri
5	Triângulo Mineiro/Alto Paranaíba
6	Central Mineira
7	Metropolitana de Belo Horizonte
8	Vale do Rio Doce
9	Oeste de Minas
10	Sul/Sudoeste de Minas
11	Campo das Vertentes
12	Zona da Mata

Figure 1. Spatial distribution of positive and negative cases in the municipalities of Minas Gerais, Brazil.

covered only a single city in a region that had Q fever outbreaks. Based on classes of IgG and IgM, the current study evidenced three groups of past exposure to *C. burnetii* among the analysed sample: chronic Q fever in an older exposure, Q fever in the past without becoming chronic and chronic Q fever more recently.

Interestingly, the current study showed that rural residence was considered to be a risk factor for *C. burnetii* infections, as previously found by other researchers.[10] Thus, two spatial *C. burnetii*-seropositive clusters were found within a significant area by Scan, and there was also a probable relationship between the Scan result and the livestock concentration by area. The large contingent of livestock animals registered in all the municipalities of this study, added to the fact that Brazil has the second largest livestock population and the most commercial one in the world, along with this state having one of the largest livestock populations in Brazil,[24,26] reinforces that this may be an appropriate scenario for human occurrences of *C. burnetii*. This scenario of risk was demonstrated by other findings made by our team,[19] who detected *C. burnetii* DNA from raw cow's milk artisanal cheese, which is produced by almost 10% of rural family-based processing agroindustries in the same state in Brazil, suggesting that this pathogen is widely present among the respective dairy cattle herds. This reinforces the zoonotic potential of *C. burnetii*, corroborating other authors.[5,8,29]

The results obtained by qPCR and the IFI suggest that the patients in this study did not have any evidence of active Q fever or recent exposure at the time of blood collection, as no *C. burnetii* DNA was detected[12] and they all had antibody titers below the cut-off point necessary to consider the disease active or recent exposure using a single sample.[5,28] We did not find any PCR-positive samples like Mares Guia et al.,[11] who found samples from suspected dengue fever patients, perhaps because, unlike the current study, outbreaks of *C. burnetii* were occurring at the site of their study.

Although molecular diagnosis of *C. burnetii* by PCR is an important diagnostic tool widely used in several countries around the world,[5,12] its use is still scarce in Brazil and there are no regulations that control its use in this country. However, the method has been used for research purposes in Brazil and other countries in South America. Lemos et al.[16] reported the first molecular evidence of *C. burnetii* in Brazil. Lamas et al.[23] evaluated the heart valves of 51 patients with endocarditis and negative blood cultures, finding one positive. Mares-Guia et al.[11] analysed 272 febrile patients in one municipality, finding 9 (3.3%) positive. Orrego et al.[30] investigated Q fever in 143 farmers, finding 25.9% positivity. Additionally, it is noteworthy that the present study, to the authors' knowledge, is the most robust in South America to use molecular methodology in an attempt to detect *C. burnetii*.

This study has limitations that should be discussed, as they may have a small effect on the interpretation of its results. The samples used were those that were available, which were sent by several municipalities in Minas Gerais for the diagnosis of dengue

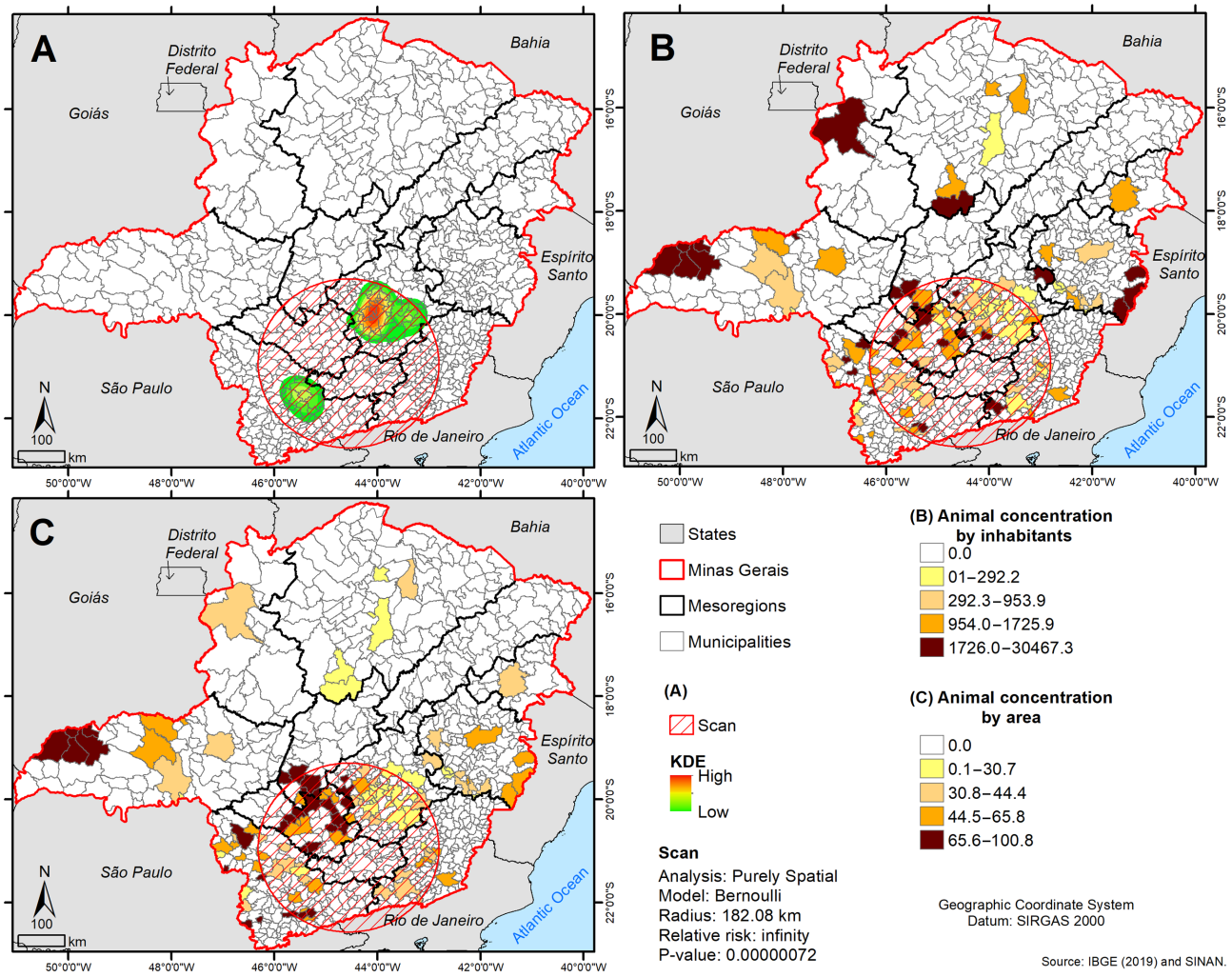


Figure 2. (A) Kernel density estimation (KDE) and spatial scanning (Scan); map of spatial distribution of the number of animals concentration by (B) inhabitants and (C) area in the municipalities of Minas Gerais, Brazil.

fever, instead of being chosen randomly. Thus, not all municipalities in the state were represented in this sample. Even so, this was the most robust national survey in terms of sample size and spatial distribution in the country. An additional limitation of this study is that a single sample was analysed, and it was not possible to evaluate seroconversion. However, the methodologies described in the work conducted by Dupont et al.,[28] Lamas et al.[17] and Eldin et al.[5] made it possible to infer the infection activity for *C. burnetii* at the time the samples were collected, as well as the exposure chronology.

This research was conducted through cooperation between the health (Fiocruz, Funed), agriculture (Embrapa) and education (UFJF) sectors, bringing together researchers, laboratories, financial resources and efforts to research *C. burnetii* in a multidisciplinary way, in order to support related public policies. Finally, as a knowledge transfer, we made a specific recommendation to the Ministry of Health of Brazil and related bodies about including Q fever in the practice of differential diagnosis of acute febrile diseases and of endocarditis with negative haemoculture in Brazil. We think this is an important step for this disease to no longer be neglected and under-reported, making its diagnosis possible.

In conclusion, the *C. burnetii* seroprevalence found indicates that human exposure to this pathogen is occurring in this state of Brazil, and is possibly being neglected. This reinforces the need for greater dissemination and awareness about the occurrence of this zoonosis in Brazil on the part of healthcare authorities, healthcare professionals and the population. Additionally, a joint cooperation with investigation, control and prevention measures encompassing humans, animals and environmental domains of this disease with a One Health approach is essential in this country. This study also established baseline figures for *C. burnetii* seroprevalence in humans in Minas Gerais, Brazil, allowing the monitoring of trends, setting of control targets and, in future, even more representative longitudinal and risk analysis studies.

Table 4. Head numbers of bovine animals, goat and sheep (livestock) in the municipalities and the concentration of these livestock animals per territorial area and per 1000 inhabitants

	Size of herds					
Municipality of residence	Total heads of bovine animals, goat and sheep (livestock)	Bovine animals' heads (%)	Goats' heads (%)	Sheep heads (%)	Heads of livestock/km^2 of territorial area	Heads of livestock/1000 inhabitants
Arcos	36 697	99.59	0.14	0.27	71.97	915.32
Belo Vale	14 052	99.75	0.00	0.25	38.40	1821.39
Brumadinho	15 075	97.88	0.40	1.72	23.58	375.91
Campos Gerais	25 167	97.96	0.13	1.91	32.71	874.64
Cristina	24 345	99.82	0.05	0.13	78.20	2376.98
Divinópolis	49 257	99.33	0.26	0.40	69.56	206.76
Lima Duarte	31 553	98.60	0.06	1.34	37.18	1889.63
Mariana	14 288	97.77	1.12	1.11	11.96	235.29
Oliveira	42 156	96.18	0.05	3.76	46.98	1011.25
Pedro Leopoldo	12 645	98.81	0.16	1.03	43.18	196.78
Rio Piracicaba	12 946	98.68	0.36	0.96	34.70	902.85
Sabará	2394	93.94	3.05	3.01	7.92	17.56
Santa Bárbara	4539	99.29	0.44	0.26	6.63	144.90
Santana do Deserto	6261	99.62	0.00	0.38	34.28	1574.70
São José da Barra	11 755	99.53	0.47	0.00	38.13	1582.95
Sarzedo	908	96.25	0.99	2.75	14.61	27.72
Três Corações	45 275	99.39	0.21	0.40	54.68	569.63
Varginha	16 947	99.73	0.00	0.27	42.86	125.02

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Data availability: All data will be available if requested to the corresponding author.

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