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Spotted Fever in the Morphoclimatic Domains of Minas Gerais State, Brazil

Emília de Carvalho Nunes[1], Nicole Oliveira de Moura-Martiniano[2], Ana Íris de Lima Duré[3], Felipe Campos de Melo Iani[3], Stefan Vilges de Oliveira[4], Flávio Luis de Mello[5] and Gilberto Salles Gazêta[2]*

[1] Programa de Pós Graduação em Biologia e Comportamento Animal, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil, [2] Laboratório de Referência Nacional em Vetores das Riquetsioses, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [3] Laboratório de Riquetsioses e Hantavirose, Fundação Ezequiel Dias, Belo Horizonte, Brazil, [4] Faculdade de Medicina, Universidade Federal de Uberlândia, Uberlândia, Brazil, [5] Escola Politécnica, Universidade Federal do Rio deJaneiro, Rio de Janeiro, Brazil

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***Correspondence:**
Nicole Oliveira de Moura-Martiniano
nicmoura@ioc.fiocruz.br

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In Brazil, the tick-borne rickettsiosis known as Spotted Fever (SF) has been recorded from 59% of the Federative Units, however, the knowledge of the epidemiology and dynamics of human infection remains incipient in certain areas, complicating appropriate public health actions to inform the general population and control the disease. Here, we improved the interpretation of epidemiological information of SF cases recorded for an important endemic area. A descriptive epidemiological study was carried out based on records in the SINAN (Notifiable Diseases Information System) SF case databases. Data analysis was performed using Python programming language, Pandas library and Qgis map making. To evaluate the sociodemographic, clinical, assistance, laboratory and epidemiological characteristics, simple and relative nominal values of occurrences, means and standard deviations, and molecular analyzes were performed to identify the bioagent present in biological samples collected during each case investigation. Of the 298 confirmed cases, 98 resulted in death, the number of cases increased from 2011, and the disease scenario had 32.8% lethality. Overall, 207 cases involved men, and lethality was higher in this group. The most affected age group was 30 to 59 years old. The majority of patients reported having had contact with animals such as ticks, capybara and domestic animals such as dogs and cats. The results corroborate existing studies in areas of severe SF cases in Brazil. Despite reports of SF cases from the Cerrado Biome, analyses show that serious cases occur in anthropized areas of the Atlantic Forest biome, and in a transition area between this and the Cerrado. Complex, longitudinal, multidisciplinary studies, with an eco-epidemiological focus, should be carried out to allow the construction of algorithms capable of predicting, in time and space, the risk factors associated with severe cases and deaths from SF, with the aim of avoiding their expansion.

Keywords: rickettsiosis, epidemiology, biomes, *Rickettsia rickettsii*, tick-borne disease

INTRODUCTION

In Brazil, Spotted Fever (SF) has been recorded from 59% of the Federative Units (16/27), with the Southeast and South regions of the country reporting the most cases (1). Three epidemiological scenarios are recognized for SF in Brazil. The first, with severe cases and deaths, occurs in anthropized areas in the Southeast and part of the South (northern Paraná). The bacterium *Rickettsia rickettsii* is the etiological agent and the tick *Amblyomma sculptum* the vector. In this scenario, horses and capybaras are important for the maintenance of *A. sculptum* in nature. Furthermore, in these areas, capybara is the main amplifier of *R. rickettsii*. The second scenario, also with serious cases and deaths, is restricted to the metropolitan region of São Paulo, in areas of Atlantic forest fragments and their surroundings, where *R. rickettsii* is transmitted by *Amblyomma aureolatum*. Here, humans become infected when dogs, with free access to the environment, carry the infected vector to their homes and humans. The third scenario involves less severe cases that occur mainly in areas with Atlantic forest fragments, or in their surroundings, in areas of the South, Southeast and Northeast regions, where *Rickettsia parkeri* strain Atlantic forest is transmitted by *Amblyomma ovale* (2–9). A probable fourth scenario may occur in the Pampa biome, involving *R. parkeri* sensu stricto, *Amblyomma tigrinum* and dogs. The cases are considered mild and the probable site of infection (PSI) appears to be the rural environment or forest area/forest edge (7, 10).

However, knowledge of the epidemiology and dynamics of human infection remains insipient in the various PSIs across the country (2), complicating appropriate public health actions to inform the general population and control the disease. In addition, studies progressed, different species of Rickettsia spp./Spotted Fever group, related to different species of ticks, have been detected in Brazil, whether or not they are linked to SF outbreaks (6, 7, 11–15). This has demonstrated the great biodiversity of Rickettsiae and potential vectors, and highlighting the potential complexity of the mechanisms by which these microorganisms might circulate within the country, and the danger for the appearance of new outbreaks.

The State of Minas Gerais has the third largest number of confirmed SF cases, ranking second in Brazil for the number of SF deaths (1). The disease has been known in the State since the 1930s, occurring in several regions, and showing a variety of clinical symptoms. Different species of infected Rickettsiae and ectoparasites have already been reported for the State (1, 16–19). However, in most instances, the circulating bioagent is not identified, nor are studies undertaken that would allow a greater understanding of the eco-epidemiological aspects. Accordingly, this article aims to improve interpretation of the epidemiological information of SF cases recorded for the Minas Gerais State using case records from January 2007 to November 2019, and to understand the vectors and etiological agents involved in the disease cycle within the State. In this sense, the interpretation described here contributes to a better understanding of the behavior of the disease, and can contribute positively to Brazilian epidemiological surveillance programs.

METHODS

A descriptive epidemiological study was carried out using SF case records in the databases using the Notification and Investigation forms (NIF) placed on SINAN (Notifiable Diseases Information System)between January 2007 and November 2019. Confirmed cases of the disease were analyzed over the study period, that is, cases in which the symptoms and epidemiological history matched the definition of a suspected case and when the Rickettsia infection of the spotted fever (SFG) group was established. The study was carried out by analyzing cases that occurred in the 82 municipalities of the Minas Gerais State that had confirmed cases between 2007 and 2019, noting whether the municipalities those where patients resided, and also whether the probable infection sites of the confirmed SF cases occurred in these municipalities (**Figure 1**).

Descriptive Study

An epidemiological study of confirmed SF cases was carried out between January 2007 and November 2019, in the State of Minas Gerais. The SINAN database and Technical reports of the investigations carried out by epidemiological surveillance teams of the Municipal Health Secretariats and the State Health Secretariat were used as data sources.

Data analysis was performed using the Python 3.6.8 programming language, Pandas 0.24.2 library, and using Qgis® 2.18.11 software to map cases of the disease and their locations in morphoclimatic domains of the State. For the evaluation of sociodemographic, clinical, care, laboratory, and epidemiological characteristics, we calculated simple and relative nominal values of occurrences, means, and standard deviations.

The variables associated with confirmed cases were analyzed using the following classification (1): General data on the origin of patients (Municipality of Notification); Individual data (age, sex and ethnicity); clinical data (main signs and symptoms); epidemiological data (specifically related to risk exposures); Conclusive data (evolution of 104 individual cases).

Molecular Studies

A total of 304 blood samples from human patients and 1,612 samples of *A. sculptum* ticks, collected in places with suspected or confirmed cases of FM, during case investigation and environmental surveillance from suspected SF cases in the State of Minas Gerais, from 2017 to 2018, were analyzed. The sampling units for collection of potential ectoparasite vectors consisted of specimens from the same host or environment. Adult vectors were packaged and processed individually, nymphs in pools of 10 individuals and larvae in pools of 100 individuals.

Genomic DNA was extracted from samples using the DNeasy Blood and Tissue Kit (Qiagen) and subjected to quantitative Polymerase Chain Reaction (qPCR), using KAPA SYBR FAST qPCR Kit (Sigma-Aldrich) and oligonucleotides to detect Rickettsiae: *gltA* (RpCS.877P/RpCS.1258N) (20), *htrA* (17k-5/17k-3 (21), and *ompA* (Rr 190.70p/Rr 190.602n) (22).

Samples positive for at least one of the genes in the qPCR were subjected to amplification reactions of fragments of rickettsial

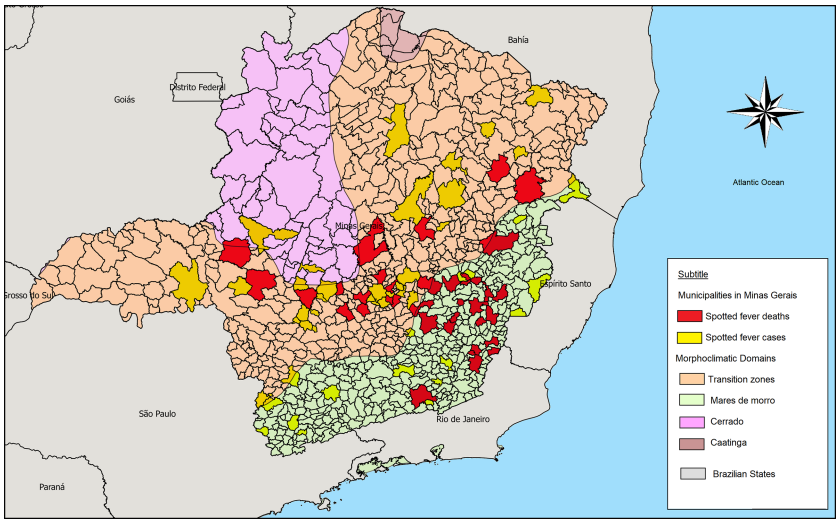

FIGURE 1 | Municipalities in the State of Minas Gerais with cases and death records due to spotted fever between 2007 and 2019 (November).

genes *gltA* (CS-78/CS-323 (23) and CS-239/CS-1069 (21)), *sca4* (D1738F/D2482R) (24) and *ompA*(Rr 190.70p/Rr 190.602n) (22). In all amplification reactions, 300 ng of *R. parkeri* DNAg was used, as a positive control, and ultra-pure water free of DNase and RNase, as a negative control.

Amplicons of the expected sizes were purified using the Wizard SV Gel and PCR Clean-Up TM System Protocol Kit (Promega), following the manufacturer's guidelines, and subjected to a sequencing reaction using Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems). Nucleotide sequences were read by an automatic ABI 3730xl DNA analyzer (Applied Biosystems) from the Sequencing Platform (PDTIS) of the Oswaldo Cruz Foundation. Obtained Sequences were edited using the SeqMan™ II program (DNASTAR package, Lasergene), and identity of the sequences was assessment *via* comparative analysis with existing rickettsial sequences held in GenBank (BLASTn). Phylogenetic inferences were made using Maximum Likelihood, with an evolutionary model GTR+G, selected through the Bayesian Information Criterion, and indicated by the MEGA 7.0 22 program. Internal branches support values were calculated using a 1,000 replica bootstrapping procedure.

Ethical Considerations

Data collection in this study obeyed Resolution 466/2012 (Ministry of Health of Brazil), guaranteeing the confidentiality of information and non-disclosure of individual patient data.

RESULTS

Between 2007 to 2019 (November), 298 cases of SF were confirmed in the State of Minas Gerais, Brazil. Of these, 98 ended in deaths. An increase in the number of cases was observed from the year 2011 on (**Figure 2A**). The SF scenario in the State had a lethality of 32.8%, with the Municipality of Belo Horizonte having the highest number of cases: 63, and the Municipality of Juiz de Fora the highest lethality: 48.1%. Overall, 207 (69.4%) cases involved males, with lethality being higher in this group, 78.8% (n = 78). The age group most affected was 30 to 59 years old, the average age of individuals in cases that died was 39 years old, with a standard deviation of 18.7 (**Table 1**). The average age of individuals in cases of recovery was 31.6 years, with a standard deviation of 20.6. The greatest number of cases occurred between August and November, the most severe cases that died, during September and December (**Figure 2B**).

Most patients reported having had contact with wild animals such as ticks, capybara, and domestic animals such as dogs and cats. In cases that ended in death, contact with dogs and cats was most common (89 cases) followed by contact with ticks (74 cases) and horses (35 cases). Contact with capybaras ranks lowest in contact records. This was true both for patients who recovered and those who did not (**Figure 2C**).

In qPCR screens, 99 samples (human blood and ticks) were positive for the presence of *Rickettsia* spp. These subsequently underwent conventional PCR to search for rickettsial genes. Of these, 85 samples were positive for at least one of the searched-for genes; it being possible to obtain partial nucleotidesequences of the following—*gltA* in 85 samples **(MT957958-MT958042)**, *ompA* in 66 samples **(MT958043-MT958108)** and from the *sca4* gene 56 samples 175 **(MT958109-MT958164)** (**Table 2**).

BLAST analyzes showed that all sequences obtained in this study were identical and showed 100% similarity to *R. rickettsii* cepa Brasil (CP003305) sequences, and other strains of this species available in Genbank. Accordingly, sequences from three samples were selected for the phylogenetic reconstruction generated from comparison of concatenated partial sequences of the *gltA*, *ompA*, and *sca4* genes (of 1,106, 491, and 704 bp,

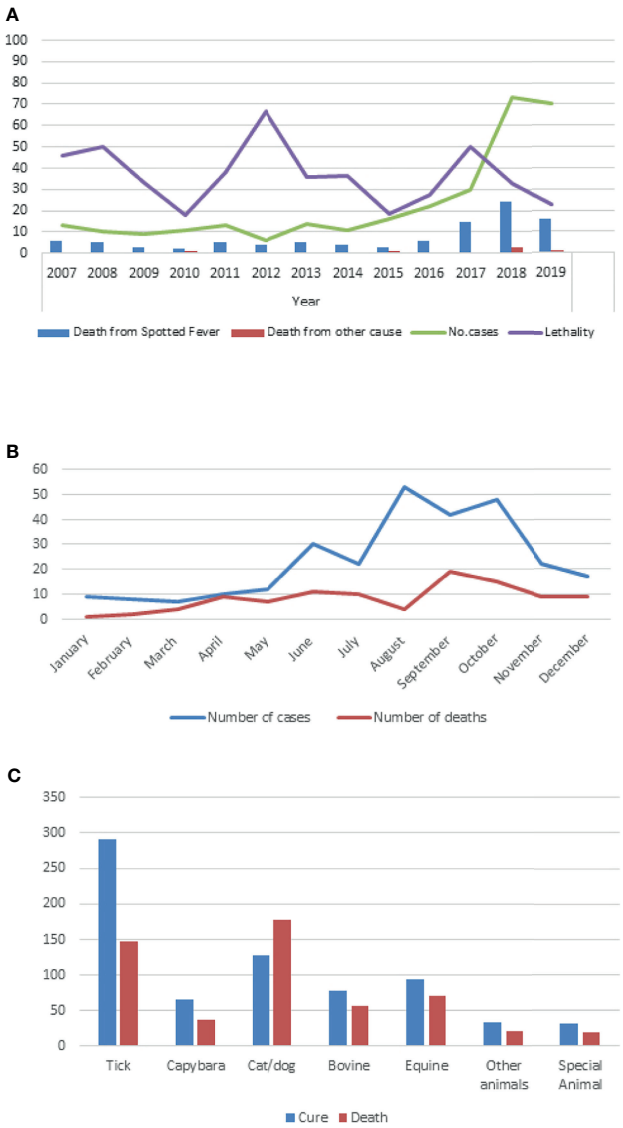

FIGURE 2 | Epidemiological dynamics of Spotted Fever in the State of Minas Gerais, 2007–2019 (November): **(A)** Absolute number of reported cases, deaths, and SF lethality rate; **(B)** Monthly distribution of SF cases; **(C)** Contact with animals × disease evolution.

respectively), demonstrating that the sequences from Minas Gerais obtained here are phylogenetically related to the *R. rickettsii* group (**Figure 3**).

None of the samples (human blood or ticks) tested positive for the presence of Rickettsia spp. came the from Cerrado biome.

DISCUSSION

In 2007, SF began to be recorded on SINAN and in 2011, training on rickettsiosis environment surveillance began in the country as did the incorporation of molecular diagnostic techniques, which enabled the unambiguous identification of deaths from the disease. This timeline may explain the increase in recorded mortality rates after 2011, observed in the present study (1).

There was a considerable increase in the number of SF cases in the State from 2014. This was because, in June of that year that, Ordinance No. 1,271 made the immediate notification of SF compulsory, with other rickettsioses disease being notifiable within 24 h (1). The increase in the number of notifications is the result of efforts promoted by SUS (Health Unic System), which develops continuous training processes and improves epidemiological surveillance network structure (22). As pointed out in the present study, the increase in SF lethality in recent years in both Brazil, and in the State of Minas Gerais, shows the severity of the recent cases (**Figure 2A**). The present study demonstrated that the majority of SF victims, fatal or not, are

TABLE 1 | Absolute and relative frequency of confirmed cases and deaths from Spotted Fever, based on individual, demographic and epidemiological variables from 2007 to 2019 in the State of Minas Gerais, Brazil.

	Cases		Deaths	
	(n)	%	(n)	%
Women				
<1 year	2	0.66	0	0
1 - 5 years	7	2.3	0	0
6 -10 years	4	1.33	0	0
11 - 15 years	4	1.33	2	2.04
16 - 20 years	3	1	1	1.02
21 - 29 years	8	2.67	3	3.06
30 - 39 years	11	3.67	5	5.1
40 - 49 years	9	3.01	5	5.1
50 - 59 years	8	2.67	4	4.08
60 - 69 years	7	2.34	1	1.02
70 - 79 years	6	2	0	0
> 80 years	1	0.33	0	0
Men				
<1 year	2	0.66	0	0
1 - 5 years	14	4.68	6	6.12
6 - 10 years	22	7.35	4	4.08
11 - 15 years	17	5.68	3	3.06
16 - 20 years	15	5.01	4	4.08
21 - 29 years	20	6.68	9	9.18
30 - 39 years	25	8.36	4	4.08
40 - 49 years	40	13.37	20	20.4
50 - 59 years	33	11.03	15	15.3
60 - 69 years	14	4.68	6	6.12
70 - 79 years	1	0.33	2	2.04
> 80 years	4	1.33	4	4.08
Ethnic group				
White	97	32,5	29	30,58
Black	28	9,39	11	11,22
Yellow	1	0,33	0	0
Brown	117	39,26	45	45,91
Indigenous	0	0	0	0
Information refused	25	8,38	12	12,24
Ignored	21	7,04	2	2,04
Education				
illiterate	2	0,67	0	0
To school grades 1[a]–4[a]	15	5,03	5	5,10
4th grade completed	16	5,36	8	8,16
To school grades 5[a]–8[a]	31	10,40	10	10,20
Basic education completed	11	3,69	2	2,04
Highschool incomplete	15	5,03	9	9,18
Highschool complete	23	7,71	8	8,16
Higher level education incomplete	3	1,00	0	0
Higher level education completed	5	1,67	0	0
Ignored	89	29,86	39	39,79
Information refused	39	13,08	13	13,26
Does not apply	28	9,39	5	5,10
Probable infection location				
Urban	192	62,31	58	59,18
Rural	64	23,10	29	29,59
Peri Urban	5	1,80	3	3,06
Ignored	4	1,44	4	4,08
Information refused	12	4,33	5	5,10

male, which is in agreement with the results for the disease in Brazil (2). The most affected ethnic groups were brown, followed by whites. However, it is worth mentioning that, on very dark skins, the maculating rash is not easily identified, which can cause difficulties in the diagnosis in this portion of the population (23). In MG State, cases are concentrated in the portion of the population with the lowest level of education, up to incomplete elementary school. In the current study, it was notable how cases were concentrated in urban areas, corroborating findings similar studies in several regions of the country. This demonstrates a potential urbanization of the disease, which has been occurring in regions that had not previously been considered to be at risk of transmission, which suggests that the disease is now occurs not just rurally, but also in peri-urban and urban areas, including public parks (24–26) (**Table 1**).

The greatest number of cases was recorded between the months of August and November, a period in which the highest density of *A. sculptum* nymphs (27, 28) is generally recorded. This may be linked to disease transmission to humans since the nymphs' bite is less painful than that of an adult, and may, indeed, be imperceptible.

In Brazil the SF epidemiological scenario is diverse, involving different species of vectors and Rickettsiae, with cases ranging from moderate to severe, and with deaths occurring. For example, in degraded areas and the Cerrado bioma, in the southeastern region and part of the southern region (northern Paraná state), the most severe form of the disease occurs, and *R. rickettsii*, *A. sculptum*, capybaras and horses may be involved in the epidemic cycle, especially in rural and peri-urban environments. Capybaras occur in several biomes in preserved and anthropized areas including urban areas of Minas Gerais as observed in Juiz de Fora and Belo Horizonte municipalities. In the present study, contact with capybaras did not appear as a relevant factor in the suspicion of FM, both in mild cases and in cases that progressed to death, as was also noted in RJ and Paraná (29, 30). However, direct contact with capybaras is not necessary for human tick bite to occur. Capybaras can be shy and cautious in hostile environments and spread ticks, infected or not, in the environment without being seen. Serious cases and deaths are also associated with the transmission of *R. rickettsii* by *A. aureolatum* in a less anthropized area of the Atlantic Forest, in the metropolitan region of São Paulo, with the infected tick vectors when these ticks are carried by dogs into the anthropic environment, especially households close to the forests, and, this close relationship between humans and hosts, especially dogs, is characterized as one of the main risk exposure factors (2–4, 30, 31).

Moderate cases are associated with the transmission of *R. parkeri* by *A. ovale*, in an area with less anthropic impact on the Atlantic forest biome in the South, Southeast and Northeast regions of the country, especially in the coastal areas. In addition, there is evidence that *A. tigrinum* can participate in the transmission of *R. parkeri* in the Pampa biome, in southern Brazil. In these scenarios, dogs can take the infected tick to the anthropic environment or humans become infected when entering natural foci (2, 3, 5, 6, 32, 33). However, in other areas of the national territory, such as in the Cerrado biomes (Midwest region), Amazon, and Caatinga, the epidemiological scenarios are not defined, and there may be clinical variation, absence of species known as Rickettsiae vectors or pathogenic *Rickettsia* (2, 4, 20, 34).

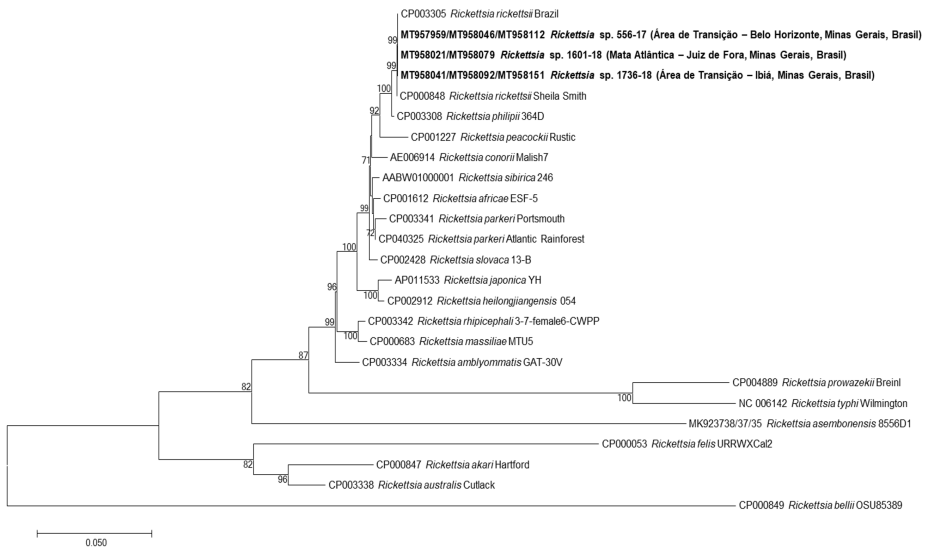

FIGURE 3 | Phylogenetic inferences from the comparison of the concatenated partial sequences of the *gltA*, *ompA*, and *sca4* genes (of 1,106, 491, and 704 bp, respectively) of human patients' blood, from suspected Spotted Fever cases in the State of Minas Gerais, between 2017 and 2018, using the maximum likelihood method of 1,000 replicated trees. As an evolutionary model, the GTR + G + I algorithm was used, and bootstrap values above 70% are shown in the branches. Sequences obtained in this study are highlighted in bold, preceded by the respective GenBank accession numbers, and followed by information about the case location.

The State of Minas Gerais is characterized by having severe cases and deaths from SF spread over a large area of the State, and also both the Cerrado and the Atlantic Forest biomes. However, the analysis of the spatial distribution, according to the morphoclimatic domains, shows deaths to have occurred only in the area of the Atlantic Forest biome and in the transition area between the Atlantic Forest and the Cerrado, with no record of deaths in the area of pure Cerrado biome within the State (**Figure 1**). Likewise, in Brazil in general, by far the greatest majority of serious cases and deaths from SF are spatially distributed in the Atlantic Forest, or in transition zones between this and the Cerrado (2–4, 30, 31).

The factors associated with the absence of records for serious cases and deaths in the typical Cerrado region of Minas Gerais are not clear. However, while, it is unusual to record *R. rickettsii* in typical areas of this biome [16], *A. sculptum*, considered its main vector, is found throughout the Cerrado region and does not have its own genetic structure for its population in this region (8, 34, 35). Additionally, horses and capybaras, considered the main vertebrates in the Brazilian spotted fever epidemic cycle, are traditionally present in this biome. Therefore, all biotic predisposing factors for enzootic and epidemic cycle to occur are present in the Cerrado. However, there is no circulation of *R. rickettsii*, the determining factor in the disease cycles.

However, there are other clinical manifestations seen for Cerrado-based cases (2), signaling the possibility of the involvement of another species of *Rickettsia* in this biome.

Thus, it is possible that ecological factors are influencing the spatial distribution of *R. rickettsii* and, consequently, restricting serious cases and deaths to the most densely populated area of the State of Minas Gerais. In such locations there should be greater attention from health services to record SF cases, control and prevent them, and seek to reduce the lethality level. In addition, complex multidisciplinary, longitudinal studies, associated with eco-epidemiology, should be carried out in the search for the construction of algorithms capable of predicting, in time and space, the risk factors associated with severe cases and deaths from SF, and so avoid the expansion of this disease.

CONCLUSION

The results corroborate existing studies in areas of severe cases of DES in Brazil. Despite the case reports of SF from the Cerrado biome in MG, the analyzes show that severe cases occur in anthropized areas of the Atlantic Forest biome and in a transition area between this and the Cerrado. The finding of only *A. sculptum* in the areas of cases of the disease may suggest the strong relationship of this vector in severe cases of FS in MG. These results may suggest an eco-epidemiological scenario, apparently more similar to Brazilian cases of spotted fever related to A. aureolatum as a vector in other states, and, it is not possible to completely rule out other possible vectors of the disease in the state without a systematic long-term study. Complex, longitudinal, multidisciplinary studies, with an eco-epidemiological focus, should be carried out to allow the construction of algorithms capable of predicting, in time and space, the risk factors associated with severe cases and deaths from SF, in order to avoid their expansion.

TABLE 2 | *Rickettsia rickettsii* detected by analysis of partial nucleotide sequences of the *gltA*, *ompA*, and *sca4* genes from blood (human) and tick (*Amblyomma sculptum*) samples from areas of suspected Spotted Fever cases in the state of Minas Gerais, from 2017 to 2018.

Locality	Type	Sample code	Gene	Accession Number
Antônio Peçanha	Human blood	1724/18	*gltA*	MT958040
			ompA	MT958091
Belo Horizonte	Human blood	556/17	*sca4*	MT958150
			gltA	MT957959
			ompA	MT958046
			sca4	MT958112
Belo Horizonte	Human blood	825/17	*gltA*	MT957961
			ompA	MT958047
			sca4	MT958113
Alvinópolis	Human blood	849/18	*gltA*	MT957986
			ompA	MT958048
			sca4	MT958114
Belo Horizonte	Human blood	851/18	*gltA*	MT957987
			ompA	MT958049
			sca4	MT958115
Belo Horizonte	Human blood	979/18	*gltA*	MT958025
			ompA	MT958043
			sca4	MT958142
Belo Horizonte	Human blood	1327/18	*gltA*	MT958011
			ompA	MT958050
			sca4	MT958116
Belo Horizonte	Human blood	1636/18	*gltA*	MT958023
			sca4	MT958144
Belo Horizonte	Human blood	1637/18	*gltA*	MT958030
			ompA	MT958081
Belo Horizonte	Human blood	1638/18	*gltA*	MT958031
			ompA	MT958082
Belo Horizonte	Human blood	1640/18	*gltA*	MT958032
			ompA	MT958083
Belo Horizonte	Human blood	1641/18	*gltA*	MT958033
			ompA	MT958084
Belo Horizonte	Human blood	1642/18	*gltA*	MT958034
			ompA	MT958085
			sca4	MT958145
Belo Horizonte	Human blood	1647/18	*gltA*	MT958027
			sca4	MT958146
Belo Horizonte	Human blood	1648/18	*gltA*	MT958028
			sca4	MT958147
Belo Horizonte	Human blood	1649/18	*gltA*	MT958029
			ompA	MT958086
Belo Horizonte	Human blood	1853/18	*gltA*	MT958036
			ompA	MT958044
			sca4	MT958153
Belo Horizonte	Human blood	1884/18	*gltA*	MT958035
			ompA	MT958045
			sca4	MT958154
Betim	Human blood	1200/18	*gltA*	MT958006
			ompA	MT958051
			sca4	MT958117
Betim	Human blood	1201/18	*gltA*	MT958007
			ompA	MT958052
			sca4	MT958118
Caratinga	Human blood	1243/17	*gltA*	MT957976
			ompA	MT958053
			sca4	MT958119
Contagem	Human blood	846/17	*gltA*	MT957962
			ompA	MT958054
			sca4	MT958120
Contagem	Human blood	968/17	*gltA*	MT957963
			ompA	MT958055
			sca4	MT958121
Divinópolis	Human blood	961/18	*gltA*	MT957993
			ompA	MT958056
			sca4	MT958122
Divinópolis	Human blood	1139/18	*gltA*	MT958005
			ompA	MT958057
			sca4	MT958123
Divinópolis	Human blood	1217/18	*gltA*	MT958008
			ompA	MT958058
			sca4	MT958124
Florestal	Human blood	982/17	*gltA*	MT957965
			ompA	MT958059
Ibiá	Human blood	1736/18	*gltA*	MT958041
			ompA	MT958092
			sca4	MT958151
Ipatinga	Human blood	1684/18	*gltA*	MT958038
			ompA	MT958089
Ipatinga	Human blood	1786/18	*gltA*	MT958042
			ompA	MT958093
			sca4	MT958152
Itamarandiba	Human blood	1106B1/17	*gltA*	MT957970
			ompA	MT958060
			sca4	MT958125
Itamarandiba	Human blood	1106B2/17	*gltA*	MT957971
			ompA	MT958061
			sca4	MT958126
Itamarandiba	Human blood	1106C/17	*gltA*	MT957972
			ompA	MT958062
			sca4	MT958127
Itaúna	Human blood	858/18	*gltA*	MT957988
			ompA	MT958063
			sca4	MT958128
Itaúna	Human blood	1027/18	*gltA*	MT957994
			ompA	MT958064
			sca4	MT958129
Itaúna	Human blood	1028/18	*gltA*	MT957995
			ompA	MT958065
			sca4	MT958130
Itaúna	Human blood	1663/18	*gltA*	MT958037
			ompA	MT958087
			sca4	MT958148
Itaúna	Human blood	1664/18	*gltA*	MT958026
			ompA	MT958088
			sca4	MT958149
Jaguaraçu	Human blood	1420/18	*gltA*	MT958017
			ompA	MT958066
			sca4	MT958131
Juiz de Fora	Human blood	1601/18	*gltA*	MT958021
			ompA	MT958079
Juiz de Fora	Human blood	1631/18	*gltA*	MT958022
			ompA	MT958080
Juiz de Fora	Human blood	1714/18	*gltA*	MT958039
			ompA	MT958090
Matozinhos	Human blood	979/17	*gltA*	MT957964
Matozinhos	Human blood	1020/17	*gltA*	MT957966
			sca4	MT958132
Miradouro	Human blood	1366/18	*gltA*	MT958012

(Continued)

TABLE 2 | Continued

Locality	Type	Sample code	Gene	Accession Number
			ompA	MT958067
			sca4	MT958133
Pedro Leopoldo	Human blood	536/17	*gltA*	MT957958
			ompA	MT958068
Pedro Leopoldo	Human blood	1259/17	*gltA*	MT957977
			ompA	MT958069
			sca4	MT958134
Ponte Nova	Human blood	1460/18	*gltA*	MT958019
			ompA	MT958070
			sca4	MT958135
Rio Casca	Human blood	1196/17	*gltA*	MT957975
			ompA	MT958071
			sca4	MT958136
Ribeirão das Neves	Human blood	1242/18	*gltA*	MT958009
			ompA	MT958072
			sca4	MT958137
Santa Cruz do Escalvado	Human blood	1471/18	*gltA*	MT958020
			ompA	MT958073
			sca4	MT958138
São Gonçalo do Rio Abaixo	Human blood	1129A/17	*gltA*	MT957973
			ompA	MT958074
			sca4	MT958139
São Gonçalo do Rio Abaixo	Human blood	1129B/17	*gltA*	MT957974
			sca4	MT958140
São Gonçalo do Rio Abaixo	Human blood	1269/17	*gltA*	MT957978
São José do Jacuri	Human blood	584/17	*gltA*	MT957960
			ompA	MT958075
			sca4	MT958141
Ubá	Human blood	489/18	*gltA*	MT958024
			ompA	MT958076
Belo Horizonte	Vector tissue (*A.sculptum*)	674/18	*gltA*	MT957980
			sca4	MT958155
Belo Horizonte	Vector tissue (*A.sculptum*)	686/18	*gltA*	MT957981
			ompA	MT958097
Belo Horizonte	Vector tissue (*A.sculptum*)	688/18	*gltA*	MT957982
			ompA	MT958098
Belo Horizonte	Vector tissue (*A.sculptum*)	778/18	*gltA*	MT957983
			sca4	MT958156
Belo Horizonte	Vector tissue (*A.sculptum*)	779/18	*gltA*	MT957984
Belo Horizonte	Vector tissue (*A.sculptum*)	783/18	*sca4*	MT958157
			gltA	MT957985
			sca4	MT958158
Belo Horizonte	Vector tissue (*A.sculptum*)	884/18	*gltA*	MT957989
			sca4	MT958159
Belo Horizonte	Vector tissue (*A.sculptum*)	921/18	*gltA*	MT957990
			ompA	MT958099
Belo Horizonte	Vector tissue (*A.sculptum*)	922/18	*gltA*	MT957991
			ompA	MT958100
Belo Horizonte	Vector tissue (A.sculptum)	924/18	gltA	MT957992
			ompA	MT958101
Belo Horizonte	Vector tissue (A.sculptum)	1107/18	gltA	MT957996
			ompA	MT958102
Belo Horizonte	Vector tissue (A.sculptum)	1108/18	gltA	MT957997
			ompA	MT958103
Belo Horizonte	Vector tissue (A.sculptum)	1110/18	gltA	MT957998
			ompA	MT958104
			sca4	MT958160
Belo Horizonte	Vector tissue (A.sculptum)	1114/18	gltA	MT957999
			ompA	MT958105
Belo Horizonte	Vector tissue (A.sculptum)	1118/18	gltA	MT958000
			ompA	MT958106
Belo Horizonte	Vector tissue (A.sculptum)	1120/18	gltA	MT958001
			sca4	MT958161
Belo Horizonte	Vector tissue (A.sculptum)	1122/18	gltA	MT958002
			sca4	MT958162
Belo Horizonte	Vector tissue (A.sculptum)	1248/18	gltA	MT958010
			sca4	MT958163
Belo Horizonte	Vector tissue (A.sculptum)	1377/18	gltA	MT958013
			ompA	MT958107
Belo Horizonte	Vector tissue (A.sculptum)	1378/18	gltA	MT958014
			ompA	MT958108
Belo Horizonte	Vector tissue (A.sculptum)	1380/18	gltA	MT958015
			ompA	MT958094
Belo Horizonte	Vector tissue (A.sculptum)	1381/18	gltA	MT958016
			ompA	MT958095
Belo Horizonte	Vector tissue (A.sculptum)	1442/18	gltA	MT958018
			ompA	MT958096
Contagem	Vector tissue (A.sculptum)	1068.2/17	gltA	MT957967
			sca4	MT958109
Contagem	Vector tissue (A.sculptum)	1068.3/17	gltA	MT957968
			ompA	MT958077
			sca4	MT958110
Contagem	Vector tissue (A.sculptum)	1068.7/17	gltA	MT957969
			ompA	MT958078
			sca4	MT958111
Coronel Pacheco	Vector tissue (A.sculptum)	003/17	gltA	MT957979
Itabirito	Vector tissue (A.sculptum)	1124/18	gltA	MT958003
			sca4	MT958143
Itabirito	Vector tissue (A.sculptum)	1130/18	gltA	MT958004
			sca4	MT958164

(Continued)

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/MT957958–MT958164/.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

EN, NM-M, FM, and GG designed the study, interpreted the results and wrote the article. FI performed the real-time amplification screening. NM-M performed the molecular and phylogenetic analysis. AD and SO collected and analyzed the clinical and epidemiological data of the cases. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.