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Environmental and Laboratorial Investigation of Two Potential Spotted-fever Group Rickettsioses Cases in a Non-endemic Area of Brazil

Ana Paula Betaressi da Silva[1]*, Carolina de Castro Araújo Pacheco[1], Ana Carolina Mota de Faria[2], Ana Beatriz Pais Borsoi[3], Karla Bitencourth[3], Clarício Alvim Bugarim Neto[4], Waldinéa Maria da Silva[4], Diego Pereira Gonçalo Hora[4], Carlos Eduardo da Silva[4], Anderson Brandão Leite[5], Ana Íris de Lima Duré[6], Francisco Carlos Lins da Silva[7], Edivânia Izabel Santos Cavalcante Soares[7], Maria Jose da Silva Oliveira[7], Rodrigo Nogueira Angerami[8], Danielle Cristine Castanha da Silva[1], Luciana Nogueira de Almeida Guimarães[1], Luis Antonio Alvarado Cabrera[1] and Gilberto Salles Gazêta[3]

[1]Field Epidemiology Training Program (EpiSUS-FETP Brazil), Environment and Health Surveillance Secretariat, Ministry of Health, Brazil

[2]General Coordination of Zoonoses Surveillance and Vector Transmission Diseases, Department of Communicable Diseases, Ministry of Health, Brazil

[3]Laboratory of Ticks and Other Vectors, Laboratory of National Reference in Vectors of Rickettsioses, Oswaldo Cruz Foundation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

[4]Surveillance of Communicable Diseases, Alagoas Health Secretariat, Alagoas State Department of Health, Brazil

[5]Alagoas Central Laboratory of Public Health, Alagoas, Brazil

[6]Ezequiel Dias Foundation, Minas Gerais Central Laboratory of Public Health, Minas Gerais, Brazil

[7]Maragogi Health Secretariat, Maragogi Department of Health, Alagoas, Brazil

[8]Post-Graduate Program of Epidemiology of Infectious Diseases of University of Campinas, Campinas, Brazil

****Correspondence:***
Ana Paula Betaressi da Silva, RN, MBA, Specialist in Health Surveillance. SRTV PO 700, Via W5 Norte, lote D. Zipcode 70.719-040. Federal District, Brazil. Department of HIV/AIDS, Tuberculosis, Viral Hepatitis and Sexually Transmitted Infections (DATHI), Health and Environment Secretariat, Ministry of Health, Brazil, E-mail: ana.betaressi@aids.gov.br/ ana.betaressi@saude.gov.br/ anapaula_silvarp@hotmail.com

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Abstract

Objective: To describe two-rickettsiosis laboratory-positive cases, and their eco-epidemiology, in non-autochthonous area of Brazil, 2022.

Methods: Case report study with environmental investigation, from May 1st to November 14th, 2022. Data collection occurred through interviews and health records; ectoparasites and dogs' blood samples were collected, and spatial monitoring of a *Rickettsia* serologic reagent dog was performed. Software used: Epi Info 7.2.5.0, QGIS 3.16.6, Microsoft Excel® 365, GenBank, Google Earth 10.43.0.2 and Gaia GPS 2022.9.

Results: The first (1st) case presented fever, myalgia, headache, lower limb rash, hepatosplenomegaly, renal alterations, and death after 18 days of hospitalization. The second (2nd) case manifested fever, edema, abdominal pain and intermittent pain in limbs for three months. The suspicion of the 2nd case was raised due to territorial link with the 1st case. None of them used antibiotics for the disease. Among common exposure factors were the circulation in the municipality's rural area; no visitation to endemic regions; no exposure to risk activities; and dogs from the area with access to the forest. The investigation resulted in identification of *Amblyomma varium* hosted by agent similar to *Candidatus Rickettsia* andeanae and *Rickettsia amblyommatis*; dogs' blood serologically positive for *Rickettsia*; the monitored dog accessing the Atlantic Forest and human households.

Conclusion: Although the data was not sufficient to set rickettsiae species involved, and if the human cases were genuinely Spotted Fever rickettsiosis, it is certain the necessity of a sensitive health surveillance to elucidate pathogenic rickettsiae species circulation in the area, its potential transmission biocycle and epidemiology.

Keywords: Rickettsia Infections; Tick-Borne Diseases; Fatal Outcome; *Amblyomma*; Public Health

Abbreviations

Alagoas state (AL); Água Fria agrarian land reform (Água Fria); Brazilian Spotted Fever (BSF);

Ezequiel Dias Foundation laboratory (Funed); Santa Rita land reform (Santa Rita)

Introduction

Rickettsiosis is a vector-borne illness of variable clinical patterns, complex transmission cycles and epidemiological scenarios. The non-specificity of signs and symptoms in their initial phases leads to late suspicion or differentiation from other febrile syndromes such as dengue, yellow fever, leptospirosis, and exanthematous viruses [1-3].

In Brazil, the most important rickettsiosis is the Brazilian Spotted Fever (BSF). The disease, caused by *Rickettsia rickettsii* (*R. rickettsii*), takes place mainly in the Southeast region, with fatality rate close to 50%[1,4]. The prevalent eco-epidemiological scenario involves the *Amblyomma sculptum* (*A. sculptum*) tick occurring primarily in rural areas [1, 5]. A second scenario is exclusive to the metropolitan region of São Paulo, with *Amblyomma aureolatum* (*A. aureolatum*) as its vector, the infection occurs when humans enter the forest or dogs with access to it bring infected ticks to human residences [1, 2, 6].

Besides *R. rickettsii*, another pathogenic rickettsia in the country is the *Rickettsia parkeri (R. parkeri),* which is transmitted by *Amblyomma ovale* (*A. ovale*) tick [1, 2, 4, 5]. It provokes mild-moderate disease, when compared to BSF, and is found in the Atlantic rainforest in the South, Southeast and Northeast regions [1, 2, 5, 6].

The frequent clinical presentation caused by both *Rickettsiae* is fever, headache, myalgia, prostration, abdominal pain, nausea and vomiting, while petechiae, hemorrhage, hypotension, shock, neurological manifestation, respiratory distress, renal failure, hepato and splenomegaly, and high lethality are attributed to *R. rickettsii,* but not to *R. parkeri* [2].

Over the last few years, epidemiological changes in spotted fever (SF) have been reported in the Americas. In Colombia [9], Panama [10, 11] and Brazil [12, 13], for example, SF human cases, including severe cases and deaths, have been registered in non-endemic or, until then, epidemiologically silent areas, which may be related to the expansion and improvement of health surveillance, laboratory diagnostic capacity and structure, and environmental changes [1, 4, 7-9].

In this regard, rickettsiosis deaths were reported in an unprecedented state of Brazil. Thereby, to describe these two-rickettsiosis laboratory-positive cases, and understand their eco-epidemiology, from May 1st to November 14th, 2022, a field epidemiology investigation was conducted.

Methods

This study was performed in Maragogi city, Alagoas (AL) state, Northeastern Brazil (Figure 1) [10], in an area of 334.16 square kilometers (km^2), Atlantic rainforest biome, and tropical climate [10-12].

To obtain patients' data, interviews were conducted with cases families' members, employing an unstructured questionnaire, and medical records from Health Services, where the cases were attended, utilizing a semi-structured questionnaire.

Before the field team arrived, blood samples of the first case (1st case); and cerebral, lungs, pancreatic, splenic, liver, kidney, and myocardial tissue fragments from second case (2nd case) were already acquired, and sent to the Ezequiel Dias Foundation (Funed) laboratory, Brazil [13]. All samples were submitted to total automated DNA extraction employing the Magna Pure 96 equipment technique. First, DNA was done by quantitative real-time polymerase chain reaction (qPCR) of the citrate synthase gene (gltA) using CS5 and CS6 primers (CS5- GAGAGAAAATTATATATCCAAATGTTGAT and CS6- AGGGTCTTCGTGCATTTCTT; probe [5′ 6-FAM d(CATTGTGCCATCCAGCCTACGGT) BHQ-1 3′]) [7]. Further, the 1st case blood sample was also searched for immunoglobulin (IgG), by the indirect immunofluorescence assay (IFA), using *R. rickettsii* as antigen [14].

During the field investigation, animals' blood and ectoparasites were sampled from the living and surrounding areas of the 1st case family's house, in Água Fria agrarian land reform (Água Fria), Maragogi (AL) (Figure 2). There was a riparian forest and a football field near it, and in the last one, a dry ice trap and tick drag-flag technique was applied.

Concerning the 2nd case, we were able to obtain ectoparasites and blood from dogs from her daughter's and husband's house dwellings

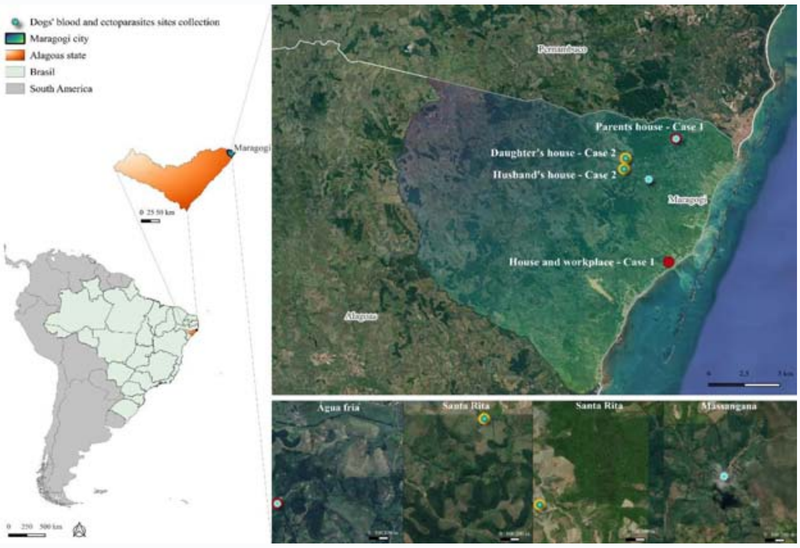

Figure 1: Visiting and living areas of the potential cases of spotted-fever group rickettsiosis, Maragogi (AL), Brazil, 2024.

Figure 2: Água Fria agrarian land reform, Maragogi (AL), Brazil, 2022.

Figure 3: Tick flag trap with dry ice technique in Massangana agrarian land reform, Maragogi (AL), Brazil, 2022.

in Santa Rita land reform (Santa Rita), Maragogi (AL). Samples were also collected from horses and grass, in a football field where equines graze, in the agrarian reform Massagana, Maragogi (AL) - along the way to Santa Rita (Figure 3) - since it had been reported dogs dying from Ehrlichiosis there.

The 2nd case's mother lived in an urban area in Matriz de Camaragibe (AL) without dogs; then, blood and ticks from stray dogs were collected near her domicile.

All collected ectoparasites were directed to the Ticks and Other Apterous Vectors Laboratory at Oswaldo Cruz Foundation (Fiocruz), Brazil, where, initially, were screened and morphologically identified [15, 16]. For molecular analysis, arthropods were submitted to DNA extraction [17] and quantification, individually (nymphs and adults) and/or in pools (larvae). Genus-specific primers (*glt*A) [13] and genus-specific groups to identify *Rickettsia spp.* and RGFM *(ompA, htrA-Nested and ompB-Nested)* [13, 18-20], respectively, were implemented in order to run PCR. Tick larvae were specifically identified through 16S rDNA gene fragment analysis [21]. After the PCR, samples were subjected to agarose gel (2%) electrophoresis, staining in ethidium bromide, and exposed to ultraviolet light [22], for DNA amplification and visualization of fragments. The positive samples were purified and sequenced using the same primers employed in PCR. The sequences obtained (PQ583811-14; PQ586212-16) had their identity compared to the one's existent at the GenBank. Subsequently, for each rickettsia marker and for 16S rDNA, the sequences were aligned managing the ClustalW algorithm in the MEGA-X software. The evolutionary relationships between rickettsias, based on concatenated alignment, and ticks were analyzed by implementing the Maximum Likelihood Method, with the aid of SeaView software, through the T92+G evolutionary model for rickettsia and GTR+G for ticks, indicated by the MEGA-X program.

Dogs' blood samples were sent to the Funed laboratory for serologic testing. Slides containing *R. rickettsii* specific antigens were produced by the institution, performing indirect IFA [14].

It is imperative to enlighten that 40 days after the 1st case death, professionals from the Alagoas Health Secretariat visited her family and collected blood from dogs residing in the settlement, as well as dogs near her store/house, in the urban area. One of the dogs from Água Fria was serologically reagent to *R. rickettsii* (IgM titration 1:64) and *R. parkeri* (IgG titration 1:256). Therefore, the team chose to attach a smartphone to this dog collar and monitor the animal for five days, utilizing the Free GAIA Global Positioning System (GPS) 2022.9 [23]. The device was recharged once a day.

To measure the distance between all visited and collection areas, geographical coordinates were captured from photos by a personal smartphone, and placed on Google Earth 10.43.0.2 for analysis.

Descriptive analyses were implemented. Other software to manage data were Epi Info 7.2.5.0, QGIS 3.16.6, Microsoft Excel® 365 Office, GenBank.

Table 1: Serological panel and molecular analysis for Spotted Fever Group rickettsiosis in dog samples from Maragogi, AL, Brazil, 2022.

Sample	SFG by PCR (N=43)	*R. rickettsii* IgG (N=46)	*R. typhi* IgG (N=46)	*R. felis* IgG (N=46)	*R. parkeri* IgG (N=1)	Spotted-fever IgM (N=1)	*Ehrlichia chafensis* IgG (N=34)
Blood collected from dogs	Non-detectable	Reagent (n=4) 1:64 (n=1) 1:128	Non-reagent (n=37)	Non-reagent (n=37)	Reagent (n=1) 1:256	Reagent (n=1) 1:64	Reagent (n=1) 1:80 (n=2) 1:60 (n=7) 1:320 (n=3) 1:640

PCR – Polimerase Chain Reaction; R. – Rickettsia; SFG – Spotted Fever Group

The study protocol was approved on October 31st, 2023, by the Research National Ethics Committee, number: 6.428.520, Brazil. Verbal consent was obtained from the patients' family members and from the dogs' owners.

Results

Case 1 (1st case)

In May 2022, a 36-year-old female, clothes trader, started with fever, headache, myalgia, petechiae, and exanthem in lower limbs. The symptoms occurred and lasted around eight days – without the possibility of identification which one(s) and on what day they occurred. After these eight days, the patient was found unconscious at her store/house with cyanotic lips, generalized rash, and livedo reticularis. She was sent to the Maragogi (AL) Emergency Unit with high-grade fever (40,1° Celsius), 121 heartbeats per minute, and 91% peripheral oxygen saturation; the diagnosis was dengue fever. Even after being transferred to a hospital, and receiving ceftriaxone and intravenously (IV) fluids, she progressed with mental confusion, dyspnea, diffuse abdominal pain, leukocytosis with left shift, anemia, increasing urea, creatinine, C-reactive protein, gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK), creatine phosphokinase MB fraction (CPKMB), lactic dehydrogenase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) serum levels.

Intensive care treatment started on the third day, still she kept presenting severe thrombocytopenia with no bleeding, refractory seizures to diazepam, hypotension, necessity of intubation, norepinephrine, albumin 20%, and piperacillin/tazobactam. Thoracic and abdominal scans showed bilateral pleural laminar effusion and thickening of the interlobular septa at the lung's basis and hepatosplenomegaly. Lumbar puncture was performed on day seven, and bacteriological and chemocytological cerebrospinal fluid showed no abnormalities. On the next day, a suspicion of SF disease was raised, resulting in a blood sample collection for qPCR and indirect IFA (IgG).

During the next days, she received ampicillin/sulbactam, meropenem and linezolid and became dialysis-dependent. Serological tests for Human Immunodeficiency Virus (HIV), antiglobulin testing (Coombs), hepatitis B surface antigen (HBsAg), hepatitis B envelope antibody (anti-HBe), total antibody hepatitis B core antigen (anti-HBc), IgM leptospirosis, IgM and IgG chikungunya, qPCR for dengue fever, qPCR Zika virus were all negative, whereas hepatitis B surface antibody (anti-HBs) and IgG hepatitis A antibody were positive.

After 18 days of hospitalization, she progressed with lower peripheral oxygen saturation, hypotension, bradycardia, and death, which occurred 27 days after the onset of symptoms.

Twenty-eight days after the sample collection, the laboratory issued detectable results for *Rickettsia* sp. by qPCR in serum (Ct= 37), collected on the 17th day of onset of symptoms, and negative IgG for the illness by indirect IFA.

Case 2 (2nd case)

In June 2022, a 52-year-old smoker and hypertensive housewife experienced abdominal pain, swelling, pain in upper and lower limbs (mentioned by her months before June 2022), and no fever. In the same month, she travelled to her mother's house, in Matriz de Camaragibe (AL), 52.4 km from hers. Approximately three to five days there, she travelled to her brother's house in Barreiros (80.8 kilometers from Matriz de Camaragibe), Pernambuco (PE) state, Brazil. One month later, she went back to her mother's house, where she remained for 30 days.

When inquired about her stay, the family reported that she started presenting fever, abdominal pain and vomiting, and was attended at Matriz de Camaragibe (AL) hospital. Eleven days later, she was readmitted referring swell and pain in her left upper limb, they administered dexamethasone and diclofenac due to arthritis hypothesis.

The next day, she returns to Santa Rita (AL), residing intermittently between her husband's and daughter's house. After nine days, the patient underwent an appointment at her neighborhood Health Center, mentioning fever, abdominal pain, nausea, diarrhea, hand and foot arthralgia, which had begun three weeks ago. Chikungunya was the hypothesis; blood was sampled to run full blood count, lipid profile, urea, electrolytes, thyroxine and thyroid-stimulating hormone, glucose, and liver blood tests; fever was mentioned, but not taken.

On that same night, she experienced epigastric pain, and self-administered a medication - which is unknown to the family. On the next day, she progressed to death at home. The body was sent to an autopsy, no macroscopic alteration was found, except pulmonary congestion. Hence, SFG rickettsiosis hypothesis was raised due to territorial link with the 1st case; and because of this, fragment samples were collected, in which brain fragments for yellow and dengue fever were negative (PCR), but spleen PCR assay fragment revealed a positive result (Ct = 35.2) for *Rickettsia* sp., fourteen days after her death.

Additionally, we identified that neither of the two women had been in SF endemic areas or done wildland activities, but they had access to agrarian reform places (rural areas), 7.8 km apart, and in both houses, dogs with access to Atlantic rainforest.

The 1st case's family revealed that the patient lived and worked in urban area, fed roaming cats there and in her parent's house, which she visited constantly. As for the 2nd case, clothes and household utensils were washed by her at a stream with Atlantic rainforest, and eventually she visited her husband's yields accompanied by their hound dogs, which hunted by themselves, if not fenced.

There were rumors of capybaras on agrarian reform lands; a field search for paw prints, destruction of vegetation, feces, fur, and dens

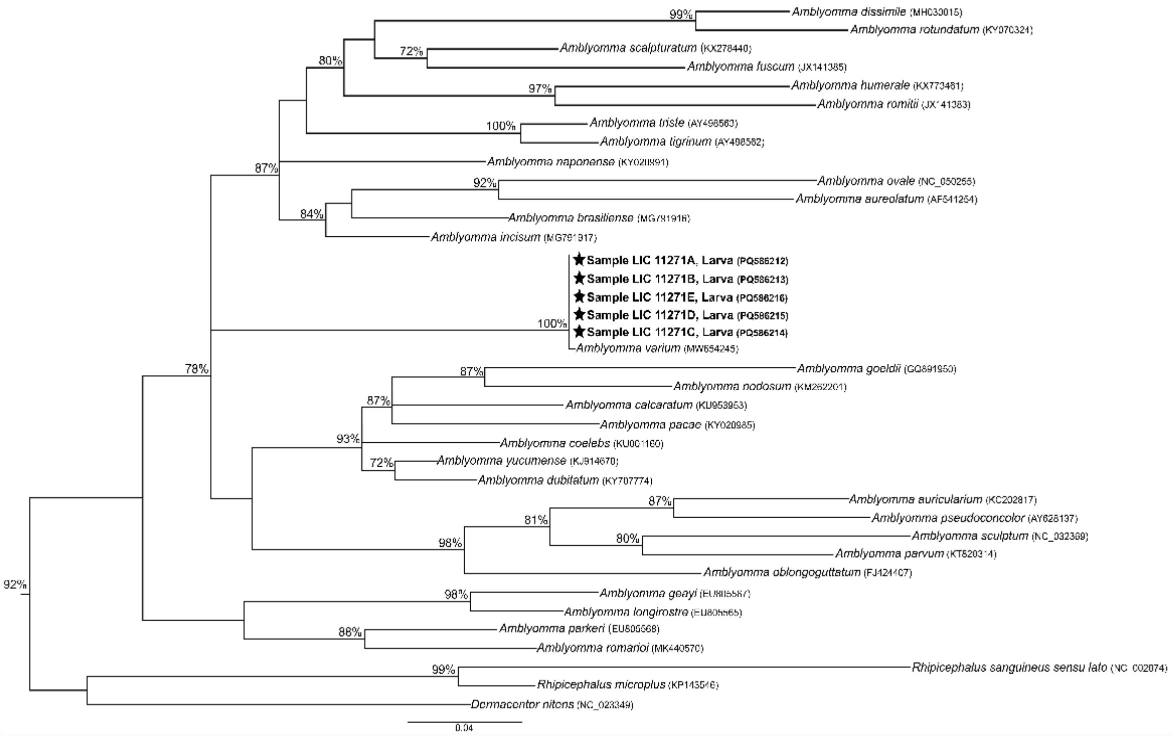

Figure 4: Supplementary Phylogenetic reconstruction, inferred by Maximum Likelihood analysis with the GTR+G evolutionary model, of a fragment of the 16S rDNA gene (464 bp) of ticks amplified from ixodids collected in Água Fria, Maragogi (AL), Brazil.

were initiated by the team, yet there was no evidence of the animal presence.

Laboratory and Environmental Findings

We collected 172 tick specimens (83 *Dermacentor nitens*, 80 *Rhipicephalus linnaei,* nine *Amblyomma* spp.) and 28 fleas (*Ctenocephalides felis*) (N= 200). Analysis of the 16S rDNA fragment indicated that, of the nine *Amblyomma* spp. larvae, five showed 99.3% (448/451) identity with *Amblyomma varium (A. varium)* (MW654245), and the other four larvae were unsuccessful in sequencing, remaining as *Amblyomma* spp. The phylogenetic reconstruction of ticks corroborated this molecular identification (Figure 4).

After the amplification method, three ticks and three fleas were found hosting rickettsias. However, there was only success in obtaining and analyzing the sequences of one flea and two tick samples. The molecular analyses resulted in: for gltA sample LIS1093A had 99.5% (397/399) identity with *R. felis* (MN726356) hosted by *C. felis* female from Santa Rita; for htrA two samples of *A. varium* larvae (LIC 11271C and D) had 100.0% identity (370/370) with several rickettsiae from the spotted fever group; and for the analyzed fragment of the ompB gene, the *A. varium* larvae sample LIC 11271C demonstrated 99.5% (408/410) identity with *Candidatus* Rickettsia andeanae (*Ca.* R. andeanae) (GU395297) and 98.7% (405/410) identity with *Rickettsia amblyommatis* (*R. amblyommatis*) (CP015012). All these tick larvae samples were from Água Fria. The phylogenetic inference of rickettsiae endorsed that the LIS1093A flea sample was infected with *R. felis* and for *A. varium* larvae it was not possible to conclude whether the infection was by *Ca.* R. andeanae or *R. amblyommatis* in LIC 11271C or another rickettsia from the SFG in LIC 11271 D (Figure 5).

Overall, 46 dogs' blood samples were obtained. Of these, five canines (5/46) were positive for *R. rickettsii* IgG in the serological panel; in which four presented IgG titration 1:64 and one 1:128. The ticks-larvae *A. varium-host-Ca R. andanae/R. amblyommatis*, belonged to this dog reagent to *R. rickettsii* IgG titration 1:128, which lived at Água Fria, while the monitored dog, that was already serological reagent for *R. rickettsii* IgG titration 1:64, *R. parkeri* IgG titration 1:256 and SF IgM titration 1:64 in July 2022, was tested again and revealed IgG titration <1:64 and negative PCR, respectively (Table 1).

When it comes to the outcome from GAIA GPS [23], the animal was monitored during 83 hours and 17 minutes, resulting in 20.9 km of peregrination. Several residences, peridomiciliar areas, yields, Atlantic rainforest, and dams were explored by the dog, almost reaching the border to PE. Although the monitoring lasted five days, we were not able to recover data from day four.

Discussion

Considering the laboratory, clinical and environmental context, the 1st case symptoms, evolution, and circumstances, except the leukocytosis, are compatible with the known disease [1-4, 7, 24-29]. However, SFG rickettsiosis mimics other hemorrhage fever illnesses [1-3], and atypical presentations may foster potential confounding [28]. Whereas knowing tick exposure or travel supports the diagnosis and aids differentiating it from other conditions, in multiple times it is not noticiable [28]. In this way, laboratory and clinical patterns may be of support to discern them.

For instance, fever accompanied by headache, myalgia, abdominal pain evolving with rash, petechiae, hypotension, thrombocytopenia, renal failure, hepatocellular injury (elevated bilirubin and

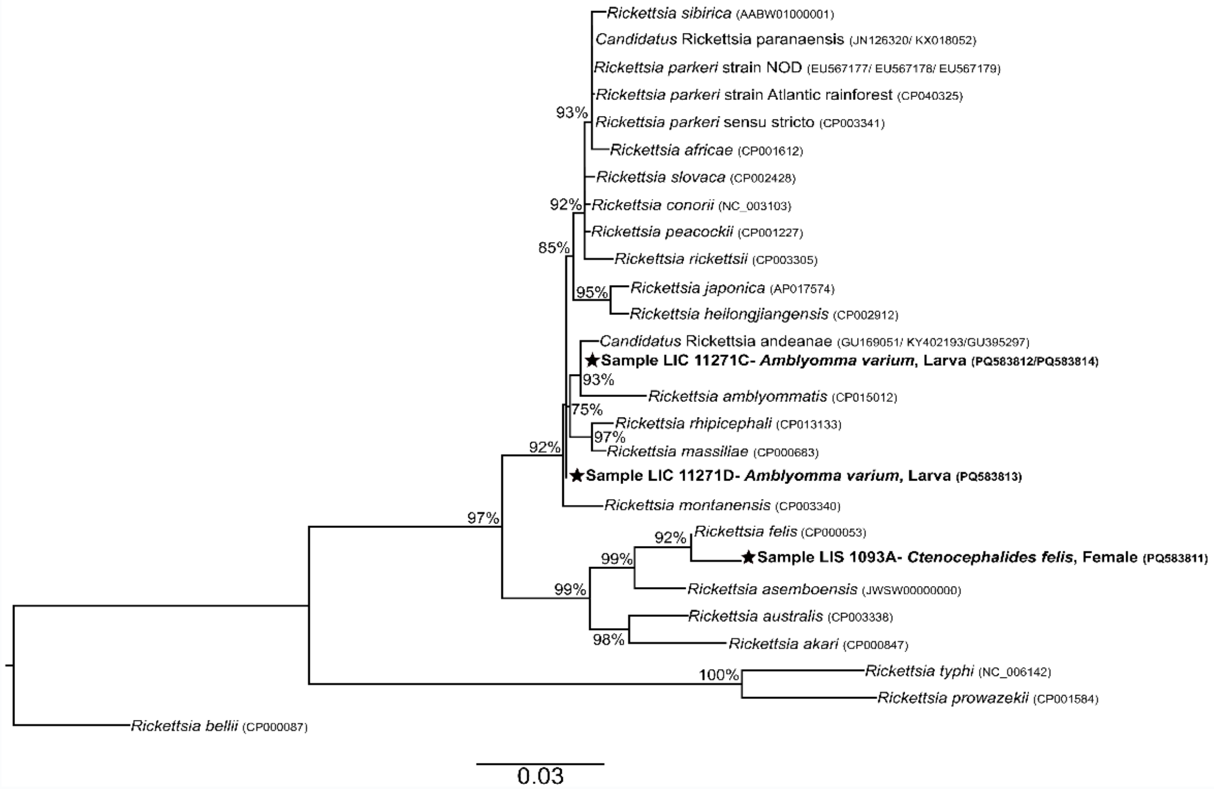

Figure 5: Concatenated phylogeny, inferred by maximum likelihood analysis with T92+G evolutionary model, of a fragment of the gltA, htrA ompB genes of rickettsiae (406 + 370 + 422 bp) detected in Ctenocephalides felis and *Amblyomma varium* collected in Maragogi, (AL), Brazil. The numbers in the branches indicate statistical support values (70% “cut-off”).

transaminase levels), neurological complications are reported in BSF cases with progression to severe forms and deaths [25, 28, 29], and observed in our 1st case.

Laboratory, because her advanced time in suspicious and death, and our 2nd case post-mortem hypothesis, it was not possible to collect a second sample for IgG comparison; but having been the first sample collected in a late immunological period, it would be expected that IgG was reagent. Complementary, the first line of antibiotics given to our 1st case had no anti-rickettsial activity and was not the ones preconized for the illness and have proven to be unsuccessful. Doxycycline, or chloramphenicol[1], might reduce the potential lethality of SFG infection, if administered immediately after the suspicious and, preferably, within five days of the onset of symptoms [3, 25, 30, 31].

Regarding environmental characteristics, they likely involve a potential vector and, possibly, the occurrence of rickettsiae [1, 6] once the cases’ living/visiting areas encompass Atlantic rainforest and feature potential competent amplifiers and hosts, such as horses and dogs; and thus, favorable parasitism, and potential infection, if confirmed competent infected vectors in the region [1, 6, 30, 32], and considering the role that dogs play in human dwellings since some rickettsiae species can be transmitted from wild cycles [1, 6, 30, 33].

Given these circumstances and the importance of acknowledging the ecological scenario of every rickettsiosis of public health interest [6], the team monitored the serologically positive dog to recognize its behavior and mobility pattern. The results corroborate with an eco-epidemiological scenario compatible with the risk of rickettsiae occurrence potentially associated with ticks [1-3, 7, 34], particularly in this specific location where capybaras are absent, but dogs with SFG serological evidence are present.

Concurrently, potential relation between SFG disease by *R. rickettsii* using dogs as hosts is supported by other countries such as the United States of America and Mexico, whereby Rocky Mountain spotted fever (RMSF) reached epidemic levels, with sustained transmission by brown dog ticks (*Rhipicephalus sanguineus s.l.)* [25]. Interestingly, there were cases among RMSF patients [25] that did not travelled preceding the illness, but relatives that often visited Mexico endemic areas, brought pet dogs with them across the border [25].

In European countries, Turkey, for example, reported a connection between Mediterranean SF and *Rickettsia conorii subsp. Conorii*, using *Rhipicephalus sanguineus s.l.* [27], while in Spain and Germany, SFG rickettsiosis, benign outcome, were associated with fleaborne *R. felis*, though all of them were treated with doxycycline [35, 36].

Differently from the majority SFG rickettsiosis lethal cases [4], in Brazil, a SFG fatal isolated human case, with identification of genus *Rickettsia,* was reported in PE state [8], a non-autochthonous area and AL neighbour state [37].

In our research, we were able to identify genus *Rickettsia* infecting *A. varium* larvae, albeit not able to determine, if by *Ca*. R. andeanae or *R. amblyommatis* (LIC 11271C) or another SFG rickettsia species (LIC 11271 D). One of our *A. varium* samples showed 98.7% similarity to *R. amblyommatis,* which is a SFG rickettsia prevalent in the Americas and has been detected in 34 tick species [38, 39].

In 2019, in Mexico, both of them were identified in *Rhipicephalus sanguineus s.l.,* which in turn, parasitizes dogs [40], though with undetermined pathogenicity for humans [38, 39]. In other Latin American countries, *R. amblyommatis* has been found through the years, but its pathogenicity for people is still to be elucidated [32, 38, 39, 41, 42].

The same applies to *A. varium,* this particular tick was reported being host to *R. rickettsii,* in Costa Rica [43] while in the Northern Brazilian territory, *A. varium* nymphs were identified parasitizing humans [44]. Moreover, although not well established, other types of ticks such as *Amblyomma parvum* (*A. parvum*) have demonstrated ability to bite humans in Northeastern Brazil, and potential for rickettsiae, as *Ca.* R. andeanae and *R. amblyommatis* [45], resulting in equal questioning about these ticks, their eventual role in the disease, and its potential widespread dissemination, which is a major public health concern [38, 42, 46].

Specifically related to our 2nd case, she showed no clinical evidence compatible with SFG rickettsiosis. Thus, our team believes her demise could not have been the result of the illness itself, but while infected by it, if it was a true positive laboratory result.

Finally, our findings highlight the importance of considering potential pathogenic SFG rickettsiosis in the area once there are favorable environment, clinical symptoms, and laboratory indications of the disease. Initially, BSF was first described in 1929, decades later (2003) a new pattern with uncommon benign evolution cases, and in other state, was found [2]. At first, because the cases were only confirmed serologically, there was no possibility to identify the rickettsia involved, yet with the strengthening of laboratory and research, it was possible to discover the *R. parkeri* species; and then, propose specific recommendations for surveillance and treatment [31, 47].

Conclusion

Unfortunately, the existing data was not sufficient to conclude which rickettsiae species, whether pathogenic or not, were potentially infecting the two cases, and if they were truly SFG rickettsiosis subjects; therefore, whether they had any association with the clinical conditions presented. If the species found were, in fact, *Ca.* R. andeanae and *R. amblyommatis*, the species infecting the ticks analyzed would probably be unlikely the etiological agents causing the severe clinical manifestations and evolution to death observed in the patients, especially due to the lack of definitive evidence that establishes the two species as known pathogenic for humans.

Nonetheless, in light of the available information, it is reasonable to assume that this illness and its bio-cycle may be affected by the local ecology, species or strain virulence, epidemiological risk factors, and human personal characteristics [4, 6], and despite inconclusive, there is some clinical evidence in the 1st case, laboratory, and risk for a possible vector exposure and rickettsial infection, in both cases, in the investigation we carried out.

For this reason, there is a demand for an active and sensitive epidemiological surveillance, under One Health approach, to detect early possible suspected cases compatible with rickettsiosis, and any possible rickettsiosis that is still occurring silently in the area in question; a laboratory investigation protocol with appropriate samples collected timely and in number; and more studies related to possible vectors and hosts that may be participating in a possible transmission cycle of a known or unknown rickettsiosis, that would be candidates of an already known or emerging rickettsiosis in the region.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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