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# SHORT REPORT
# A cluster of *Rickettsia rickettsii* infection at an animal shelter in an urban area of Brazil

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## SUMMARY

*Rickettsia rickettsii* infection is being increasingly recognized as an important cause of fatal acute illness in Brazil, where this tick-borne disease is designated Brazilian spotted fever (BSF). In this study we report five fatal cases of BSF in employees of an animal shelter in an urban area in the municipality of Rio de Janeiro in southeast Brazil after a natural disaster on 11 January 2011. Four of the cases occurred from 27 January to 11 April 2011, while the fifth fatal case was identified in April 2012. Three cases were confirmed by molecular analysis and two by epidemiological linkage. An investigation of BSF was performed in the animal shelter, and blood samples were collected from 115 employees and 117 randomly selected dogs. The presence of high levels (1024–4096) of antibodies against spotted fever group rickettsiae was found in three (2·6%) employees and 114 (97·5%) dogs. These findings emphasize the need to consider BSF as a possible cause of undifferentiated febrile illness, especially dengue and leptospirosis, in patients occupationally exposed to dogs heavily infested by ticks, mainly working at kennels and animal shelters that have inadequate space for the animals housed and frequently providing an environment conducive to exposure to pathogens such as *R. rickettsii*.

**Key words**: Animal shelter, Brazil, cluster, fatal cases, *Rickettsia rickettsii*, spotted fever.

Employees and/or volunteers who work in animal shelters are at risk for occupational exposure to tick-borne pathogens, including spotted fever group rickettsiae (SFGR). Although SFGR are not primarily considered agents specifically associated with occupations, cases may occur in people who are occupationally exposed to animals, especially dogs and ticks [1–4].

SFGR are Gram-negative bacilli belonging to the alpha-1 subgroup of the class Proteobacteria, which contains more than 25 described species or strains that are recognized as emerging or re-emerging pathogens [5]. *Rickettsia rickettsii* is the most important

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species in SFGR within the American continents, and it is the causative agent of Rocky Mountain spotted fever (RMSF) and Brazilian spotted fever (BSF) [6].

BSF is the most frequently reported tick-borne disease, and since 2001, it has been considered a reportable disease in Brazil [7–10]; in fact, between 2001 and 2013, over 1100 cases have been reported, and four states (Minas Gerais, Rio de Janeiro, Santa Catarina, São Paulo) account for over 80% of all reported cases (Brazilian Ministry of Health, unpublished data).

Here, we report a cluster of fatal BSF cases in employees working at an animal shelter in an urban area in Rio de Janeiro, where the five cases were previously diagnosed as dengue and leptospirosis.

*Case 1*. A 44-year-old man who worked as a veterinary care assistant became febrile on 21 January 2011. On 27 January, he sought medical care at a public health unit for treatment and was diagnosed with dengue. He presented with fever, myalgia, headache, and asthenia. Supportive treatment was initiated, but he developed haemorrhagic phenomena and died 24 h after hospitalization. Results of laboratory analysis included a leukocyte count of 7900/mm$^3$, with 36% bands, platelet count of 60 000/mm$^3$, and haemoglobin level of 11·9 g/dl (normal range 13–18 g/dl). No specific laboratory tests were performed during his hospitalization.

*Case 2*. A 52-year-old man who worked as a stonemason presented to another public health unit on 3 February 2011 with a history of 7 days of fever (39·5 °C), severe malaise, myalgia, headache, vomiting, asthenia, and swelling. The haemogram and blood biochemistry examinations were unremarkable. Based on the clinical findings, he was diagnosed with dengue, and supportive therapy was initiated. Two days later, the patient remained febrile and again sought medical attention. The laboratory analysis showed haemoglobin level of 15·0 g/dl, leukocyte count of 11 500 cells/mm$^3$ and thrombocytopenia (54 000 platelets/mm$^3$, normal range 140000–450000 platelets/mm$^3$). Dengue and leptospirosis serological tests were negative. Ultimately, he developed pulmonary and renal failure and died 48 h after admission. Clinical samples for laboratory analysis were not available for rickettsial tests.

*Case 3*. A 52-year-old man who worked in the animal crematorium sought medical attention on 16 March 2011 at a public health unit. He presented with complaints of headache, fever, chills, myalgia, calf pain, nausea, vomiting, and diarrhoea. Blood samples were collected and revealed thrombocytopenia (60 000 platelets/mm$^3$), high levels of alanine aminotransferase (171 U/l, normal <40 U/l), aspartate aminotransferase (257 U/l, normal <40 U/l), urea (102 mg/dl, normal <39 mg/dl), creatinine (3·6 mg/dl, normal <1·3 mg/dl), creatine phosphokinase (1184 U/l, normal <174 U/l), and C-reactive protein (215 mg/l, normal <3 mg/l). He was treated for leptospirosis with 1 500 000 IU of aqueous crystalline penicillin G, but no improvement occurred. Two days later, he was transferred to a referral hospital where his condition continued to deteriorate, and he died 72 h later from multiple organ failure. Serum collected on day 4 of the illness was negative for dengue and leptospirosis. An immunofluorescence commercial assay (IFA; Scimedx™, Scimedx Corp., USA) for antibodies against *R. rickettsii* was performed considering a cut-off titre at 64. The serum sample was IgM reactive (class-specific immunoglobulin M, titres of 128), and IgG non-reactive (titres <32). A culture for *Leptospira* was negative. A polymerase chain reaction (PCR) assay on the serum sample targeting the type II citrate synthase (*gltA*, 381 bp) and outer membrane protein A (*ompA*, 532 bp) genes was evaluated via the National Reference Laboratory for Rickettsioses Brazil and segments of rickettsial genes were amplified [8–11].

*Case 4*. A 36-year-old woman, a general services assistant, was taken to an emergency unit on 11 April 2011 with a high fever, dehydration, and hypovolaemic shock. She had experienced a 5-day history of fever, severe malaise, headache, myalgia, arthralgia, and retro-orbital pain. No exanthema was observed. She was transferred to a public hospital with a diagnosis of dengue. Supportive treatment was initiated, but the patient's condition continued to deteriorate, and she died of multiple organ failure 6 days after the onset of the clinical manifestations. Laboratory findings included leukocyte count of 2900 cells/mm$^3$ and thrombocytopenia (21 500 platelets/mm$^3$). No dengue NS1 antigen was detected in her serum sample. The serum sample was assayed by PCR for both the *gltA* and *ompA* genes, which confirmed the BSF diagnosis, but sequencing was not performed due to the lack of a sufficient clinical sample.

After the confirmation of BSF in these cases, an epidemiological investigation was performed at the animal shelter where the patients worked to identify the origin of the outbreak. In June 2011, serum

Table 1. *Qualitative assessment of IgG antibody serum reactivity and geometric mean titre of 117 dogs determined by indirect imunofluorescence assay to* Rickettsia rickettsii

| Titres | No. of dogs (%) |
|---|---|
| 16 | 3 (2·6%) |
| 64 | 50 (42·7) |
| 128 | 13 (11·1) |
| 256 | 22 (18·8) |
| 512 | 3 (2·6) |
| 1024 | 10 (8·6) |
| 2048 | 13 (11·1) |
| 4096 | 3 (2·6) |
| Geometric mean titre (range) | 189 (16–4096) |

For calculation of geometric mean titre estimates, a titre of <32 was assigned a value of 16, and a titre of ⩾4096 was assigned a value of 4096.

samples were collected from 115 employees and 117 dogs that were randomly selected. Specific-IgG IFA was detected in the serum samples from three (2·6%) employees, with titres of 64, 128 and 512, and 114 (97·5%) dogs, with titres of 64–4096 (Table 1). Two blood samples from IFA-negative dogs were evaluated by routine PCR assay and the result was negative. No dogs presented with clinical manifestations of illness. Tick samples were not available for analysis, because insecticides had previously been applied at the animal shelter to control for ectoparasites on the dogs. Control measures were adopted, and information about the nature, transmission route, and clinical manifestations of BSF was disseminated to the employees.

Although control measures and strategies for preventing exposure to tick-borne diseases had been implemented at the animal shelter, another case developed. Specifically, on 12 April 2012, an employee responsible for cleaning the kennels, a 47-year-old-woman (case 5) who sought medical care at a referral hospital presented with influenza-like symptoms and exanthema. The laboratory analysis revealed a haemoglobin level of 14·1 g/dl, leukocyte count of 7580 cells/mm$^3$, platelet count of 30 000, alanine aminotransferase 326 U/l, aspartate aminotransferase 250 U/l, urea 126 mg/dl, creatinine 4·4 mg/dl and creatine phosphokinase 342 U/l. She died within the first day of admission to the hospital, 4 days after the onset of the illness. IgM and IgG against *R. rickettsii* were non-reactive in serum samples collected on 17 April, but a PCR assay of autopsy samples from the liver, brain, heart, spleen, and kidney, collected 24 h after her death, confirmed an SFGR infection based on the presence of both the *gltA* and *ompA* genes.

DNA sequencing in cases 3 and 5 were identified as *R. rickettsii* by sequencing of the genes coding for the *ompA* gene (100% similarity): case 3 (KJ184538) and case 5 (KJ433988). The nucleotide sequences of the *gltA* fragments from case 3 (KJ184537) and case 5 (KJ433987) had 100% and 99% sequence similarity to the homologous fragment of *R. rickettsii* that was isolated from Brazil (GenBank accession no. CP003305).

In this study, we describe a cluster of *R. rickettsii* infections at an animal shelter in an urban area in the municipality of Rio de Janeiro, Brazil. This animal shelter, maintained by a non-governmental organization (NGO) has a constructed area of about 5400 m$^2$, housing over 4000 dogs and 800 cats, among other animals such as birds, rabbits, and horses. The area available for animals is greater, but due to lack of resources, the NGO has failed to build the necessary kennels and catteries. Thus, hundreds of dogs are concentrated in the old shelter, sleeping on a cement floor. In addition, in this shelter the entry of animals is not limited or controlled. In this context, every day this NGO rescues about 10 animals on the streets and receives 50 other animals from different municipalities of the state of Rio de Janeiro. Consequently, this large number of animals in a confined space increases the risk for infectious diseases which is difficult to control in the animal shelter. Under this scenario, it may be assumed that the high prevalence of the seropositive reactions to SFGR in dogs and the occurrence of fatal BSF cases in the present study were probably caused by the entry of infected animals into a shelter with unsuitable sanitary conditions and overcrowding.

Spotted fever caused by *R. rickettsii* remains the most severe of all tick-borne rickettsioses and continues to cause significantly high mortality in Brazil, where this zoonosis is frequently associated with misdiagnosis, especially with diseases such as dengue, meningococcaemia, and leptospirosis. In this study, all of the cases were diagnosed as dengue or leptospirosis, and the specific antibiotic therapy was not initiated within 5 days of the onset of illness, which undoubtedly led to the fatal outcomes. These five patients died a short time after admission (24–72 h) – three cases were confirmed by PCR assay of blood or autopsy samples and two were clinically diagnosed. Unfortunately, although BSF is a reportable disease, a lack of prompt

initiation of the anti-rickettsial treatment has been frequently observed in Brazil, where BSF has wrongly been approached as a diagnosis of exclusion.

Family clusters are a well-recognized feature of rickettsial disease caused by *R. rickettsii* and other SFGR, but few cases associated with occupational exposure have been described [7, 12–14]. However, studies demonstrate that workers occupationally exposed to tick bites are under significantly increased risk of other SFGR infections [4]. In this BSF cluster, three cases were confirmed by PCR analysis and two others by an epidemiological link to an animal shelter, where animals infested with ectoparasites are often gathered. Some studies have shown that animal shelter workers are suspected of having a high risk of tick-borne diseases. Many shelters do not have adequate areas to house the animals, which are often infested with ectoparasites, especially ticks, increasing the risk of transmission of tick-borne agents not only for employees, but also among the animals themselves [1–4].

Three employees with no history of acute febrile illness had serological evidence of BSF infection. This finding associated with the rate of SFGR serum prevalence in the dogs, which is considerably higher than rates previously reported in the United States and Europe [15, 16], provides more evidence that this cluster was almost certainly associated with exposure to the dogs at the animal shelter. The occurrence of the fifth BSF case, 12 months later than the fourth case, suggests that the focus of *R. rickettsii* had persisted in the animal shelter [17, 18].

Several studies in Brazil and other regions of the world have shown that dogs are an intermediary source for human tick infestation and can act as a sentinel for BSF [15, 16, 19, 20]. Therefore, the risk of human disease is increased at animal shelters, where sick and flea- and tick-infested animals are often housed in confined spaces.

It is a limitation of this study that no tick specimens were available for identification and molecular analysis. Although the use of insecticides at the animal shelter prevented studying the ticks, the presence of anti-SFGR antibodies in the employees and the elevated titres in 97·5% of the dogs confirmed the circulation of SFGR at the animal shelter. Similarly, it has become impossible to identify what were the type(s) of rickettsia(s) responsible for the high canine serum reactivity detected in this study, the insecticide application at the shelter prior to June 2011 made it impossible to identify species of ticks involved in the outbreak. In previous studies in the state of Rio de Janeiro *R. rickettsii* has been detected in *Amblyomma sculptum* (formely *Amblyomma cajennense*), *Amblyomma dubitatum* and *Rhipicephalus sanguineus*.

Since the first BSF cases were identified in 1941, this zoonosis has often been described in the state of Rio de Janeiro, with the highest number of confirmed cases in the north-west and mountainous regions. Since 2005, when five patients with BSF (two fatal cases) were identified in the mountainous region of Rio de Janeiro (state), this area has been considered an important BSF-endemic region. Although it cannot be confirmed, it is possible that this BSF animal shelter cluster in the urban area of Rio de Janeiro (municipality) is associated with dogs rescued from this mountainous region, ~100 km north of Rio de Janeiro, where torrential rains caused the worst natural disaster in Brazil on 11 January 2011, 10 days before the occurrence of the first fatal (probable) case of BSF.

Although the risk factors for outbreaks after natural disasters such as floods are mainly associated with the displacement of the human population, the transfer and dispersion of sick or ectoparasite-infested animals to other regions also needs to be considered. Thus, this fatal BSF cluster may have been caused by dogs infested with ticks or infected by *R. rickettsii* coming from the mountainous region of Rio de Janeiro (state), where BSF cases are monitored, to an urban area that was previously disease-free.

Control measures and strategies for preventing exposure to tick-borne diseases were implemented at the animal shelter, but the last fatal BSF case occurred 1 year after the first case. This observation reflects the persistence of infected ticks at the animal shelter and reinforces the need for maintaining surveillance for BSF in this urban area in Rio de Janeiro (municipality).

Our results emphasize the importance of appropriate empirical anti-rickettsial treatment and including BSF in the differential diagnosis of acute febrile diseases, especially in high-risk patients such as those employed at animal shelters. It is also important to consider that the animals rescued and transferred during natural disasters, such as the flooding in Rio de Janeiro (state) on 11 January 2011, can lead to new outbreaks of BSF or other zoonotic diseases associated with sick animals or their infected ectoparasites.

Finally, as it is highly likely that several zoonoses, especially tick-borne diseases, will continue affecting the human population, surveillance and response

systems for these zoonoses need to be strengthened, particularly with regard to natural disasters, when the displacement of human populations and animals can generate new foci of zoonoses in previously non-endemic areas.

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## DECLARATION OF INTEREST

None.

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