Acta Tropica 167 (2017) 18–20

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short communication

"Candidatus Rickettsia asemboensis" in *Rhipicephalus sanguineus* ticks, Brazil

Bruno Dall'Agnol[a,b], Ugo Souza[a], Anelise Webster[a], Bárbara Weck[a], Bárbara Stenzel[c], Marcelo Labruna[d], Guilherme Klafke[a], João Ricardo Martins[a], Carlos Alexandre Sanchez Ferreira[b], José Reck[a,*]

[a] *Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Fundação Estadual de Pesquisa Agropecuária (FEPAGRO), Eldorado do Sul, RS, Brazil*
[b] *Faculdade de Biociências (FABIO), Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil*
[c] *Centro Estadual de Vigilância em Saúde (CEVS), Porto Alegre, RS, Brazil*
[d] *Universidade de São Paulo (USP), São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 9 December 2016
Accepted 12 December 2016
Available online 13 December 2016

Keywords:
Rickettsia felis-like
Spotted fever
Rio Grande do Sul
Flea-borne

ABSTRACT

"Candidatus Rickettsia asemboensis" is an obligate intracellular bacterium of the Rickettsiales order, genetically related to species belonging to the *Rickettsia felis* group, agents of flea-borne spotted fever. Here we report for the first time the detection of "Ca. R. asemboensis", a flea-associated organism, in *Rhipicephalus sanguineus* ticks. It is the first occurrence of this emerging bacterium in Brazil, which increases the geographical distribution of this *R. felis*-like agent.

1. Introduction

Rickettsia felis and related *Rickettsia* species are worldwide distributed emerging pathogens. Recently, there is an increasing number of reports around the world implicating these agents as a human pathogens. The syndromes associated to *R. felis* and its related species are also referred as flea-borne spotted fever (Angelakis et al., 2016).

"Candidatus Rickettsia asemboensis" is a recently described bacterium of the Rickettsiales order. It is genetically closely related to the human pathogen *Rickettsia felis*, and it has been found in endemic areas of flea-borne spotted fever (Jiang et al., 2013). Until now, it was only described infecting fleas associated to domestic animals, rodents and from human households, such as *Xenopsylla cheopis*, *Synosternus cleopatrae*, *Pulex irritans*, *Ctenocephalides felis* and *Ctenocephalides canis*. In addition to the site of first description (Asembo, Kenya), this microorganism has been additionally reported in South Africa, Israel, Ecuador, Colombia and United States (Jiang et al., 2013; Oteo et al., 2014; Rzotkiewicz et al., 2015; Faccini-Martínez et al., 2016; Billeter et al., 2016; Kolo et al., 2016).

According to the concept used for novel/unclassified *Rickettsia* spp., the potential of unknown or novel bacterial species as disease agents is never ruled out when other species from the same genus are pathogenic, particularly for vector-borne organisms (Labruna et al., 2007). In recent decades, diseases associated with rickettsial bacteria are considered as emerging and re-emerging diseases worldwide, particularly for those species belonging to the Spotted Fever Group (which includes *R. rickettsi* and *R. parkeri*) and Transitional Group *Rickettsia* (which includes *R. felis* and "Ca. R. asemboensis") (Parola, 2011; Sahni et al., 2013). Here, we report the detection of "Ca. R. asemboensis" for the first time in Brazil. Moreover, it is the first evidence of "Ca. R. asemboensis" in ticks.

2. Materials and methods

From February to March 2015, we performed tick collections on stray dogs from Tapes municipality (30°40′25"S 51°23′56"W), Rio Grande do Sul state, Southern Brazil. This area was chosen because cases of fever of unknown origin were recently reported, including one suspected human spotted fever fatal case.

Ticks identification was performed according Barros-Battesti et al. (2006). Total genomic DNA was extracted from samples

* Corresponding author at: Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Estrada do Conde, 6000, Eldorado do Sul, 92990-000, RS, Brazil.
E-mail address: jose.reck@gmail.com (J. Reck).

http://dx.doi.org/10.1016/j.actatropica.2016.12.008
0001-706X/

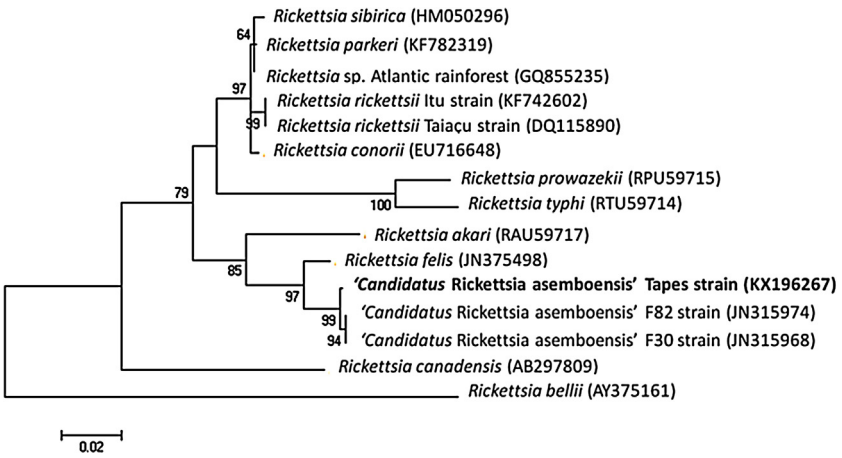

Fig. 1. Phylogenetic analysis of the *gltA* gene was performed using MEGA 7 software, using the Maximum Likelihood algorithm and Tamura-3-parameter with Gamma distribution as the DNA substitution model and 500 bootstraps for the phylogeny test.

according to the protocol published by Aljanabi and Martinez (1997). Samples were screened for *Rickettsia* spp. using a PCR assay for a 401-bp fragment of the citrate synthase (*gltA*) gene (Labruna et al., 2004). PCR amplification targeting was then performed for the Outer Membrane Protein A (*ompA*) gene, directed for Spotted Fever Group (SFG) *Rickettsia* (Oteo et al., 2004). To allow for the identification of the *Rickettsia* found, we performed three other PCR reactions, one directed to another *gltA* fragment (834 bp) (Labruna et al., 2004) and two other for the *htrA* gene (434 and 549 bp) (Labruna et al., 2004).

Sequencing of PCR products for the *gltA* and *htrA* genes was performed. Contig sequences were assembled with Geneious R9 software (Biomatters Ltd., Auckland, New Zealand), and the consensus sequences were submitted to a BLAST algorithm. The phylogenetic tree of the *gltA* gene was building using MEGA 7 software (Kumar et al., 2016). This study was approved by Ethics Committee of IPVDF (approval no. 13/2013).

3. Results and discussion

In 2015, some cases of humans showing long-term fever of unknown origin were reported in Tapes. One case fatality was registered. The fatal victim reported a "bug" bite some days before the onset of clinical findings. Local health authorities considered that spotted fever was the presumptive diagnosis associated to the fatal case. Then, we investigated the presence of *Rickettsia* spp. in ticks from dogs of this locality. A total of 63 *Rhipicephalus sanguineus* ticks (two larvae, 30 nymphs, 15 females and 16 males) were collected from 14 stray dogs. No fleas or other ectoparasites were found in sampled dogs.

One *R. sanguineus* male tick was positive in the screening PCR for *Rickettsia* spp. for *gltA* gene. The sample was negative in the PCR for the *ompA* gene, indicating that it was not SFG *Rickettsia*. The sample was positive in all three PCR reactions (*gltA* fragment 834 bp and *htrA* fragment 434 and 549 bp). BLAST analysis showed 99% and 100% identity with "Ca. R. asemboensis" isolate F30 (GenBank: JN315968 and JN315969) for the *gltA* and *htrA* genes, respectively. The sequences of *gltA* and *htrA* genes from samples of this work were deposited in Genbank under accession numbers KX196267 and KX196268, respectively. The phylogenetic tree of the *gltA* gene (Fig. 1) showed that the DNA sequence of "Ca. R. asemboensis" from Tapes, Brazil, was close to that of "Ca. R. asemboensis" from Kenya, and both were placed together with *R. felis* and *R. akari* in a clade of the Transitional Group *Rickettsia*. The sequence of "Ca. R. asemboensis" is also highly similar (99%) to that of *Rickettsia* sp. strain RF2125, a member of the *R. felis*-like genotype group that circulates in fleas from Uruguay (Venzal et al., 2006).

This is the first report of "Ca. R. asemboensis" infection in ticks. Also, it is the first detection of "Ca. R. asemboensis" in Brazil. This rickettsia is genetically similar to *Rickettsia felis*, a flea-associated *Rickettsia* considered pathogenic for humans (Raoult et al., 2001). Regardless of the vast literature on Spotted Fever Group *Rickettsia* spp. from Brazil (for a comprehensive review see Szabó et al., 2013), data on *R. felis*-related species are scarce. The detection of *Rickettsia* spp. in *R. sanguineus* draws attention since it is the most widespread tick in the world (Dantas-Torres, 2010; Szabó et al., 2010). In this sense, further studies are needed to address the potential involvement of ticks in "Ca. R. asemboensis" infection and transmission.

It is possible that "Ca. R. asemboensis" could be present in other South American countries, as well as in other Brazilian regions. These data increase the geographical distribution of this *R. felis*-like agent. Further studies must address the association of "Ca. R. asemboensis" with Brazilian cases of rickettsiosis and infection prevalence.

Acknowledgments

We thank to staff of Secretaria Municipal da Saúde de Tapes, Rio Grande do Sul, Brazil for help in field work. We also thank to Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

References

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25 (22), 4692–4693.
Angelakis, E., Mediannikov, O., Parola, P., Raoult, D., 2016. *Rickettsia felis*: the complex journey of an emergent human pathogen. Trends Parasitol. 32 (7), 554–564.
Barros-Battesti, D.M., Arzua, M., Bechara, G.H., 2006. Carrapatos De Importância Médico-Veterinária Da Região Neotropical: Um Guia Ilustrado Para Identificação De Espécies, Vox/ICTTD-3/Butantan, São Paulo.
Billeter, S.A., Diniz, P.P., Jett, L.A., Wournell, A.L., Kjemtrup, A.M., Padgett, K.A., et al., 2016. Detection of *Rickettsia* species in fleas collected from cats in regions endemic and nonendemic for flea-borne Rickettsioses in California. Vector Borne Zoonotic Dis. 16 (3), 151–156.
Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasites Vectors 3, 26.
Faccini-Martínez, Á.A., Ramírez-Hernández, A., Forero-Becerra, E., Cortés-Vecino, J.A., Escandón, P., Rodas, J.D., et al., 2016. Molecular evidence of different *Rickettsia* species in Villeta, Colombia. Vector Borne Zoonotic Dis. 16 (2), 85–87.
Jiang, J., Maina, A.N., Knobel, D.L., Cleaveland, S., Laudisoit, A., Wamburu, K., et al., 2013. Molecular detection of *Rickettsia felis* and *Candidatus* Rickettsia

asemboensis in fleas from human habitats, Asembo, Kenya. Vector Borne Zoonotic Dis. 13 (8), 550–558.
Kolo, A.O., Sibeko-Matjila, K.P., Maina, A.N., Richards, A.L., Knobel, D.L., Matjila, P.T., 2016. Molecular detection of zoonotic Rickettsiae and *Anaplasma* spp. in domestic dogs and their ectoparasites in Bushbuckridge, South Africa. Vector Borne Zoonotic Dis. 16 (4), 245–252.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7), 1870–1874.
Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., et al., 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42 (1), 90–98.
Labruna, M.B., Pacheco, R.C., Richtzenhain, L.J., Szabó, M.P., 2007. Isolation of *Rickettsia rhipicephali* and *Rickettsia bellii* from *Haemaphysalis juxtakochi* ticks in the state of São Paulo, Brazil. Appl. Environ. Microbiol. 73 (3), 869–873.
Oteo, J.A., Portillo, A., Blanco, J.R., Ibarra, V., Santibáñez, S., 2004. *Rickettsia africae* infection. Three cases confirmed by PCR. Med. Clin. (Barc.) 122 (20), 786–788.
Oteo, J.A., Portillo, A., Portero, F., Zavala-Castro, J., Venzal, J.M., Labruna, M.B., 2014. 'Candidatus Rickettsia asemboensis' and *Wolbachia* spp., *Ctenocephalides felis* and *Pulex irritans* fleas removed from dogs in Ecuador. Parasit. Vector 7, 455.
Parola, P., 2011. *Rickettsia felis*: from a rare disease in the USA to a common cause of fever in Sub-Saharan Africa. Clin. Microbiol. Infect. 17 (7), 996–1000.
Raoult, D., La Scola, B., Enea, M., Fournier, P.E., Roux, V., Fenollar, F., et al., 2001. A flea-associated *Rickettsia* pathogenic for humans. Emerg. Infect. Dis. 7 (1), 73–81.
Rzotkiewicz, S., Gutiérrez, R., Krasnov, B.R., Morick, D., Khokhlova, I.S., Nachum-Biala, Y., et al., 2015. Novel evidence suggests that a '*Rickettsia felis*-like' organism is an endosymbiont of the desert flea, *Xenopsylla ramesis*. Mol. Ecol. 24 (6), 1364–1373.
Sahni, S.K., Narra, H.P., Sahni, A., Walker, D.H., 2013. Recent molecular insights into rickettsial pathogenesis and immunity. Future Microbiol. 8 (10), 1265–1288.
Szabó, M.P., de Souza, L.G., Olegário, M.M., Ferreira, F.A., de Albuquerque Pajuaba Neto, A., 2010. Ticks (Acari: Ixodidae) on dogs from Uberlândia, Minas Gerais, Brazil. Transbound. Emerg. Dis. 57 (1–2), 72–74.
Szabó, M.P., Pinter, A., Labruna, M.B., 2013. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front. Cell. Infect. Microbiol. 3, 27.
Venzal, J.M., Pérez-Martínez, L., Félix, M.L., Portillo, A., Blanco, J.R., Oteo, J.A., 2006. Prevalence of *Rickettsia felis* in *Ctenocephalides felis* and *Ctenocephalides canis* from Uruguay. Ann. N. Y. Acad. Sci. 1078, 305–308.