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Diversity and molecular characterization of novel hemoplasmas infecting wild rodents from different Brazilian biomes

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ABSTRACT

Although hemoplasma infection in domestic animals has been well documented, little is known about the prevalence and genetic diversity of these bacteria in wild rodents. The present work aimed to investigate the occurrence of hemotrophic mycoplasmas in wild rodents from five Brazilian biomes, assessing the 16S rRNA phylogenetic position of hemoplasma species by molecular approach. Spleen tissues were obtained from 500 rodents, comprising 52 different rodent species trapped between 2000 and 2011. DNA samples were submitted to previously described PCR protocols for amplifying *Mycoplasma* spp. based on 16S rRNA, followed by sequencing and phylogenetic inferences. Among 457 rodent spleen samples showing absence of inhibitors, 100 (21.9%) were PCR positive to *Mycoplasma* spp. The occurrence of hemotropic mycoplasmas among all sampled rodents was demonstrated in all five biomes and ranged from 9.3% (7/75) to 26.2% (38/145). The Blastn analysis showed that amplified sequences had a percentage of identity ranging from 86 to 99% with other murine hemoplasmas. The ML phylogenetic analysis of 16S rRNA gene of 24 positive randomly selected samples showed the presence of ten distinct groups, all clustering within the *Mycoplasma haemofelis*. The phylogenetic assessment suggests the circulation of novel hemoplasma species in rodents from different biomes in Brazil.

1. Introduction

Hemotropic mycoplasmas are epicellular erythrocytic bacteria attach to red blood cells of a wide variety of wild and domestic animals, including humans. Although hemoplasma infection in domestic animals has been well documented, little is known about the prevalence, genetic diversity, and routes of transmission, interactions among bacteria-host-vectors, and the impact of infection on wild animal's health [1–3]. Blood-sucking arthropods (ticks, fleas and lice) are responsible for the transmission of hemotropic mycoplasmas among animals [4,5].

Phylogenetic analyses have identified two major clusters of hemoplasmas, comprising *Mycoplasma suis* and *Mycoplasma haemofelis* groups. Several hemoplasma species infecting wild and domestic animals were included in these two clusters [6,7]. Phylogenetic approaches have been showing the circulation of a wide and diverse number of genotypes and/or *Candidatus* to new hemoplasma species in wild animals, including some with zoonotic potential [8,9].

Rodentia is the most diversified and widespread order of mammals. A wide number of species is found in different habitats, including semi-aquatic, terrestrial and semi-fossorial ones in Brazil [10]. Rodent species may act as reservoirs for diverse zoonotic pathogens [11–13]. However, the zoonotic potential of rodent hemoplasmas has not yet been established [14].

Recently, *Mycoplasma haemomuris*, one of two haemotropic species of the *Mycoplasma* genus currently recognized in rodents, has ben separated in two subgroups genetically distinct: '*Candidatus M. haemomuris* subsp. musculi', detected in small Japanese field mouse (*Apodemus argenteus*) an endemic species from Japan [15] and '*Candidatus M. haemomuris* subsp. ratti', detected in *Rattus rattus* [16]. According to the authors, the separation of *M. haemomuris* in two species depends mostly on the host species where these strains have been detected rather than the geographical

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localization where positive animals have been sampled. Experimental and/or field studies have incriminated lice (*Polyplax serrata* and *P. spinulosa*) as vectors of *M. coccoides and M. haemomuris* among rodents [14,17].

Considering the large number of hemotropic mycoplasma species, the crescent number of *Candidatus* to new species recently described in different species of mammals and the wide spectrum of rodents distributed around the world, the diversity of hemoplasmas infecting rodents are presumably more complex than is currently supposed.

Regarding the occurrence of hemotropic mycoplasmas in rodents in Brazil, novel hemoplasmas have been reported in capybaras in the state of Paraná [18]. Besides, hemoplasmas have been detected in several other wild animals in Brazil, including wild carnivores [19], cervids [20] and non-human primates [21,22].

The present study aimed to investigate the occurrence and assessment of the 16S rRNA phylogenetic position of hemoplasma species infecting wild rodents from different Brazilian biomes through molecular approach.

2. Material and methods

2.1. Distribution and rodent species sampled

Between 2000 and 2009, different rodent species (52) were trapped in five Brazilian biomes (Fig. 1) (http://www.informativoflorestal.com.br). Animals were caught using Tomahawk and Shermann "live-traps" during previous studies performed by the Laboratories of Trypanosomatid Biology and of Biology and Parasitology of Wild Mammals Reservoirs Laboratories, Oswaldo Cruz Institute, Rio de Janeiro, Brazil [23–25]. Euthanasia of animals was performed for taxonomic identification and/or diagnosis of parasites. Rodents were chemically immobilized using an association of ketamine hydrochloride (100 mg/mL) and acepromazine (10 mg/mL) intramuscularly. When the death of anesthetized animal did not occur after total blood collection, then euthanasia was performed through the application of Potassium Chloride 19.1% via intracardiac (2 mL/kg).

Spleen tissues from 500 rodents were collected and stored in DNase and RNase-free microtubes, containing ethanol and stored at −20 °C until DNA extraction. Sampling procedures were approved by Brazilian Institute for the Environment and the Natural Renewable Resources (IBAMA), (IBAMA/CGFAU/LIC 3665-1) and Oswaldo Cruz Foundation (FIOCRUZ) ethics committee (P0007-99; P0179-03; P0292/06; L0015-07).

2.2. PCR assays

DNA was extracted from 10 mg of each rodent spleen tissue using the DNeasy® Blood & Tissue Kit (Qiagen®, Valencia, California, USA), according to manufacturer's instructions. DNA concentration and absorbance ratio (260/280) nm were measured using a spectrophotometer (Nanodrop, Thermo Scientific, USA). Microtubes containing ultra-pure sterile water were intercalated between each series of twenty rodent spleen samples and submitted to DNA extraction.

In order to discard the presence of PCR inhibitors, each sample of spleen extracted DNA was used as a template in an internal control PCR targeting the mammal IRBP gene ("Interphotoreceptor Retinoid Binding Protein") as previously described [26]. Negative samples to IRBP gene-PCR were subsequently submitted to another internal control PCR targeting the GAPDH gene as previously described [27]. Positive samples to above described internal

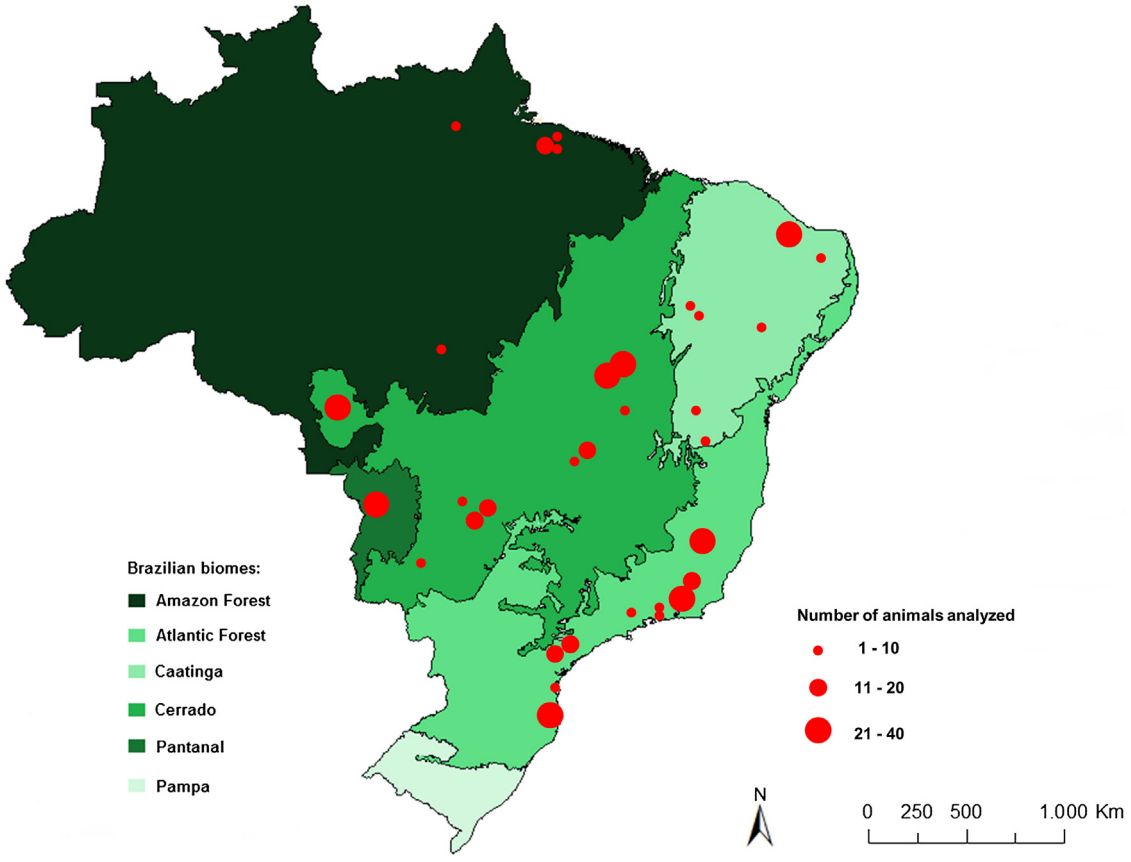

Fig. 1. Distribution of rodents sampled in different Brazilian biomes.