RESUMO SIMPLES - PROTOZOÁRIOS E AGENTES TRANSMITIDOS POR VETORES DE IMPORTÂNCIA EM SAÚDE

OCCURRENCE AND MOLECULAR IDENTIFICATION OF ANAPLASMATACEAE AND MYCOPLASMATACEAE BACTERIA IN CAPYBARAS (HYDROCHOERUS HYDROCHAERIS) FROM BRAZILIAN SPOTTED FEVER ENDEMIC AND NON-ENDEMIC AREAS

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Capybaras (Hydrochoerus hydrochaeris), important tick hosts, can serve as reservoirs for pathogens, including members of the families Anaplasmataceae and Mycoplasmataceae. In São Paulo state, they have been linked to the transmission of Brazilian Spotted Fever (BSF), caused by Rickettsia rickettsii and transmitted by Amblyomma sculptum. This study, which aims to detect and

molecularly identify bacteria from the families Anaplasmataceae and Mycoplasmataceae in capybaras, was submitted to and approved by the Ethics Committee on the Use of Animals of the University of São Paulo (CEUA/USP), São Paulo, SP, Brazil, under protocol no. 5948070314/2014. The sampling areas were categorized as endemic (with BSF cases reported in the last 10 years), non-endemic with low diversity, and non-endemic with high diversity. The endemic areas included Sobrado Velho (Americana/SP), USP Campus (ESALQ) in Piracicaba/SP, and Araras/SP. The non-endemic areas with low diversity were USP Campus in Pirassununga/SP, USP Campus in Ribeirão Preto/SP, and Tatuí/SP. The selected non-endemic areas with high diversity were the Pantanal of Poconé (Poconé/MT) and the Pantanal of Nhecolândia (Corumbá/MS). In each location, one sampling event was conducted per year for four consecutive years, totaling four captures per area and 155 samples collected by the end of the expeditions. Between 5 and 10 mL of venous blood were obtained from the capybaras, placed in EDTA tubes, and stored at −20 °C. DNA was automatically extracted using the commercial Extracta Kit (Loccus). For the detection of Anaplasma/Ehrlichia, all 155 samples were subjected to PCR using the primers EHR16SD and EHR16SR (300 bp), which target conserved regions of the 16S rDNA gene. For the detection of Mycoplasma spp., a preliminary PCR was performed on 20 samples using the primers Myco 322s and Myco 938as (600 bp), targeting the 16S rRNA gene. Negative controls (ultrapure water) were included in all reactions, and positive controls (Ehrlichiacanis and Mycoplasma spp.) were included in their respective assays. PCR products were resolved on agarose gels, stained with ethidium bromide, and visualized using a photodocumentation system. PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA). Of the 155 capybara blood samples analyzed, 84 tested positive for bacteria from the family Anaplasmataceae. In the state of São Paulo, 67 samples were positive, 39 of which originated from areas endemic for BSF. The proportion of positive samples by municipality was as follows: Americana (60%), Araras (71.4%), Avaré (64.3%), Piracicaba (73.3%), Pirassununga (80%), Ribeirão Preto (86.7%), and Tatuí (20%). In the Pantanal, a region considered non-endemic for BSF, 17 samples (20.2%) were positive, including 14 from Poconé and 3 from Corumbá. From the positive samples, six were selected for sequencing, each corresponding to one of the following municipalities: Americana, Araras, Piracicaba, Ribeirão Preto, Corumbá, and Poconé. Of these, five (Americana, Araras, Piracicaba, Ribeirão Preto, and Poconé) were identified as Anaplasma sp., showing 100% identity with

sequence MW785881.1, previously reported in a capybara from Curitiba, Brazil. The sample from Corumbá was identified as Ehrlichia sp., exhibiting 99.4% identity with OR854331.1, described in a capybara from French Guiana. Regarding the detection of Mycoplasma spp., 19 of the 20 samples analyzed tested positive (95%), a notably high prevalence; however, further analyses and confirmatory detection assays are needed to validate this finding. The detection of Anaplasmataceae in 54.2% of capybara samples, in both BSF-endemic and non-endemic areas, indicates a widespread circulation of these zoonotic agents. The high positivity in municipalities of São Paulo and in the Pantanal suggests that these bacteria are not restricted to regions with Rickettsia rickettsii transmission, reinforcing the importance of capybaras as hosts—and potential reservoirs—of pathogens relevant to public health.

Palavras-chave: hemoparasites; ticks; zoonoses; rodents.