Primary Isolation of Spotted Fever Group Rickettsiae from *Amblyomma cooperi* Collected from *Hydrochaeris hydrochaeris* in Brazil

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This paper reports the first isolation of a spotted fever group rickettsia from an Amblyomma cooperi *ixodid collected from a capybara (*Hydrochaeris hydrochaeris*) in an endemic area of spotted fever in the County of Pedreira, State of São Paulo, Brazil. Isolation was performed in Vero cell culture and submitted to immunofluorescence, using antibody from* Rickettsia rickettsii*-positive human serum.*

Key words: Rickettsia - *Amblyomma cooperi* - isolation - capybara (*Hydrochaeris hydrochaeris*) - Brazil

Since humans are accidental victims of spotted fever, a tick-borne rickettsiosis, the epidemiology of this exanthematic febrile disease depends primarily on the species and ecology of the tick vector involved (Brezina et al. 1973, Burgdorfer 1975). In Brazil, most human cases of this rickettsiosis described have been limited to the southeastern region states where dozens of confirmed cases have occurred in the last two decades (Gonçalves et al. 1981, Lemos 1991, Souza et al. 1991, Dietz et al. 1992, Melles et al. 1992). In Brazil, *Amblyomma cajennense* is considered the most important ixodid vector and although the studies performed by the 1950's identified other arthropod vectors as potential vector, naturally infected ticks were rarely reported (Dias 1938, Dias & Martins 1939, Travasssos & Vallejo-Freire 1944-1945, Magalhães 1952, Aragão & Martins 1961, McDade & Newhouse 1986).

Considering the limited available information on this rickettsiosis in Brazil, principally in relation to the vectors involved in perpetuating it in foci, we designed a project in an endemic area in order to expand knowledge on this zoonosis. This paper provides partial results of attempts at isolating rickettsiae from ticks collected in the County of Pedreira, State of São Paulo.

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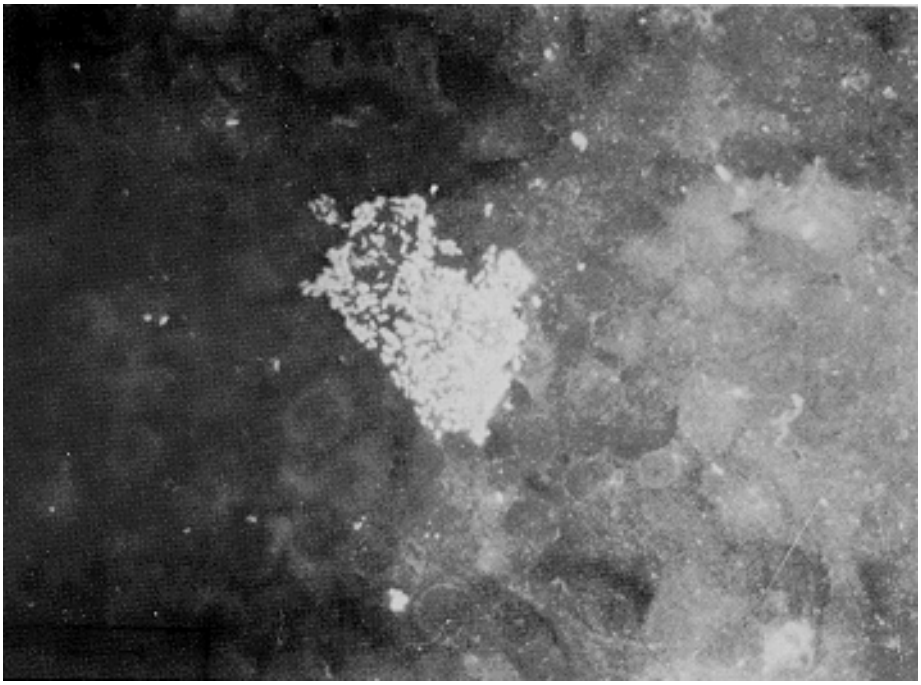
MATERIALS AND METHODS

Three female *A. cooperi* tick specimens were collected from a capybara (*Hydrochaeris hydrochaeris*) captured at the workers' settlement of the Nadir Figueiredo factory located in the County of Pedreira (22°44'21" S, 46°54'27" W) in October 1994. After cleaning with 3% hydrogen peroxide, 10% formaldehyde solution, 70% alcohol, and sterile distilled water, the ixodidae were stored in test tubes containing Snyder solution at -70°C until the moment of inoculation in cell culture.

The initial inoculum was prepared with the triturated ticks in 1.0 ml of BHI (brain-heart infusion). The suspension was centrifuged at 700 X g for 10 min at O°C and 0.2 ml of the supernatant was inoculated in a confluent monolayer of Vero cells on circular slides adapted to the flat-bottomed tubes. Prior to inoculation, the cell cultures were maintained with Eagle growth medium without antimicrobials for 24-48 hr. The tubes containing the inocula were centrifuged at 1500-1800 X g at 25°-30°C for 1 hr. After decantation of the inoculum, 1.0 ml of modified Eagle minimum medium was added containing 5% bovine fetal serum, 10 µg/ml of gentamicin, 100 µg/ml of vancomycin, and 2.5 µg/ml of amphotericin B. Following incubation for five days at 37°C, the second passage was performed with one of the three tubes into new cell culture tubes, using growth medium with one third the dosage of antimicrobials used in the previous stage. Assessment of the inoculated cell cultures for presence of infection was performed on



Evidence of rickettsial multiplication in Vero cell culture after inoculation of triturated ticks - three female *Amblyomma cooperi* (indirect immunofluorescent staining, magnification, X 400).

the slides from the other two tubes, using Giménez stain (Elisberg & Bozeman 1979) and the immunofluorescence reaction prepared with standard *Rickettsia rickettsii*-positive human serum.

RESULTS AND DISCUSSION

A rickettsia strain from the spotted fever group was isolated, and despite the absence of a cytopathic effect on the culture cells, the immunofluorescence reaction and Giménez stain were considered positive. In the immunofluorescence reaction, we were able to observe the presence of microorganisms in the form of intracellular bacteria, including intranuclear ones, and a fluorencent intensity of 4 + (Fig.).

The presence of a rickettsia strain from the spotted fever group isolated from *A. cooperi* suggests that this rickettsia, as yet unidentified, is prevalent at the endemic region in the County of Pedreira. The fact that this tick species almost exclusively infests the capybara, a very abundant animal in the region, leads us to believe that this animal is important in perpetuating rickettsiosis there.

Further information will be obtained subsequently when we evaluate the isolate genetically, correlating it with other strains isolated in Brazil.

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