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Experimental Leptospirosis in Capybaras (Hydrochaeris hydrochaeris) Infected with Leptospira interrogans Serovar Pomona

Author(s): Maria Fernanda Vianna Marvulo, D.V.M. M.S., Jean Carlos Ramos Silva, D.V.M. Ph.D., Patrícia Marques Ferreira, D.V.M. PhD., Zenaide Maria de Morais, Biol., Andrea Micke Moreno, D.V.M. Ph.D., Daniela Sabatini Doto, D.V.M. M.S., Renata Paixão, D.V.M. M.S., Maria Regina Baccaro, D.V.M. Ph.D., Silvio Arruda Vasconcellos, D.V.M. Ph.D., and José Soares Ferreira Neto, D.V.M. Ph.D.


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EXPERIMENTAL LEPTOSPIROSIS IN CAPYBARAS (HYDROCHAERIS HYDROCHAERIS) INFECTED WITH LEPTOSPIRA INTERROGANS SEROVAR POMONA


Abstract: Capybara (Hydrochaeris hydrochaeris), the largest rodent in the world, is widely distributed in South America. These animals live in areas with abundant water, which makes them a potential reservoir for Leptospira. The objective of this study was to investigate seroconversion, leptospiremia, and leptospiruria in capybaras experimentally infected with a virulent strain of Leptospira interrogans serovar Pomona. Seven capybaras were used: one control and six infected. Agglutinins against serovar Pomona were initially detected in serum 6 or 7 day after inoculation with Leptospira (10^9–10^11 organisms, given i.v.), peaked (titer, ~3,200) between 9 and 27 day, and were still present at 83 day (end of study). The earliest and latest isolation of leptospires from the blood was from 2–12 day and from urine, 9–19 day after exposure. However, polymerase chain reaction and isolation results from kidney and liver samples were negative for leptospires. The control animal tested negative on all diagnostic tests. Hence, the capybara can serve as a host for Leptospira.

Key words: capybara, experimental infection, Hydrochaeris, Leptospira, leptospirosis.

INTRODUCTION

Leptospirosis, a disease caused by bacteria of the genus Leptospira, is a widespread zoonosis with a large range of wildlife and domestic animal hosts. Affected animals have various clinical signs; infection is detected by serologic tests and isolation or detection of Leptospira in biologic samples. The epidemiology of Leptospira infection is very complex, because it involves more than 200 pathogenic Serovars and numerous maintenance hosts.

Maintenance hosts, or reservoirs, act as a source of infection and can excrete leptospires in the urine for prolonged intervals. They are difficult to identify, because they can be persistently infected without clinical signs and have a low serologic response. Several synanthropic and sylvatic rodents can act as efficient Leptospira reservoirs and are sources of infection for other wildlife, domestic animals, and humans.

Capybara (Hydrochaeris hydrochaeris) is the largest rodent in the world and a widespread species in South America. However, reports regarding the importance of this animal in the epidemiology of leptospirosis are rare. Capybaras could be efficient reservoirs for Leptospira, because they live in areas with abundant water, an important environmental factor for disease transmission. Antibodies against Leptospira, as well as the isolation of the agent from kidneys of this species, were reported. Although serologic and bacteriologic results suggest that capybara is susceptible to Leptospira infection, there is no information regarding Leptospira shedding in urine from these animals. Therefore, the objective of the current study was to determine the antibody response, the duration of leptospiremia, and, most important, the duration of leptospiruria in capybaras experimentally infected with a virulent strain of Leptospira interrogans serovar Pomona.

MATERIALS AND METHODS

Animals

Seven capybara (H. hydrochaeris) cubs, 2–4 mo old and 10–15 kg in body weight, were used. Capybaras were kept in individual pens, with solid floors and an effluent drain in the back of
the pen. They were fed fruit, tubers, and grass, with ad libitum access to water. This study was approved by the Ethics Committee of the Faculdade de Medicina Veterinária e Zootecnia of the University of São Paulo (São Paulo, Brazil).

**Restraint**

Capybaras were physically and chemically restrained, which involved, respectively, the use of a squeeze chute and intramuscular injection of a combination of 1.5 mg/kg ketamine (Vetaset, Fort Dodge, Animal Health, Campinas, SP, Brazil) and 0.5 mg/kg xylazine (Rompun, Bayer, HealthCare, São Paulo, SP, Brazil).

**Bacteria/inoculum**

The inoculum used was *L. interrogans* serovar Pomona (strain Fromm, Laboratórios Salsbury, Ltda). Live organisms were obtained through maceration of liver from hamsters (*Mesoeci cetus auratus*) previously inoculated with this strain. The concentration of organisms in the inoculum was determined with a Petroff-Hauser chamber, in accordance with the manufacturer’s instructions. The organisms were suspended in Ellinghausen-McCullough-Johnson-Harris medium (DIFCO, Detroit, Michigan 48201, USA) before inoculation.

**Experimental design**

To ensure that none of the animals had been previously exposed to the agent, all animals underwent three consecutive tests with 21-day intervals. These tests consisted of an anti-leptospiral antibody assay using a microagglutination test, leptospiral culture, and polymerase chain reaction (PCR) of blood and urine. The study was composed of a preliminary study and an experiment. For the preliminary study, the objective was to find the dose that causes infection without killing the animal. Three animals, female 1, female 2, and male 3, were given i.v. injections of $2.3 \times 10^9$, $2.3 \times 10^{10}$, and $2.3 \times 10^{11}$ *Leptospira*, respectively. In the absence of a published reference on leptospirosis in capybaras, the i.v. route was used to enhance the efficiency of the experimental inoculation. Throughout this study, the day of inoculation was designated day 0. Blood and urine samples were collected on day 2, 7, 10, 12, 14, 19, 22, 26, 33, 37, 43, 51, and 65. A necropsy was performed on all animals that died or were euthanatized at the conclusion of the experiment (day 65). For experiment 1, because all doses tested in the preliminary study resulted in infection, the lowest dose was used. Three animals, male 4, male 5, and male 6, were inoculated by i.v. injection of $10^9$ *Leptospira*. Capybara male 7 served as the control and was inoculated with the inoculum containing no *Leptospira*. Blood and urine samples were collected on day 2, 6, 9, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, and 83. A necropsy was performed on all animals that died during the experiment or that were euthanatized at the conclusion of the experiment (day 83).

**Clinical evaluation and sampling**

Animals were evaluated on a daily basis for clinical signs indicative of leptospirosis, including fever, prostration, apathy, jaundice, and hemorrhage. Blood was collected by jugular venipuncture. Placement of a urethral catheter was required for urine collection. At necropsy, kidney and liver samples were aseptically collected on sterile petri dishes. Necropsies were performed on all animals that died or were euthanatized to determine macroscopic and microscopic alterations and to collect samples of kidney and liver.

**Laboratory procedures**

The presence of antibodies against *Leptospira* was investigated using the microagglutination test against a collection of 25 live antigens (Andaman, Australis, Autumnalis, Bataviae, Bratislensis, Bratislava, Butembo, Canicola, Castellosis, Copenhageni, Cynopteri, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Panama, Patoc, Pomona, Pyrogenes, Sentot, Shermanni, Tarassovi, Whitcombii, and Wolfii). The presence of *Leptospira* was investigated through isolation and PCR. Isolation was performed on semisolid, nonselective Fletcher medium (DIFCO), which was incubated at 28°C in room air for 6 wk, with weekly macroscopic and microscopic evaluations. Bacterial DNA was extracted from urine and tissue (liver and kidney) samples as described by Boom et al. and from blood samples as described by Sambrook et al. For PCR examinations of blood, urine, and tissue samples, *Leptospira* primers previously published by Merien et al. were used. Electrophoresis on agarose gel 1.5% was used to separate PCR products (10 μl), which were stained with ethidium bromide (10 μg/ml) and photographed under ultraviolet light. A 100–base pair molecular weight marker was used. Whitin-Starry silver staining was used to identify *Leptospira* in tissue samples.
RESULTS

In the preliminary study, animals 1 and 3 died on day 22 and 26, respectively, whereas animal 2 was euthanatized on day 65. In experiment 1, animals 4 and 5 died on day 48 and 55, and the remaining two animals were euthanatized on day 83. None of the animals had clinical signs of leptospirosis.

Agglutinins against *Leptospira* were initially detected between day 2 and 7, peaked between day 9 and 27, and persisted until day 83 (Tables 1, 2). Leptospiremia was confirmed (on one to three occasions) in five animals (Tables 1, 2). Leptospiruria was confirmed in four animals, between day 9 and 43 (Tables 1, 2). There were no gross or histologic lesions suggestive of leptospirosis, and Whartin-Starry silver staining was negative in all cases. *Leptospira* were not isolated or detected in liver or kidney samples.

DISCUSSION

To the authors’ knowledge, this is the first report to document experimental infection of capybaras with *Leptospira*. This study demonstrated that the capybara is susceptible to *L. interrogans* serovar Pomona and that this species experiences the classical leptospiremic and leptospiruric phases, similar to those previously described in cattle, pigs, dogs, and other animals.\(^1,8,29\) It was noteworthy that *Leptospira* was not detected in the blood after day 12. Similar results were described in other species experimentally infected with various serovars; 4–5 day in cattle infected with serovar Hardjo\(^5\); 5 day for striped skunk (*Mephitis mephitis*) infected with serovar Pomona\(^17\); 10 day for common opossum (*Didelphis marsupialis*) infected with serovar Balcanica\(^7,11\); 10–12 day in sheep infected with serovar Hardjo\(^2\); 14 day in dogs infected with serovars Canicola and Icterohaemorrhagiae\(^13\); 14 day for coyotes (*Canis latrans*) infected with serovars Pomona, Canicola, and Copenhageni\(^16\); and 15 day for common brush-tail opossum (*Trichosurus vulpecula*) infected with serovar Balcanica.\(^11\)

In capybara, leptospiral shedding in urine was detected as early as day 9 and as late as day 43. Because leptospiruria was intermittent, it may continue for longer than 43 day after inoculation, with alternating phases of excretion and latency. Intermittent leptospiral shedding in urine has also been reported in cattle for serovar Hardjo\(^5,27\) and in the common brush-tail opossum for serovar Balcanica.\(^7,11\) Leptospiral shed-
Ding in urine has also been reported in coyotes for serovars Pomona, Canicola, and Copenhageni; in striped skunk, common raccoon (Procyon lotor), and woodchuck (Marmota monax) for serovar Pomona; and in the Norway rat (Rattus norvegicus) for serovar Icterohaemorrhagiae.

Agglutinins against serovar Pomona were initially detected in blood serum 6 or 7 day after inoculation, peaked (titer, 3,200) between 9 and 27 day, and were still present at 83 day (end of study) in animals that survived. The dynamics of agglutinin response observed in this experiment were similar to those reported for cattle, sheep, common brushtail opossum, Norway rat, and Cuban hutia (Capromys pilorides).

In conclusion, it has been demonstrated that the capybara is susceptible to Leptospira. Antibody responses and leptospiremic and leptosporuric phases in this species were very similar to those reported for other domestic and wild species. Capybara can shed Leptospira in urine and may serve as a source of infection for other animals.

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LITERATURE CITED


Table 2. Serum antibody titers and detection of Leptospira in blood and urine samples (experiment 1) in three capybara (animals 4–6) experimentally infected with 10⁹ Leptospira interrogans serovar Pomona and one control (animal 7).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Interval after inoculation (day)</th>
<th>Titer</th>
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<th>Urine</th>
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<tr>
<td>4</td>
<td>0 2 6 9 13 20 27 34 41 48 55 58 62 69 76 83</td>
<td>0 0 800 3,200 1,600 1,600 800 1,600 1,600 1,600 ND ND ND ND</td>
<td></td>
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<tr>
<td>5</td>
<td>0 2 6 9 13 20 27 34 41 48 55 58 62 69 76 83</td>
<td>0 0 800 3,200 1,600 1,600 800 1,600 1,600 1,600 ND ND ND ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0 2 6 9 13 20 27 34 41 48 55 58 62 69 76 83</td>
<td>0 0 1,600 800 1,600 1,600 3,200 1,600 1,600 1,600 ND ND ND ND</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>0 2 6 9 13 20 27 34 41 48 55 58 62 69 76 83</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Animals 4 and 5 died on day 48 and 55, respectively, whereas animals 6 and 7 were euthanatized on day 83.

ND, not determined; I, positive isolation and polymerase chain reaction.


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