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Review

FIRST ISOLATION OF *LEPTOSPIRA INTERROGANS* FROM *CONEPATUS CHINGA* IN ARGENTINA.

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Abstract: To identify carriers of *Leptospira* spp. in Buenos Aires province, Argentina, we trapped wild animals: 10 *Didelphis albiventris,* five *Chaetophractus villosus,* two *Lycalopex griseus* and three *Conepatus chinga*. Blood samples from these animals were tested by microscopic agglutination test (MAT), and one (5%: one *Conepatus chinga*) was positive for *Leptospira* Castellonis, Canicola, Grippotyphosa, Hardjo and Icterohaemorrhagiae serovars, at titers of 1:50 and 1:200. Kidneys of all animal were cultured, and one isolate of *L. interrogans* from a *Conepatus chinga* was obtained. Hamsters inoculated with this isolate strain died six days later, with several pulmonary macroscopic lesions at autopsy; the histology study revealed congestion in liver, and congestion and hemorrhage in kidney and lungs. Isolated strain was identified by serologic and molecular methods as *Leptospira interrogans* serogroup Canicola, with a genetic profile similar to that of Hound Utrecht IV strain.

Keywords: *Conepatus chinga, Leptospira* Canicola, molecular genotipification

INTRODUCTION

Wildlife species, commonly rodents, are the most important reservoirs of leptospires in nature (Scialfa *et al*., 2010; Reilly *et al*., 1970; Vanazco *et al*., 2003). In this country, *Leptospira interrogans* serogroup Canicola.was first isolated from humans and dogs in Buenos Aires province in 1943 (AAVLD, 2006); and subsequently was also isolated from cattle and *Chaetophractus villosus* of Buenos Aires province (AAVLD, 2006). More recent studies have allowed the isolation of *Leptospira interrogans* serogroup Canicola from *Didelphis albiventris* (Brihuega *et al*., 2007).

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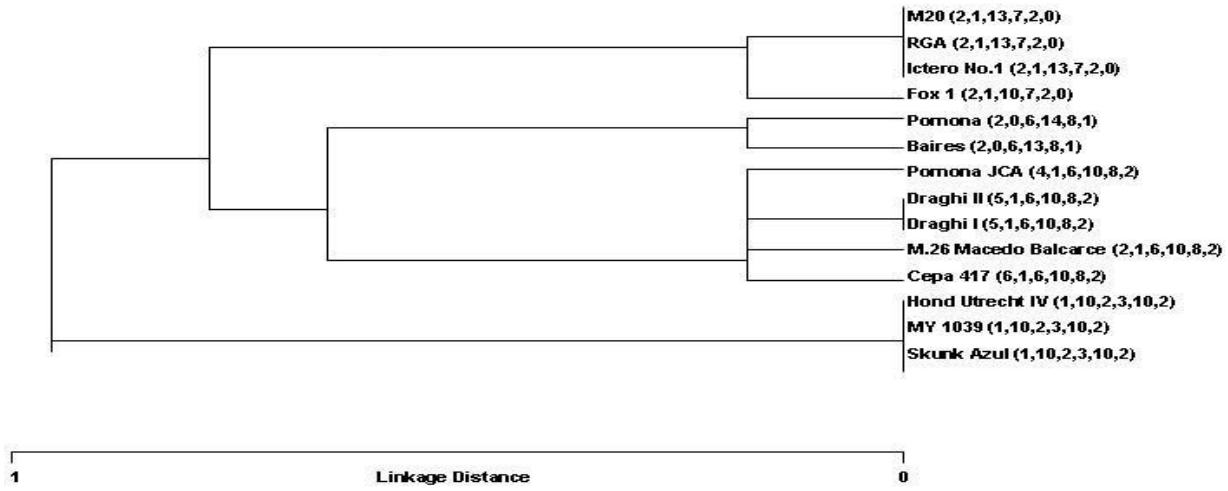
Figure 1: Macroscopic evidence of hemorrhagic scattered ecchymoses and petechiae on the pulmonary wall of hamsters inoculated with *Leptospira interrogans* Canicola isolated from *C. chinga.*

Figure 2: Genetic relationships among Argentine reference strains of *L. interrogans* and the new genotype isolated from *C. chinga* (Skunk Azul) using UPGMA clustering analysis. This analysis was conducted using MEGA version 5.



This report describes the first isolation and identification of a *Leptospira* strains recovered from *C. chinga* trapped in Buenos Aires province, Argentina. This strain, the first to be reported from the skunk, was serologically analyzed and its pathogenicity was established. Wild mammals were caught in November 2010, using 29 Tomahawk live-capture traps in the forest area of Azul, Buenos Aires province, Argentina. This region is characterized by extensive cattle ranching and croplands, which restrict native vegetation to the edge of the road. Traps were baited with animal fat and checked every morning for three consecutive days. Captured animals were euthanized according to Animal Welfare Committee of the Veterinary Sciences Faculty, National University of the Center of Buenos Aires province, Argentina (Number interne dispatch: 13). None of the animals trapped were endangered. Animals were classified as adults or juveniles based on genital development and body size. Necropsies were performed using appropriate biosafety measures. Blood samples were collected for serological analysis by cardiac puncture. Kidney tissue samples were obtained aseptically for culture isolation of leptospires. The microscopic agglutination test (MAT) was carried out with a battery of 10 leptospires (serovars Canicola, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes and Wolffi of *L. interrogans*; Castellonis and Tarassovi of *L. borgpetersenii*; and Grippotyphosa of *L. kirschneri*) maintained in Ellinghausen-McCulough-Johnson-Harris (EMJH medium: Difco Laboratories, Detroit Michigan USA). The serum titer which was the highest dilution agglutinated 50% of the antigen and titers above 1:50 were considered positive. Kidney tissue from each animal was crushed aseptically into transport medium (buffered solution pH 7.2, containing 200 µg/ml of 5-fluorouracil as selective agent) for 2 hrs. This suspension was diluted (1:10 and 1:100) with sterile phosphate-buffered saline, and 0.5 ml of each dilution was inoculated in EMJH medium. Cultures of this kidney were incubated at 28° C

for 90 days and leptospiral growth was monitored weekly using dark field microscopy. Kidney tissue removed was placed in buffered formalin for 48 hrs. After gradually dehydration with alcohol, paraffin was used over the samples and dried in an oven at 56º-58ºC. The blocks thus obtained were cuts by sliding inclined plane microtome and the sections obtained were drying for two hours, withdrew and proceeded to dew axing with xylene. Finally, they were stained with hematoxylin and immersed in eosin contrast. Pathogenicity testing was carried out by intraperitoneal inoculation of two young hamsters (each weighing about 50 g), using 0.5 ml of the fluid culture isolation. Two control hamsters were injected with 0.5 ml of EMJH medium whereas two other animals were injected with 0.5 ml of EMJH medium containing 10^8 leptospires/ml. Aseptic autopsies of dead hamsters were carried out and kidney and hepatic tissue suspensions were collected for culture isolation. Kidneys, lungs, and livers from all animals were removed and fixed in 10% buffered formalin. Tissue sections were processed by routine histologic methods and stained with hematoxylin-eosin. The strains isolated were identified by serologic and molecular methods. MATs were performed with rabbit hyperimmune sera prepared in the reference centers Institute Superiore Di Sanitá (Rome, Italy) and the Royal Tropical Institute (KIT: Amsterdam, the Netherlands). Serovars representative of nine serogroups of *Leptospira* were used: Canicola, Icterohaemorrhagiae, Pomona, Pyrogenes, Hardjo, Hebdomadis, Wolffi Tarassovi, Castellonis and Grippotyphosa. were found (Figure 1). Structural alterations were observed in the histologic exams of kidney, lung and liver tissues. The renal histopathology showed marked congestion and hemorrhage, with infiltration of mononuclear cells, and presence of red blood cells in tubules spaces. Microscopic examination of the lung sections revealed congestion and severe hemorrhage,and emphysema. The alveolar and interalveolar capillaries were distended and engorged with red blood cells and protein material deposition. The liver was markedly congested. The histopathology study of renal Argentina. The role in the epizootiology of leptospirosis was demonstrated whit the tissue from skunk with isolation of a strain of *Leptospira* showed Genotyping of the strain isolated from *L. griseus* was performed by multiple-locus variable-number tandem repeat analysis (MLVA) using the primers flanking the VNTR4, VNTR7, VNTR9, VNTR10, VNTR19, VNTR23 and VNTR31 loci (Majed *et al*., 2005). Tandem Repeats Finder programmer was used to define exactly the copy number of each VNTR locus. UPGMA (unweighted pair group method with arithmetic mean) clustering analysis was performed using the Sequence Type and Recombinational Test software on genotype scores from a combined data set of the Argentine genotypes and the genotypes of the reference strains listed (Majed *et al*., 2005). During the study period 20 asymptomatic animals were captured: 10 *Didelphis albiventris,* five *Chaetophractus villosus,* two *Lycalopex griseus*, and three *Conepatus chinga*. None of the examined mammals displayed physical evidence of illness or gross pathological lesions. The capture rate achieved was 22% (total animals trapped/total trap night x 100). All animals were tested by MAT, and one of them (5%: one *C. chinga*) was positive with titers of 1:50 for *L.* Icterohaemorrhagiae-Hardjo, 1:100 for *L.* Ballum-Grippotyphosa and 1:200 for *L.* Canicola. No antibodies against the Hebdomadis, Pomona, Pyrogenes, Wolffi and Tarassovi serovars were detected. Kidneys from 20 animals were cultured, and leptospires was recovered from an adult male skunk located 36º51.305'S, 59º41.961'W at approximately 486 m elevation. Growth was observed after 29 days of culture medium (EMJH: dilution 1:100). The hamsters inoculated with the isolated strain died six days post inoculation, and macroscopic lesions in the lungs evidence of exposure to serovars Pomona, Grippotyphosa and Ballum (Richardson *et al*., 2003; McKeever *et al*., 1958) and the isolation of *Leptospira interrogans* serogroup Canicola from skunks (Roth *et al*., 1961). Possible sources of interstitial nephritis. Isolated strain was identified by serologic and molecular methods as *L. interrogans* serogroup Canicola, with a genetic profile similar to that of Hound Utrecht IV strain (Figure 2). This is the first report of isolation of a strain of *L. interrogans* from *C. chinga* in

infection for skunks in the region are interactions with other wild animals,
or water contaminated by wild or domestic mammals, however, the possibility of oral infection of carnivores in nature was demonstrated (Reilly *et al*., 1970). During a serological survey of *Leptospira* Pomona suggest the possible transmission between cattle and skunks (Schowalter *et al*., 1981).The public health significance of human's infection with *Leptospira interrogans* serogroup Canic**ola** has been established, and in this region is the serovar most frequently detected in serologic surveys of humans by our laboratory. We consider that skunks present a little direct risk to humans, however can cause human illness indirectly by transmission of leptospires through dogs, who are frequently infected whit *Leptospira interrogans* serogroup Canicol**a** in this country.

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