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Leptospira interrogans serogroup Icterohaemorrhagiae isolated from a white-headed marmoset (*Callithrix geoffroyi*) in the U.S.

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Introduction: Leptospirosis is a global zoonotic disease. Rodents and other animals are chronic carriers that excrete pathogenic *Leptospira* in their urine and contaminate the environment. Infection is acquired via direct or indirect contact with the urine of carrier animals. Rodents are an important reservoir of serogroup Icterohaemorrhagiae (1) which can cause acute infection in other animals and humans. Humans may develop severe forms of the disease such as Weil's disease and pulmonary hemorrhage syndrome (2). Monkeys are susceptible to experimental leptospirosis. Naturally acquired, acute leptospirosis in non-human primates is uncommon, however, clinical signs of infection are similar to symptoms that are observed in human cases (3). There is limited published data available on *Leptospira* infection in non-human primates (4). Here, we describe the isolation of *L. interrogans* serogroup Icterohaemorrhagiae from a white-headed marmoset within a U.S. zoo.

Methods: A veterinarian from a U.S. zoo submitted a urine sample from a white-headed marmoset (*Callithrix geoffroyi*) male, aged 11 years, to the National Veterinary Services Laboratories (NVSL), USDA, Ames, Iowa, for diagnostic testing. The submission form did not include information indicating clinical symptoms the animal may have been experiencing; however, it did state that there had been a history of leptospirosis previously in the primate population. Urine was collected for culture purposes; 500 µl was immediately inoculated into 5 mL of Hornsby-Alt-Nally (HAN) transport media and transported via overnight delivery services at ambient temperature to the NVSL. Upon arrival, a 200 µL aliquot from HAN transport media was used to inoculate two tubes of 5 mL HAN liquid media and incubated at 29°C and 37°C in 5% CO₂ (5). A *Leptospira* isolate was recovered from the urine specimen, serogrouped by the microscopic agglutination test (MAT) using a panel of polyclonal rabbit reference antisera, and the species identified by whole genome sequencing. Using kSNP 3.0, a reference-free phylogenetic analysis tool, assembled genomes were compared using the maximum likelihood output.

Results: *Leptospira* was only recovered from HAN media incubated at 37°C in 5% CO₂. This isolate was designated strain NVSL-7814. Serogrouping of strain NVSL-7814 by MAT with 18 reference antisera indicated that the isolate belonged to serogroup Icterohaemorrhagiae. Molecular typing indicated *L. interrogans*. Phylogenetic analysis based on complete whole genome sequence demonstrated clustering with reference strains of *L. interrogans*, including strains of serovar Copenhageni and Icterohaemorrhagiae, and *L. interrogans* serogroup Icterohaemorrhagiae isolated from a rodent in the U.S.

Conclusion: This is the first report of *Leptospira* isolated from a naturally infected non-human primate in the U.S. and highlights the importance of culture and concomitant genotyping and serotyping to accurately classify leptospires. Rodents are a principal reservoir host of the Icterohaemorrhagiae serogroup. The monkey may have been infected with *Leptospira* by direct or indirect contact with rodent urine. This information may also be used to design efficacious vaccine and diagnostic strategies in order to limit animal disease and reduce zoonotic risk.