

TITLE: DETECTION OF *Leptospira interrogans* SEROVAR Canicola IN SEMEN AND URINE OF DOGS EXPERIMENTALLY INFECTED WITH LO4 STRAIN USING THE POLYMERASE CHAIN REACTION TECHNIQUE.

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ABSTRACT:

Detection of *Leptospira interrogans* serovar Canicola was investigated in 40 male Mongrel dogs, 10-15 kg body weight. All animals were obtained from the Zoonosis Control Center, São José do Rio Preto city, São Paulo state. No antibody titers were detected against 24 pathogenic serovars using a microscopic agglutination test (MAT) performed twice at a 7 day interval, and negative PCR results were found in urine and semen samples tested at the same time as well. Each animal was infected with a 4mL inoculum identified as L04 which consisted of 10⁹ leptospires/mL, administered subcutaneously at the cervical dorsal region. Semen, urine and blood samples were collected on days 0, 3, 5, 7 and 10 and samples collection was repeated every 5 days up to 45 days. Semen and urine samples were evaluated by PCR technique, and DNA extraction was performed using the guanidine isothiocyanate method. A MAT was performed to detect anti-*Leptospira* antibodies in dogs serum samples. Higher temperature (40°C) was the only clinical sign observed in infected dogs at 3 and 7 days after inoculation (DAI). Positive serum reactions were observed from 5 DAI and the highest antibody titer was 2,560. Leptospires DNA was detected in semen samples from 3 DAI, but serological titers were still low, between 10 and 20 or negative. Leptospires DNA was detected in urine samples form 15 DAI, when serological titres were over 100, revealing that leptospires release from dogs semen may precede leptospiruria. Sperm quality tests of infected dogs revealed motility decrease and a raise in percentage of abnormal spermatozoa. Results obtained in this study showed that dogs experimentally infected with LO-4 serovar Canicola eliminated leptospires early in semen, previously to leptospiruria and sera positive reactions detected by serological assays.

Keywords: *Leptospira*, dogs, semen, urine, PCR

DEVELOPMENT AGENCY: Fapesp

Research Project carried out according to the Ethical Principles on Animal Experimentation, adopted by the Brazilian College of Experimentation (COBEA) and approved by the Ethics and Animal Welfare Commission (CEBEA), Faculty of Agrarian and Veterinary Sciences, Unesp, Campus de Jaboticabal, SP.