

TITLE: USE OF GENITAL SAMPLES (CERVICOVAGINAL MUCUS AND UTERINE FRAGMENT) TO DIAGNOSE LEPTOSPIRAL CARRIERS IN LIVE CATTLE

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ABSTRACT:

Bovine genital leptospirosis (BGL) is a chronic disease caused by several reproductive disorders in ruminants. For many years, genital tract infection has been considered a secondary effect of renal infection. Due to that, urine is worldwide used as a conventional sample to molecular diagnosis of bovine leptospirosis. However, it is known that leptospire belonging to serogroup Sejroe (adapted to cattle) have preference for colonizing reproductive organs. Therefore, this study aimed to analyze genital samples such as cervical-vaginal mucus (CVM) and uterine fragment (UF) from live subfertile cows through molecular diagnosis. Procedures were approved by Ethical Committee for Animal Use of Federal Fluminense University (protocol 1025/2017). Urine, UF and CVM samples were collected from ten cows with history of reproductive failures. Urine was obtained through urethral sounding, while CVM was collected with cytology brush in vaginal fornix. Uterine biopsy was proceeded through transcervical collection, using a Yeoman forceps and the obtained UF was sectioned. All samples were cultured in T80/40LH media at field and properly transported to molecular analysis. PCR was performed targeting *lipL32* gene, exclusive of pathogenic leptospire. Samples positive in *lipL32*-PCR were submitted to a nested PCR targeting *secY*, nucleotide sequencing was proceeded with its amplicon. Due to low sensitivity, cultures were all negative. However, in molecular analysis, six out of ten (60%) cows had positive samples in *lipL32*-PCR: in four cows, *Leptospira* spp. DNA was detected in uterine fragment, while the other two cows had CVM as a positive sample. Surprisingly, all urine samples tested were *lipL32*-PCR negative. Nucleotide sequencing showed sequences with 100% of similarity with *L. interrogans* serogroup Sejroe serovar Hardjo, *L. interrogans* serogroup Icterohaemorrhagiae and *L. noguchii* serogroup Australis. Despite the low number of animals, all cows of this study were only diagnosed as carriers through the testing of genital samples. Therefore, the use of urine samples alone to molecular diagnosis could lead to misdiagnosis. Other leptospire than Sejroe strain were diagnosed, reinforcing that cattle could carrier incidental leptospire. This is the first study regarding bovine genital leptospirosis in alive cows, and its findings reinforces the importance of reproductive tract as an extra-renal site for *Leptospira* spp. colonization.

Keywords: *Leptospira* spp., molecular diagnosis, PCR, nucleotide sequencing, cattle.

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