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Optimization of PCR for the detection of pathogenic Leptospira in bovine semen

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Introduction: Bovine leptospirosis is a global zoonotic disease. Bovine leptospirosis can significantly impact production on infected farms due to infertility, abortions, stillbirths, weak offspring, and decreased milk production and growth rates. Cattle are recognized as a reservoir host for *L. borgpetersenii* serovar Hardjo; and transmission occurs via urine, semen, and uterine discharges (1). Our previous studies have detected leptospires by PCR and culture in urine samples collected from beef steers, dairy cows and bulls (2,3,4). Currently, the routine diagnosis of bovine leptospirosis for the export/import of bovine semen relies on the microscopic agglutination test (MAT), a serological assay that detects agglutinating antibodies in serum from animals exposed to leptospires. However, a positive MAT titer in bovine sera does not differentiate antibodies present due to infection versus immunization, nor diagnose shedding of leptospires from urine or semen (1). In this study, we apply and optimize our PCR protocols (5) to directly detect pathogenic *Leptospira* in bovine semen samples.
Methods: *L. borgpetersenii* serovar Hardjo strain TC129 isolated from a beef steer, strain S014J isolated from a beef bull, and *L. santarosai* serogroup Pyrogenes strain DCP-017 isolated from a dairy cow were used to spike bull semen samples, which did or did not contain semen extender. DNA was extracted from spiked semen samples before or after dilution (1:2, 1:3, 1:5, 1:10) with PBS or 1% bovine serum albumin (BSA) using the Maxwell RSC Purefood Purification Pathogen kit to determine the lower limit of detection. Lower levels of detection were also determined using spiked semen that was frozen at -80? for 24 hours prior to extraction of DNA. All experiments were performed twice, independent of each other. An internal positive control was used on all samples in the PCR.
Results: Spiked semen that was not diluted prior to DNA extraction was negative for target DNA and an internal positive control. Spiked semen that was diluted 1:5 or 1:10, in either PBS or BSA, prior to extraction of DNA was optimal for detection of target DNA and an internal positive control. The lower limit of detection for leptospires in semen containing extender was 10 leptospires/ml, compared to semen without extender which was 100 leptospires/ml. No difference in lower limits of detection was observed between fresh versus frozen samples, or between different strains of leptospires.
Conclusion: The development of optimized protocols to evaluate bovine semen for the detection of pathogenic *Leptospira* by PCR and determination of lower limits of detection is essential to standardize diagnostic assays. Future studies will include the use of clinical samples and will compare the detection of *Leptospira* in bull urine compared to bull semen, to determine prevalence of shedding, and to differentiate shedding from colonized renal tubules versus the genital tract.