The detection of *Tritrichomonas foetus* in bovine semen with centrifugation and PCR

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Bovine trichomoniasis caused by *Tritrichomonas foetus*, is a true venereal disease of cattle that is spread only through coitus. Diagnostic techniques have recently improved with the emergence of a more sensitive and specific polymerase chain reaction (PCR) techniques. This is an improvement from traditional culture methods, but better diagnostic and collection methods are still needed given the serious consequences of inaccurate diagnosis. The objective of the study was to determine if a *T. foetus* infection could be detected using PCR in pre-seminal fluid and a seminal sample collected from known positive bulls.

Mature beef bulls (n=20) of various breeds from several south Florida ranches that were previously diagnosed to be positive for *T. foetus* by routine culture and real-time PCR on preputial smegma at an external state diagnostic laboratory were used for this study. These bulls underwent routine electroejaculation, and a dry preputial scraping sample was collected from each bull using a 52.5 cm infusion pipette with a flex adaptor and a 20 ml syringe. The samples collected from urethral emissions were fractionated into a pre-seminal sample and seminal sample based on gross appearance. The bulls all achieved an erection and the penis extended completely outside of the sheath. The preputial sample was immediately suspended in 2 ml of trypticase-yeast extract-maltose medium (TYM) with agar (Diamond’s medium). The samples were transported to the laboratory in a commercial incubator at 37°C. Samples from each bull were centrifuged at 4000g for nine minutes at room temperature and the resulting pellet was used for DNA isolation prior to processing for conventional PCR. Analytical sensitivity and specificity of preputial scrapes were calculated based on previous confirmation of *T. foetus* positive status using an online calculator (http://vassarstats.net/clin1.html). Overall, 13 of 20 bulls were positive by traditional preputial scraping resulting in a test sensitivity of 0.65 (95% CI: 0.41-0.84). Four of these 13 positive bulls were also positive on the pre-seminal sample. One bull tested positive on the pre-seminal sample, but was negative on preputial scraping. The test sensitivity and specificity for detection of *T. foetus* by conventional PCR in pre-seminal fluid was 0.30 (95% CI: 0.1-0.61) and 0.86 (95% CI: 0.42-0.99), respectively. None of the semen samples were found to be positive for *T. foetus*. The combined sensitivity for the preputial scrape and pre-seminal fluid in this study was [1 - (1- 0.308) x (1- 0.65) = .758], suggesting that the diagnosis of *T. foetus* in infected bulls can be improved by additionally testing pre-seminal fluid during routine collection of a preputial scrape.

**Keywords:** Bulls, *Tritrichomonas foetus*, PCR, centrifugation, pre-seminal, seminal