Real-time PCR assay for trichomonosis on pooled direct preputial samples

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Introduction

Tritrichomonas foetus is recognized as important cause of reproductive loss in beef herds. Strategies for the control of trichomonosis (trich) in beef herds are based on identifying and eliminating infected bulls. The sensitivity of conventional PCR methods is sufficient for use of detecting trich in pooled preputial samples from up to five bulls. A real-time PCR (RT-PCR) assay for trich detection has shown improved sensitivity, compared with that of the conventional PCR assay. Previous research from our group demonstrated that pools of samples from up to 25 bulls, enriched by culture, could be tested for trich with the RT-PCR assay without a significant decrease in sensitivity. The objective of the present study was to evaluate the sensitivity of a commercially available RT-PCR assay for the detection of trich in pooled samples of direct preputial samples from five or 10 bulls.

Materials and Methods

One thousand bulls from 23 locations in Saskatchewan were sampled by means of a 25-inch plastic pipette (Continental Plastics, Cambridge, ON, Canada) attached to a 20 mL syringe. Briefly, the pipette was inserted in the prepuce and the plastic sheath was pulled back. The scrape was performed by moving the pipette back and forth 10 times while applying 15 mL of suction. Two samples were collected from each bull and placed alternatively into an InPouch™ TF (Biomed Diagnostics, San Jose, California, USA) or a 4-mL cryovial containing 2 mL of phosphate buffered saline (PBS). Pouches were closed as per the manufacturer's instructions and placed in a warm cooler (approx. 77°F (25°C)), and cryovials were placed in a styrofoam box with ice packs. All samples were transported to the lab within four to seven hours. Upon arrival to the lab, pouches were placed in an incubator at 90°F (32°C) and cultured for seven days, and cryovials were stored at –112°F (–80°C) until analysis. Additionally, once a week, two samples were collected and processed by the method described above from one naturally infected and three artificially infected bulls housed at the WCVM Animal Care Unit. All pouches were examined microscopically (100X) on days 1, 3, 5, and 7. Samples collected in PBS were thawed and a 200 µL aliquot was submitted for individual RT-PCR testing to a commercial diagnostic lab (Prairie Diagnostic Services, Saskatoon, SK). Once samples were tested individually by both culture and RT-PCR assay, negative samples were blocked by order of sampling and randomly divided into groups of five and 10 samples, ensuring that every pool had one positive sample. Pools were made by including fixed aliquots (200 µL) to a total volume of 1 mL or 2 mL for pools of five and 10 bulls, respectively. One hundred pools were made for each ratio. The RT-PCR assay used was that described by McMillen *et al* and DNAeasy Blood & Tissue kit (Qiagen Inc., Toronto, ON) was used for DNA extraction. Statistical analysis of data was performed by PROC GENMOD (SAS version 9.2, SAS Institute, Cary, NC, USA).

Results

Preliminary results estimated a sensitivity of 94.1% (95% confidence interval, 73.0% to 98.9%) and 88.2% (95% confidence interval, 65.6% to 97.6%) for detection of trich in pools of five or 10 preputial samples, respectively (n = 17 per group). The sensitivity did not differ significantly between pool ratios ($P = 0.55$). Also, there was no significant effect of order of sample in the likelihood of obtaining a positive result ($P = 0.48$).

Significance

Preliminary results indicated that the testing of bull preputial samples in pools of five or 10 via a RT-PCR assay may be a viable diagnostic screening method for the detection of *T. foetus* carrier bulls. Furthermore, this method would allow producers to routinely screen bulls at an affordable cost and increase the feasibility of large epidemiological studies and planned surveillance.