Sensitivity of a Real Time PCR Assay for Bovine Trichomonosis in Pooled Preputial Samples

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Introduction

Tritrichomonas foetus has long plagued the beef industry all around the world (Campbell *et al*, 1993). Control of trichomonosis (trich) in beef herds is mostly based on eliminating infected bulls (Clark *et al*, 1983). Polymerase chain reaction (PCR) methods are gradually replacing traditional culture in the detection of trich in preputial scrape samples. A previous study showed a sensitivity and specificity of 100% when pools of five individual samples were tested using conventional PCR (Kennedy *et al*, 2008). The use of a real time polymerase chain reaction (RT-PCR) based assay has been shown to improve sensitivity and specificity over conventional PCR and culture methods (McMillen *et al*, 2006). The objective of the present study was to evaluate the sensitivity of a commercially available RT-PCR assay for trich in pooled preputial samples at different ratios.

Materials and Methods

A total of 147 steers at the University of Saskatchewan feedlot were sampled by means of a 25 in (63.5 cm) 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 forward 10 times while applying 15 ml of suction. Upon completion the pipette was withdrawn and rinsed into the upper chamber of an InPouch™ TF (Biomed Diagnostics, San Jose, California, USA). Pouches were closed as per manufacturer instructions and transported to the lab in a warm cooler (77 to 98.6°F or 25 to 37°C) within four hours. Pouches were placed in an incubator at 98.6°F (37°C) and cultured for seven days. In addition, once a week two samples were collected and processed by the method described above from a naturally infected bull housed at the WCVM Animal Care Unit. All pouches were examined microscopically (100X) on days 1, 3, 5, and 7. On day 3 a 500µl aliquot was taken from all samples and sent for individual RT-PCR testing to commercial diagnostic lab (Prairie Diagnostic Services, Saskatoon, SK). Samples yielding a positive result were subjected to quantification. Upon day 3 of culture for the infected bull and day 7 for the steers all samples were frozen in a -112°F (-80°C) freezer until later use. Pools were made by including fixed aliquots of known positive and negative samples in ratios of 1/2, 1/3, 1/5, 1/10, 1/15, 1/20, and 1/25 to a total volume of 0.5 ml. Thirty-one pools were made for each ratio. Positive samples used were quantified previously and evenly distributed so as to assure that all ratios contained high (10^7 organisms/ml) and low concentration (10^3 organisms/ml) samples. The RT-PCR assay used was that described by McMillen *et al*. Statistical analysis of data collected was performed by Proc Genmod using SAS software, Version 9.2 (Statistical Analysis Systems; SAS Institute, Cary, NC, USA).

Results

Sensitivity and 95% confidence intervals for each pool ratio were: 96.8% (83.8-99.4) for pool ratios 1/3 and 1/5; 93.5% (79.3-98.2) for pool ratios 1/2, 1/15, 1/20, and 1/25; and 90.3% (75.1-96.6). There were no significant differences (P=0.39) among any of the pool ratios evaluated. Thirteen of the total 217 pools tested were negative, nine of these negative testing pools contained the same positive sample. The media in this positive sample turned green in color, produced a large amount of gas and had a low concentration of trich (10^3 organisms/ml) which may suggest the presence of PCR inhibitors.

Significance

In conclusion, the use of pooled samples for the detection of *Tritrichomonas foetus* carrier bulls appears to be relatively sensitive in pools of up to 25 bulls. Furthermore, this strategy provides the possibility of screening large number of groups at a more affordable cost. However, individual characteristics of each sample included in a pool may, by the presence of inhibitors, alter the result of the test. Thus, further research is needed to investigate the use of pool samples without prior enrichment by culture.