Clinical sensitivity and specificity of a quantitative PCR assay for bovine genital campylobacteriosis in bulls

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Introduction

Bovine genital campylobacteriosis occurs worldwide wherever natural service is used.The disease is transmitted by carrier bulls and characterized by early pregnancy loss and infertility. Despite the challenge of maintaining viability of the *Campylobacter fetus* subsp *venerealis* (Cfv) during transport and avoiding overgrowth of contaminants, culture remains the "gold standard". Several PCR tests have been developed for the detection of Cfv, and primers VenSF/VenSR have been extensively tested and recognized worldwide. Recently, our research group at WCVM adapted these primers to a SYBR Green qPCR platform.This assay presents a high analytical sensitivity and provides a fast and effective technique, suitable to be used as a screening test for the detection of carrier bulls. The objective of the present study was to determine the clinical sensitivity and specificity of the RT-PCR assay in direct preputial samples.

Materials and Methods

Preputial samples were collected from 150 virgin bulls, three naturally infected, and 10 artificially infected bulls. Samples were collected weekly from all infected bulls. Samples were collected by means of the aspiration method. The scrape was performed by moving the pipette back and forth 10 times while applying 15 mL of suction. Upon completion, the pipette was withdrawn and flushed into phosphate buffered saline (PBS). An aliquot was withdrawn and inoculated into modified Weybridge transport media if transport was to be > 2 hours or PBS if transport was < 2 hours. The remaining sample contained in PBS was stored at –112°F (–80°C) until qPCR testing. Culture of samples with extended transport was performed with Skirrow or CSA agar. Conversely, samples processed within two hours were cultured directly on Skirrow agar and on 5% blood agar plates with a 30-minute passive filtering step that used a 0.65 µm filter (Millipore, Billerica, MA, USA). All plates were incubated at 98.6°F (37°C) under microaerobic conditions with the GasPak™ EZ Campy Pouch™ System (BD diagnostics, ON, Canada). Plates were examined after 72 hours of incubation and colonies consistent with *Campylobacter fetus* morphology were subjected to a conventional multiplex PCR assay to determine species and subspecies. From samples that were stored in PBS, DNA was extracted by the heat lysis method and tested in duplicate by qPCR assay. Bayesian latent class analysis was performed to obtain specificity and sensitivity estimates.

Results

A total of 140 virgin bulls were tested by both culture and qPCR assay. All virgin bulls were considered to be truly "negative" to Cfv because of the lack of exposure to breeding. Crude specificity estimates were 100% (95% confidence interval [CI], 97.3% to 100%) and 90% (95% CI, 83.9% to 93.9%) for culture and qPCR assay, respectively. A total of 54 samples were collected from three naturally infected and three artificially infected bulls. Bulls were considered truly "positive" if the last sampling was positive by means of any of the tests performed. Crude sensitivity estimates were 92.6% (95% CI, 82.4% to 97.1%), 64.6% (95% CI, 50.4% to 76.6%), and 92.6% (95% CI, 82.4% to 97.1%) for blood agar, Skirrow, and qPCR assay, respectively.

Significance

Preliminary results obtained indicate that the use of a qPCR assay as a screening test on direct preputial samples is highly sensitive. Although the sensitivity for the qPCR assay did not differ from that of culture in blood agar with the use of filters, care must be taken with interpretation because the transport times those samples were subjected to are unlikely to occur for samples collected in private practice. Conversely, samples for qPCR assay do not require special transport media (can be transported with ice packs). The present study is ongoing, with sample collection to be completed shortly, and final sensitivity and specificity estimates to be calculated.