"Evaluating the effect of transport time and sample pooling on a real time PCR assay for Bovine Genital Campylobacteriosis"

CONTROLLING REPRODUCTIVE DISEASE IN BEEF HERDS

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RESEARCH INSTITUTION:WESTERN COLLEGE OF VETERINARY MEDICINE
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Background: Reproductive failure is extremely costly for producers, but in many cases the root cause is never found. One cause of reproductive failure is vibrio, caused by Campylobacter fetus venerealis, a sexually transmitted bacterium. Vibrio infections cause infertility, early embryonic death and sporadic late term abortions. Infections will usually manifest as a longer calving interval, or more open cows than usual. Newly infected cows will often conceive, but the resulting pregnancy is commonly lost between 40 and 70 days after breeding. Cows that have aborted may start to cycle again, but experience temporary infertility for one to five months as they clear the infection. Infected bulls will show no clinical signs or changes in semen quality. Testing for vibrio has traditionally been very difficult as the bacteria are quite fragile and often do not survive the trip to the laboratory for culture.

Previous research (0009-038) evaluated and optimized a new field collection protocol and PCR (polymerase chain reaction) test for vibrio in beef bulls, and also piloted the test under a range of sampling conditions in the field. This test, while not perfect, improved the chance of finding the bacteria from 38% with the traditional culture test to 85% or more with the PCR test. The potential for a false positive result in a clean or disease-free bull was less than 15%.

Objectives: The objectives of this project were to determine the feasibility of pooling samples to decrease testing costs, as well as to determine the impact of delay in transport time and temperature conditions on the sensitivity of the PCR test, or its ability to find infected bulls.

What they did: To study the effectiveness of pooling, samples were collected from 176 virgin bulls, expected to be vibrio negative, and eight known vibrio positive bulls. The sampling techniques used are the same as for trichomonaisis (Tritrichomonas foetus). Pools of samples were made for the study by combining positive and negative samples in ratios of 1:3, 1:5, and 1:10.

The effect of transport time and temperature was assessed by collecting repeated samples from the vibrio positive bulls. Sample material from each collection was immediately transported to the lab for the traditional culture analysis. The remaining sample from each bull was split and tested using a series of time and temperature scenarios that could be expected during field application of the test.

The researchers also investigated the difference between their test results and that of a commercial diagnostic laboratory using a slightly different procedure.

What they learned: Samples pooled in the 1:1, 1:3, 1:5 (positive:negative) ratios showed no differences in test sensitivity. Pool ratios of 1:10 were less sensitive than the other pool ratios.

Storing samples at 4°C or 30°C for 96 hours did decrease the sensitivity of the PCR test compared to those samples stored at 30°C for two or 48 hours. Short term storage of samples at -20°C for two hours also demonstrated a decrease in sensitivity.

The alternate DNA extraction method used at the commercial diagnostic lab resulted in lower sensitivity of the PCR test.

What it means: While there was no consistent pattern of temperature difference and transport delays on the efficacy of the test, the results suggest that samples traveling short distances should be refrigerated. If longer delays are expected the samples should be frozen at -20°C prior to shipping.

Previous research demonstrated the value of pooling samples for trichomonaisis. It is possible to achieve similar cost savings by pooling samples of at least five bulls intended for vibrio testing. Serial sampling and testing (three consecutive tests over time) is recommended for high risk bulls whether or not the samples are pooled before a bull should be reported as negative for vibrio.

This project addressed some of the outstanding questions about how well collected samples intended for the PCR test for vibrio would perform given different transport times and storage temperatures, helped to optimize testing protocols, and provide confidence in a relatively new diagnostic test.

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