A more efficient screening test for trichomoniasis

Project Title: Investigating Reproductive Failure in Western Canadian Cow-Calf Herds

Researchers:
Steve Hendrick, Ph.D.  steve.hendrick@usask.ca
Alvaro Garcia Guerra, Steve Hendrick, Ph.D., Cheryl Waldner, Ph.D., Bonnie Chaban, Ph.D., and Janet Hill, Ph.D. (Western College of Veterinary Medicine, University of Saskatchewan)

Background:
Trichomoniasis (trich, pronounced “trick”) and other venereal diseases can result in large numbers of open cows at the end of the breeding season, and cause enormous economic losses in the cow-calf sector. Good diagnostic tests are available for trich, but these tests require that bulls be tested three times, one week apart, with no breeding activity in between.

Some diagnostic laboratories have moved to polymerase chain reaction (PCR) assays for detecting trichomonosis rather than traditional culture methods. These DNA-based PCR tests are very sensitive, meaning that if trich is present, the test will very likely detect it. However, PCR tests can be less specific, meaning that they can confuse other organisms with trich. Because closely related microbes often have similar DNA sequences, this can result in a ‘false positive’ diagnosis, where a ‘clean’ bull is mistakenly diagnosed as infected. If two microbes share a DNA sequence (e.g. trich and a closely related but harmless trichomonad found in feces), the PCR test may detect the trichomonad, mistake it for trich, and produce a false positive result. The specificity of PCR trich tests need to be verified.

Because PCR assays are quite sensitive, it should be possible to “pool” samples collected from several individuals without missing infected bulls. Individual samples would still be collected, but would be pooled together and tested as a group. Pooling strategies would make testing for trichomoniasis more affordable and feasible during routine breeding soundness examinations.

Objectives:
To compare what percentage of samples test positive for trichomoniasis when pooled in groups up to 25 bulls per sample.
**What They Did:**

Steers and virgin bulls (which could not have been infected with trich) were sampled and tested for trichomoniasis and were used as the source of negative samples in the pools. Positive samples were collected from one infected bull. Pooled samples were then made at the following seven ratios: 1/2 (where 1 sample was from a positive bull and 1 sample was from a negative steer or virgin bull), 1/3, 1/5, 1/10, 1/15, 1/20, and 1/25 (where 1 sample was from a positive bull and 24 negative samples were from steers or virgin bulls). All pooled samples resulted in an equal volume, and a total of 31 replicates were for each pooling ratio. Each of the 217 pools was then tested with real-time PCR.

**What They Learned:**

The percentage of pooled samples that tested positive were:

- 96.8% for pool ratios 1/3 and 1/5;
- 93.5% for pool ratios 1/2, 1/15, 1/20 and 1/25; and
- 90.3% for pool ratios 1/10.

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 colour, produced a large amount of gas and had a low concentration of trich.

**What it means:**

Pooled sampling for trichomoniasis testing is an effective screening tool. However, the optimal pool size will depend on the expected number of positive bulls. With more positive bulls, smaller pools should be used. Three (3) tested samples per bull is still recommended before considering them negative.

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**For More Information Contact:**

Beef Cattle Research Council  
#180, 6815 - 8th St. NE  
Calgary, AB T2E 7H7  
Tel: (403) 275-8558 Fax: (403) 274-5686  
info@beefresearch.ca

**For More Information Visit:**

www.beefresearch.ca