Diagnostic Complications from "Fecal" Trichomonads

Clell V. Bagley, *DVM;* **Michael E. Paskett,** *BS*
Department of Animal, Dairy and Veterinary Sciences, 5600 Old Main Hill, Utah State University, Logan, Utah 84322

© Copyright American Association of Bovine Practitioners; open access distribution.

Abstract

Routine methods of culture for *Tritrichomonas foetus* cannot differentiate between it and fecal/enteric trichomonads. Fecal/enteric trichomonads can contaminate the sheath of bulls and result in their being classed as infected with trichomonads. Current Utah regulations require the slaughter of all trichomonad-positive bulls. The presence of fecal/enteric trichomonads is documented, but the extent in Utah is unknown. Culture of fecal samples from 55 bull calves in 11 herds was conducted to recover trichomonads. Typical positive trichomonad cultures were found in 23 of 55 (42%) fecal samples, and in 11 of 11 (100%) herds. Three samples with the heaviest growth of trichomonads were sent to a second laboratory for PCR and staining diagnostics. All three isolates were negative using *T. foetus*-specific PCR primers. They were also observed to have four anterior flagella and were classified as *Tetratrichomonas* species. These results indicate fecal trichomonads are common in cattle of Utah and southern Idaho. There is no evidence that this type of trichomonad is pathogenic. Regulations need to allow for further specific identification of trichomonad organisms from young bulls, and especially for valuable bulls.

Résumé

Les méthodes routinières de culture pour la détection de *Trichomonas fœtus* ne permettent pas de distinguer entre ce dernier et les trichomonas fécaux ou entériques. Les trichomonas fécaux ou entériques peuvent contaminés le fourreau des taureaux les classant ainsi infectés avec des trichomonas. Les règlements en cours en Utah exigent l'abattage de tous les taureaux qui testent positif aux trichomonas. La présence de trichomonas fécaux ou entériques est documentée, mais la prévalence en Utah n'est pas bien établie. Des cultures d'échantillons fécaux ont été faites chez 55 taureaux provenant de 11 troupeaux dans le but de déterminer la présence de trichomonas. Des cultures positives aux trichomonas ont été trouvées dans 23 des 55 (42%) échantillons fécaux et dans tous les 11 troupeaux (100%). Trois échantillons fortement contaminés avec des trichomonas ont été acheminés dans un deuxième laboratoire pour l'analyse de la réaction par polymérisation en chaîne (PCR) et le diagnostic par coloration. Les trois isolats se révélèrent négatif au test PCR avec des amorces spécifiques à *T. fœtus*. Ces pathogènes montraient quatre flagelles antérieurs et ont été classifiés dans l'espèce *Tetratrichomonas*. Ces résultats indiquent que les trichomonas fécaux sont fréquents chez les bovins de l'Utah et du sud de l'Idaho. Ces trichomonas ne semblent pas pathogéniques. Des règlements devraient permettre l'identification plus avancée des types de trichomonas chez les jeunes taureaux et plus spécialement pour les taureaux de valeur.

Introduction

Utah producers have made great progress in reducing the amount of trichomoniasis present in beef cattle. The Utah Department of Agriculture and Food reported that during the year from October 1, 2001 to September 30, 2002 only 0.35% of the 14,290 bulls tested in Utah were positive for trichomoniasis. That is a great reduction from the 5% rate of the mid-1980s.

However, an organism referred to as "fecal trich", "enteric trich", or "trich-like" has also become apparent, and has caused problems for several bull producers. The usual culture methods for trichomonads cannot differentiate between *Tritrichomonas foetus* and fecal trichomonads.[1,7] However, fecal trichomonad organisms are not a new discovery. Previous research at Utah State University and elsewhere performed in the 1950s identified these organisms in the digestive tract.[2,5,6] Four factors contribute to the current problem: 1) the "fecal trich" organism cannot be easily differentiated from *T. foetus*; 2) the "fecal trich" organism can contaminate the penis of a bull during normal mounting of other bulls; 3) this activity tends to occur as young bulls enter puberty and when closely confined; and 4) current regulations require young bulls to be tested for trichomoniasis prior to sale (and this is important to do, as some may have bred an infected cow and could be infected).

Utah Agriculture Experiment Station journal paper no. 7524

Both California and Colorado are currently using a PCR laboratory technique to differentiate between the "fecal trich" or "T. foetus-like" protozoa and *T. foetus*. Researchers at Colorado State University conducted a trial and infused pregnant cattle with a "fecal trich" organism. They found that it did not persist as an infection and did not cause reproductive problems.[a] A similar study was conducted in California where a "fecal trich" organism was given to a small number of animals without ensuing reproductive disease problems.[b]

Considerable work has also been conducted in Alberta, Canada to compare *T. foetus* and *Tritrichomonas enteris*.[7,8,9,11]

Utah regulations regarding "fecal trich" are more stringent than those of California or Colorado, and allow no exceptions. If a "trich" organism is found, all positive bulls are required to be slaughtered, and all animals associated with the "infected" animal are required to test negative three times. This causes severe financial loss for the producers. A major question that emerged is, does the "fecal trich" organism occur commonly in Utah or is it rare, and are we at risk of increasing its presence if the current regulations are changed to allow differentiation of these organisms? To answer that question, a study was implemented to culture the "fecal trich" organism from fecal samples.

There is still another unknown. Is there another trichomonad organism that could contaminate cattle and cause reproductive problems? Such an organism was isolated by USU researchers in the 1950s which did cause reproductive problems.[2,3,5] A similar organism was recently found to cause diarrhea in cats.[4] Through use of the latest methods for identification, both organisms are now considered to be the same organism as *T. foetus*.[4,12,13] If either of these organisms were present, they would appear as positives on the PCR test for *T. foetus*.[a,b]

Materials

Fecal samples were collected on October 29, 2002 from 55 bull calves which had recently entered a feedlot for growth performance testing. Eleven herds were identified from a widespread geographic area, including southern Idaho and all of Utah. Different breeds were represented among the 11 herds. Fecal samples were collected from the first five bulls of each herd as they were individually weighed.

The culture media initially used was a modified Diamond's media in test tubes, the same used at the Utah Veterinary Diagnostic Laboratory for culture of *T. foetus*. However, for this initial culture, no antibiotic or antifungal agents were added to the media. At a second culture from the same fecal samples, the usual trichomonad media was used that contained both an antibiotic and an antifungal agent.

The Diamond's media used consisted of trypicase peptone (20.0 g), yeast extract (10.0 g), maltose (5.0 g), l-cysteine HCl (1.0 g), ascorbic acid (0.2 g), potassium phosphate, dibasic (0.8 g), potassium phosphate, monobasic (0.8 g), granulated agar (1.5 g), and distilled water qs 900 ml. After autoclaving, penicillin G (200,000,000 units), streptomycin sulfate (2.0 g), bovine serum (100.0 ml) and amphotericin B (2 mg) were aseptically added. The pH was adjusted to 7.2-7.4 and dispensed in 8-10 ml amounts into test tubes.

Methods

Upon collection of the fecal sample, the plastic glove used for collection was inverted off the arm, the bull's identification number was recorded on the glove, and it was placed in an insulated container. Within one hour of collection, fecal samples were inoculated into antibiotic-free trichomonad media. A small hole was made into the plastic glove with an applicator stick and a portion of feces (approximately 1 g) was squeezed out of the glove onto a single layer of 2" X 2" gauze. The gauze was then folded and pushed down into the test tube containing culture media until the media covered the portion of gauze containing the feces.[c] The test tubes were maintained in a rack to keep them upright, placed in an insulated container and maintained slightly above room temperature.

The media was delivered to the laboratory 18 hours after inoculation. Gauze containing the fecal sample was removed from each tube and discarded. A drop of media was collected with a 9" (23 cm) glass Pasteur pipette from the middle and another from the bottom of the test tube. A new pipette was used for each tube. For microscopic examination, the drop of media was placed on a microscope slide and spread slightly with the tip of the pipette, but no cover slip was used. Each slide was examined microscopically at 100X. After all tubes were examined, they were placed in an incubator (97.7-98.6 F; 36.5-37.0 C) and re-examined 24 hours later.

From initial cultures, only two samples had trichomonad organisms present in the media (from the feces). These two positive fecal samples (#121 and #122) were then used to further refine the culture technique. A comparison was made of media (with and without antibiotics) and temperature for incubation (at incubator 97.7-98.6 F [36.5-37.0 C] and room temperature 70.0-72.0 F [21.1-22.2C]). The times from incubation to microscopic examination were 6, 24, 30 and 48 hours (Table 1).

Because of the growth patterns found for the two positive cultures, we decided to culture in media containing an antibiotic (standard Diamond's media) and to place samples in an incubator (97.7-98.6 F; 36.5-37.0 C) for the first 24 hours. We also decided to read the samples at only 24 and 48 hours.

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 1. Trial protocol to compare trichomonad growth from fecal samples at various temperatures with and without antibiotics.*

Temperature	Media with antibiotic*	Media without antibiotic*
Incubator	6	6
Room temperature	6	6

*Three fecal culture samples from each of bull samples #121 and #122. Samples were observed at 6, 24, 30 and 48 hours.

Each of the 55 fecal samples was re-cultured using the following revised protocol. Samples of feces from each bull were placed on gauze, inoculated into the trichomonad media containing antibiotic and placed in an incubator for 24 hours. The gauze and feces were then removed and the media examined from a sample obtained at the bottom or just below any cloudiness in the media. Media droplets were examined microscopically at 100X. A separate pipette was used for each test tube. The remaining sample media was then left at room temperature and re-examined at 48 hours post-inoculation. The relative number of trichomonad organisms was estimated for each drop examined by classifying them into categories:

0 = negative (no trichomonads found)
1 = positive for trichomonads, but only a few organisms were found in the entire drop
2 = organisms were present in several locations in the drop examined
3 = multiple organisms were present in most fields
4 = many organisms were present in most fields
5 = massive numbers of organisms were present in most fields

Microscopic evaluation of multiple positive samples showed it was easiest if the 10X objective was lowered completely, then raised until the first bacteria and other objects became visible and in focus. Further focusing up and down within this plane provided the best opportunity for finding the rare trichomonad organism.

Three positive culture samples with the heaviest growth of trichomonads were submitted to the California Animal Health and Food Safety Laboratory System (CAHFS) at Davis, California for specific identification by PCR and staining. They were not informed of the original source of the cultures.

Results and Discussion

At initial culture on antibiotic-free media, only two fecal samples (numbers 121 and 122) were positive. These samples were then placed onto multiple media as described and shown in Table 1. Results show the organisms grew better in the standard trichomonad media containing antibiotic when placed in the incubator for the first 24 hours (Table 2). There was little advantage in reading the samples at six or 30 hours.

Some trichomonads may require the presence of intestinal content or specific bacteria for growth.[5] However, if there is prolific bacterial growth, the growth of trichomonads is prevented or slowed, which may be due to a change of media pH or toxic compounds rather than the actual bacterial mass itself.[10]

Re-culture of the same fecal samples was successful in identifying trichomonads in 23 of 55 (42%) bulls and in 11 of 11 (100%) of the herds (Table 3).

The laboratory report from CAHFS stated: "All three isolates were identified as Tetratrichomonas species. ... They were quite fastidious and would only pass in egg shell media. In addition, there was heavy bacterial contamination ... We were able to visualize four anterior flagella in each of the isolates, which is consistent with *Tetratrichomonas* sp. They reacted in the PCR

Table 2. Growth of trichomonads from fecal samples #121 and #122 in different media in an incubator or at room temperature, at 6, 24, 30 and 48 hours.

Time of microscopic exam (hours)	Incubator				Room temperature			
	Antibiotic		No antibiotic		Antibiotic		No antibiotic	
	121	122	121	122	121	122	121	122
6	0	0	0	0	1	0	1	0
	0	0	0	0	0	0	0	0
	0	0	0	0	1	0	0	0
24	0	0	0	1	0	0	0	0
	0	2	0	1	1	0	0	0
	0	3	0	0	0	0	0	1
30	0	2	0	1	1	0	0	0
	0	3	0	2	0	1	0	0
	0	4	0	2	1	2	0	0
48	X	0	X	2	0	0	X	0
	X	2	X	2	0	0	X	1
	X	3	X	2	0	1	X	0

0 = negative (no trichomonads found)
1 = positive for trichomonads, but only a few organisms found in entire drop
2 = organisms were present in several locations in the drop examined
3 = multiple organisms were present in most fields
4 = many organisms were present in most fields
5 = massive numbers of organisms were present in most fields
X = massive bacterial growth – not read for trichomonads

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 3. List of herds and trichomonad-positive animals within the herd, by category.

Herd	Number positive	Category of amount for each bull positive
1	3	1, 2, 3
2	1	2
3	2	1, 2
4	3	1, 2, 3
5	3	1, 1, 3
6	2	1, 2
7	3	1, 2, 2
8	1	1
9	2	1, 1
10	1	1
11	2	1, 1

1 = positive for trichomonads, but only a few organisms found in entire drop
2 = organisms were present in several locations in the drop examined
3 = multiple organisms were present in most fields
4 = many organisms were present in most fields
5 = massive numbers of organisms were present in most fields

with the universal trichomonad primers but not with the *T. foetus* specific primers. RFLP analysis of the products generated with the universal primers was consistent with Tetratrichomonas species. There is still no evidence that these organisms play any pathogenic role and they are believed to be fecal in origin."

Conclusions

A trichomonad organism is commonly present in the feces of cattle from herds in Utah and southern Idaho. This organism can be cultured from feces using the same media that is used for isolation of *T. foetus*. The organism cultured was not as prolific as expected when culturing *T. foetus,* and positive samples were difficult to maintain for continued growth. There is no evidence these fecal trichomonad organisms are pathogenic.

Experienced laboratory personnel who observe a number of positive *T. foetus* samples could gain the impression that fecal trichomonad organisms were smaller and perhaps not *T. foetus*. But, that would not be confirmatory. Persons who observe only an occasional trichomonad-positive culture would only be able to classify the fecal trichomonad organism as a trichomonad. Regardless of personnel experience, further testing would be required to confirm the identification.

State regulations need to allow for further testing and specific identification of the trichomonad isolated in some cases from cattle. This is especially true for young bulls raised in close confinement and likely to be mounting one another near the time of trichomonad testing. It would also be helpful in the case of an especially valuable bull.

Footnotes

[a]Personal communication. John M. Cheney, DVM. Colorado State University Diagnostic Laboratories, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO 80523-1644.
[b]Personal communication. Richard Walker, DVM, MPVM. California Animal Health and Food Safety Laboratory System - Davis, PO Box 1770, Davis, CA 95617.
[c]Suggested in personal communication. M.J. Kennedy, BSc, PhD, MSc. Agri-Food Laboratories Branch, 601B O.S. Longman Building, 6906 - 116 Street, Edmonton, AB (Canada) T6H 4P2.

References

1. BonDurant RH, Gajadhar A, Campero CM, Johnson E, Lun Z-R, Nordhausen RW, Van Hoosear KA, Villanueva MR, Walker RL: Preliminary characterization of a *Tritrichomonas foetus*-like protozoan isolated from preputial smegma of virgin bulls. *Bov Pract* 33 (2):124-127, 1999.
2. Fitzgerald PR, Johnson AE, Hammond DM, Thorne JL, Hibler CP: Experimental infections of young pigs following intranasal inoculation with nasal, gastric, or cecal trichomonads from swine or with *Trichomonas foetus*. *J Parasitol* 44:597-602, 1958.
3. Fitzgerald PR, Johnson AE, Thorne JL, Hammond DM: Experimental infections of the bovine genital system with trichomonads from the digestive tracts of swine. *Am J Vet Res* 19 (73):775-779, 1958.
4. Gookin JL, Levy MG, Law JM, Papich MG, Poore MF, Breitschwerdt EB: Experimental infection of cats with *Tritrichomonas foetus*. *Am J Vet Res* 62(11):1690-1697, 2001.
5. Hibler CP, Hammond DM, Caskey FH, Johnson AE, Fitzgerald PR: The morphology and incidence of the trichomonads of swine, *Tritrichomonas suis* (Gruby & Delafond), *Tritrichomonas rotunda*, n.sp. and *Trichomonas buttreyi*, n.sp. *J Protozool* 7(2):159-171, 1960.
6. Jensen EA, Hammond DM: A trichomonas species from the rumen and cecum of cattle. Abstract, *J Parasit* 48(2, Sec 2):30-31, 1962.
7. Kennedy MJ, Moraiko D: Comparative growth of *Tritrichomonas foetus* and Tritrichomonas enteris grown in the InPouch TF system and Diamond's media. *Parasitology and Entomology – Research Update 1996 / 97*, Alberta Agriculture, Food and Rural Development, http://www.agric.gov.ab.ca/research/researchupdate/97insct01.html.
8. Kennedy MJ, Higgs G: Comparative studies of *Tritrichomonas foetus* and Tritrichomonas enteris by scanning and transmission electron microscopy. http://www.agric.gov.ab.ca/research/researchupdate/97insct02.html.
9. Kennedy MJ, Manninan K: Detection of bovine *Tritrichomonas foetus* by PCR. http://www.agric.gov.ab.ca/research/researchupdate/97insct03.html.
10. Lloyd D: Nitrite inhibits hydrogen production and kills the cattle parasite *Tritrichomonas foetus*. *J Appl Micro* 93(3):492-496, 2002.
11. Lun Z-R, Gajadhar A: A sensitive PCR assay for the detection of *Tritrichomonas foetus* in cattle. Abstract #33, Am Assoc Vet Parasitol, Proceedings 43rd Annual Meeting, Baltimore, MD, July 25-28, 1998.
12. OIE Manual of standards for diagnostic tests and vaccines, 4th ed, 2000. Chapter 2.3.6, Trichomonosis. Office of International Epizootics. http://www.oie.int/eng/normes/mmanual/A_00053.htm
13. Tachezy J: Cattle pathogen *Tritrichomonas foetus* (Riedmuller, 1928) and pig commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) belong to the same species. *J Eukaryotic Micro* 49(2):154-163, 2002.

© Copyright American Association of Bovine Practitioners; open access distribution.