Diagnosis of trichomoniasis in ‘virgin’ bulls by culture and polymerase chain reaction

Sarah Parker, John Campbell, Kathy McIntosh, Alvin Gajadhar

Abstract — The diagnostic test for Trichomonas foetus in bulls is microscopic examination of cultured preputial samples. Trichomonads other than T. foetus can be present in a preputial sample. Both a staining technique and a polymerase chain reaction assay were useful in differentiating between T. foetus and another trichomonad observed in samples from virgin bulls.

Résumé — Diagnostic de trichomoniase chez des taureaux « vierges » par culture et amplification en chaîne par polymérase. Chez le taureau, Trichomonas foetus est diagnostiqué par examen microscopique de cultures de prélèvements préputiaux. Des trichomonad autres que T. foetus peuvent être présents dans un prélèvement préputial. Les techniques de coloration et d’amplification en chaîne par polymérase ont été toutes deux utiles pour différencier T. foetus d’un autre trichomonad dans les prélèvements chez des taureaux « vierges ».


Trichomoniasis is a venereally transmitted disease of reproduction in cattle caused by the protozoan parasite, Trichomonas foetus. Infection is usually inapparent in bulls, but it can cause early embryonic death and abortion in cows. Bulls, most of which remain persistently infected, are the main reservoir for the parasite (1). Control programs focus on identifying and culling infected bulls and culling nonpregnant cows. Prevention of transmission of the disease through culling practices relies on the ability to identify infected animals accurately.

The currently accepted diagnostic test for T. foetus is microscopic examination following the culture, in any of a number of prescribed media, of samples collected from the prepuces of bulls or the vaginas of cows. The key morphological features of T. foetus and the characteristic ‘rolling motion’ of the live organism in culture have been described (1). Other organisms that are similar in size, shape and motility to T. foetus can be present in preputial samples (1,2). Correct diagnosis of T. foetus infection depends on proper collection and handling of samples, use of appropriate growth media and conditions, and correct identification of the organism by microscopic examination. In samples where the concentration of organisms is sufficiently high, it is possible to further characterize the organisms by phase contrast microscopy (1) or the use of staining methods (3) to aid in visualizing key diagnostic features of T. foetus. An alternative test that can be used is a polymerase chain reaction (PCR) diagnostic assay, which is of particular value if the numbers of organisms present in culture remains low (4).

In early summer of 1999, a group of 37 bulls was added to a group of 13 research bulls, 11 of which were infected with T. foetus. Of these new bulls, 24 were ‘virgin’ bulls purchased the previous fall as yearlings or calves of the year. The remaining 13 new bulls had varying years of service in community pastures that had no record of T. foetus infection. All bulls were maintained in accordance with Canadian Council for Animal Care guidelines (5) in outdoor pens at a facility designed to house bulls. Initially, 9 of these bulls (4 from a community pasture and 5 ‘virgin’ bulls) were housed in the pen with infected bulls. The remainder of the bulls were housed in 2 separate pens. Prior to their inclusion in the research program, each bull was tested for infection with T. foetus. Preputial samples were collected by using a 53-cm pipette and a 20-mL syringe. Samples were inoculated into a commercial culture kit (InPouch TF; Biomed Diagnostics, San Jose, California, USA) or transport media at the time of collection; those in transport medium were transferred to modified Diamond’s medium (MDM) (6) on the day following collection. The samples in the commercial kit and in MDM were incubated at 37°C and examined by light microscopy on days 1, 4, and 8 after collection. In samples where organisms were observed, fixed slides were prepared and stained with a modified iodine and Wright-Giemsa stain (3). All negative cultures had a portion of the sample removed at the end of the culture period from which 2 sets of 100-μL aliquots were extracted and a PCR assay was performed. The PCR amplification utilized the primers developed by Felleisen et al (7). All negative controls run with each set of PCR assays were found to be negative. Results for PCR assay were recorded as positive where an amplicon of the same size as the amplicon from the positive control, 347 base pairs (bp), was observed.

Centre for Animal Parasitology, Canadian Food Inspection Agency, 116 Veterinary Road, Saskatoon, Saskatchewan S7N 2R3 (Parker, McIntosh, Gajadhar); Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan (Campbell).

Address all correspondence and reprint requests to Dr. Sarah Parker.

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Flagellates that were similar in size to *T. foetus* and also exhibited the characteristic ‘rolling motion’ were observed in a 2nd preputial sample collected from a yearling bull (bull A). This bull was penned separately from the infected bulls. The 1st sample collected had been negative on culture and PCR assay. In the 2nd sample, numerous flagellates were found on day 3 of culture. These flagellates were indistinguishable from *T. foetus* by light microscopy; they were subsequently removed from the commercial kit and examined with phase microscopy on wet mount slides and a stained fixed slide. Although the flagellates had an undulating membrane and were similar in size to *T. foetus*, they were rounder in shape than *T. foetus* and had 4 anterior flagella. The culture medium was removed from the kit and a portion used for testing with the PCR assay. The remainder of the culture was subcultured in a fresh commercial kit and in MDM prepared in our laboratory. These subcultures were positive for flagellates by microscopic examination and moderate numbers were present. Samples for PCR assay were also taken from these subcultures. The PCR assay was negative for 10 separate 100-µL samples containing from 300 to $1.0 \times 10^5$ organisms from the original culture and subcultures. The possibility of inhibition of the PCR assay was investigated by running duplicate 100-µL samples of the cultured preputial sample that were spiked with 100 control *T. foetus* (CAPTF 122) organisms. All of the spiked samples were positive by the PCR assay. Six subsequent preputial samples from this bull did not yield further trichomonads and PCR assays of these samples postculture were also negative. The results of these tests suggest that the trichomonad isolated from this bull was not *T. foetus*. The following summer, 6 additional preputial samples were collected (2 were cultured in the commercial kit and 4 were tested with the PCR assay). All tests were negative.

One week after recovering the flagellates from bull A, trichomonads were isolated from a 2-year-old bull (bull B), which was one of the 9 bulls penned with the infected bulls. In the 3 wk preceding, 3 preputial samples had been collected from bull B at weekly intervals and cultured in the commercial kit. Culture results and results for PCR assays on those cultures had all been negative. On this sampling day, 2 preputial samples were collected: 1 was cultured in the commercial kit, the other was cultured in MDM. Trichomonads were observed in both samples. Organisms were observed through a phase microscope on the wet mount slide prepared from the sample in MDM and on a stained fixed slide. The trichomonads observed were spindle shaped, measured 10–15 µm × 15–20 µm, and had 3 anterior flagella and an undulating membrane, which is similar in morphology to *T. foetus*. The wet mount slide with the observed trichomonads from the MDM was also washed, and the washings were concentrated and used for a PCR assay. The 5 100-µL aliquots extracted and tested by PCR assay were positive. Material was removed from both the commercial kit and the tube of MDM and subcultured in a fresh commercial kit and in fresh MDM, respectively. Both subcultures were positive. The PCR assay was positive for 12 separate 100-µL samples from the subculture in the commercial kit. Three wet mount slides with observed trichomonads from the subculture in MDM were also washed and the concentrated wash material was used for a PCR assay. Three 100-µL aliquots from each of 3 slides (9 in total) were tested. Eight of these were positive by PCR assay. On each of 2 subsequent sampling days (12 and 26 d later), 2 separate preputial samples were collected and cultured in the commercial kit and MDM, respectively. Trichomonads were isolated from all 4 cultures. Aliquots of each of these samples were positive by PCR assay and stained slides showed trichomonads with 3 flagella. The next sample from bull B was collected 2 mo after the first positive sample and no trichomonads were isolated. Seven subsequent samples collected from this bull were also negative on culture, and PCR assays of postculture samples were also negative, with the exception of 1 PCR assay that showed a faint positive amplification product, 9 wk after the previous positive culture sample had been collected. This bull remained in the study group for 2 y and no further positive tests were obtained upon periodic testing. These results confirm that this bull was indeed infected with an organism likely to be *T. foetus* and suggest that the infection was subsequently cleared.

Since both bulls were considered to be virgin bulls, there was some concern regarding the origin of bull B’s infection, as well as the source and significance of the trichomonads observed in bull A. Although bull A seemed to have a non-*T. foetus* infection, the possibility that the organism was a venereal parasite of clinical significance could not be ruled out. However, it did not appear that the infection was long lasting, or even that an infection had been established, as we were able to isolate the organism from only 1 preputial sample. An organism similar to this one has been described (8), and the source of the organism is speculated to be contamination from the gastrointestinal tract, possibly due to fecal contamination either from bulls sodomizing each other or during sample collection.

Bull B was purchased as a yearling in the fall prior to his addition to the research group and was penned with other yearling bulls. There was no possibility of contact with cows at this bull station. Information provided by the breeder from whom he was purchased suggested that this bull had no prior sexual contact with cows and that the herd from which he came was not infected with *T. foetus*. The breeder had checked his cows for pregnancy each fall and no reduction in fertility or extension of the calving season had been reported. The breeder also reported that he had not recorded any escapes of his yearling bulls, which were raised at a separate site from the cow herd after they were weaned. At the time that bull B was added to the research study group, he was moved from a pen of 2-year-old bulls to the pen containing the established group of research bulls, some of which were infected with *T. foetus*. Although bull to bull transmission of this disease is an unlikely mode of transmission, it must be considered in this case. While no description of bull to bull transmission exists in the literature, Kendrick (9) hypothesized that it was a possible source of infection for 1 of 3 infected bulls in an artificial insemination stud. He speculated that the infection was acquired when infected and uninfected bulls mounted and often rubbed on a teaser bull in quick succession to...
each other. During movement through the holding pens and chute system with the other bulls, uninfected and infected, bull B was observed to perform mounting behavior.

Although unlikely, it is also possible that the organism recovered from bull B was not *T. foetus* but a morphologically and genetically similar contaminant from the gastrointestinal tract. The occurrence of *T. foetus* in the gastrointestinal tract of cattle has not been described in the literature. The rate of transmission between bulls is likely to be very low, if it exists at all; and in this research study, no other cases of *T. foetus* infection were detected in negative bulls housed with the infected bulls. This includes 6 uninfected bulls that were kept as part of the group for 2 y.

Microscopic examination of cultured preputial samples is not 100% specific. Upon observation of motile trichomonads, further alternate diagnostic techniques should be carried out to confirm that the organism is *T. foetus*. The modified staining technique, phase microscopy, and PCR assay described here were all useful in differentiating between the 2 trichomonads observed. The PCR assay may have advantages over the staining method, if few organisms are present in culture, as preparation of an acceptable fixed slide requires a high concentration of organisms. Successful phase microscopy can also require high concentrations of organisms, as visualization of the individual flagella depends on slowly motile organisms that demonstrate all flagella present, concurrently. The PCR assay can detect as few as 50 organisms/mL (4). However, reliance on PCR results alone may not be prudent. While the PCR assay was able to distinguish between the organisms recovered from these bulls, it may not be able to differentiate between *T. foetus* and other flagellated organisms that may be present in preputial samples. Work from Felleisen et al (7) testing uninfected bulls suggests that the specificity of these PCR primers is close to 100%, but further work needs to be done on this aspect with other flagellates and uninfected bulls from different populations. For the PCR assay, as for many other types of diagnostic tests, it is important to use appropriate positive and negative controls, which is why we analyzed *T. foetus*-spiked samples from bull A alongside samples that had not been spiked, in addition to negative reagent controls and a positive *T. foetus* culture control.

Trichomonads observed in cultured preputial samples should be confirmed as *T. foetus* prior to diagnosis of trichomoniasis. Sexual transmission of *T. foetus* to bull B prior to his purchase cannot be ruled out, but bull to bull transmission of disease needs to be considered as a possible source of infection for this bull. Based on the potential for bull to bull transmission of trichomoniasis, the penning of infected and uninfected bulls together should be discouraged.

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**References**