report, NCL should be considered as a differential diagnosis for adult-onset, progressive, diffuse central nervous system disease in adult dogs, especially in those cases without advanced intracranial imaging evidence of structural pathology. This is the first report describing a Labrador Retriever with NCL, and on the basis of the available information, this case most closely resembles human adult-onset NCL (Kufs disease).

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Sources and manufacturers

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Comparison of the diagnostic sensitivity of a commercially available culture kit and a diagnostic culture test using Diamond’s media for diagnosing *Trichomonas foetus* in bulls

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Abstract. A number of different culture media have been described for use in the diagnosis of *Trichomonas foetus* infection in bulls, and recently, a commercial culture kit has become available. The objective of this study was to compare the sensitivity of 2 culture-based diagnostic tests for *T. foetus* in bulls. One test used a commercial kit for transport and culture of the samples. The other test used a thioglycollate transport medium (TFTM) for transport and a modified Diamond’s medium (MDM) for culture of the samples. Twenty-one bulls infected with *T. foetus* were sampled repeatedly. On each sampling day, samples collected from the left and right sides of the bull were tested with one of the 2 diagnostic tests being compared. The effect of the type of diagnostic test on the outcome of the test was evaluated with a chi-square test for the calculated odds ratio. Because repeated tests from the same bull cannot be considered independent measures, unadjusted chi-square tests were adjusted for the effect of clustering by bull. Samples tested using the commercial kit were 6.95 times as likely to be positive as samples tested with a diagnostic test using MDM ($P < 0.001$).

Trichomoniasis is a venereally transmitted reproductive disease of cattle caused by the protozoan parasite *Trichomonas foetus*. Infection is usually inapparent in bulls but can cause early embryonic death and abortion in cows. Whereas cows tend to clear the infection, bulls can remain carriers for life and act as the main reservoir of the disease. Trichomoniasis can be a source of extensive economic loss in cow–calf operations. Control programs focus on identifying and culling infected bulls and culling open cows. Efforts

From the Centre for Animal Parasitology, Saskatoon Laboratory, Canadian Food Inspection Agency, Saskatoon, Saskatchewan S7N 2R3, Canada (Parker, Gajadhar), and the Department of Herd Medicine and Theriogenology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4, Canada (Campbell).
to identify infected animals and prevent the transmission of the disease through culling practices rely on the accuracy of methods used to identify the infected animals.

Currently, samples are collected from the preputes of bulls or vaginas of cows and cultured using a defined growth media that supports the growth of flagellated protozoa. A variety of media have been described, and estimates of diagnostic sensitivity have been obtained for some media. Diamond’s medium is commonly used for this purpose, and estimates of its diagnostic sensitivity range from 81.6% to 93.2%. It must be noted that the actual formulations of Diamond’s medium used in individual studies may have varied slightly. A commercially available culture kit has been introduced and is now widely used. Estimates of its diagnostic sensitivity range from 88.0% to 98.4%. Not all these estimates of sensitivity have been obtained with bulls of known infection status. The objective of this study was to compare the sensitivity of 2 culture-based diagnostic tests for diagnosing T. foetus in bulls. The first test used the commercially available culture kit (TF-CK), and the second test used the thioglycollate transport medium (TFTM) and modified Diamond medium (MDM) (TF-DMT) used in the Canadian Food Inspection Agency’s (CFIA) laboratory’s diagnostic T. foetus program.

In the first part of a study conducted in 2 parts, 13 bulls (Bos taurus), infected with T. foetus, originating from several different community pastures in Saskatchewan, Canada, were included and were also part of a study group used in earlier studies. Ten of the bulls were determined to be naturally infected, and 3 of the bulls were artificially infected with a T. foetus field isolate stored at the Centre for Animal Parasitology (CAP) and identified as CAPTF 13.1, as previously described. There were 8 Charolais bulls, 4 Limousin bulls, and 1 Hereford bull, ranging in age from 5–7 years. All bulls in this group had been infected for at least 1 year. The second part of the study used 8 Charolais bulls, ranging in age from 4 to 8 years. Two of them were determined to be naturally infected, and 6 were artificially infected with 3 T. foetus field isolates: CAPTF 119.1 (3 bulls), CAPTF 61.2 (2 bulls), and CAPTF 112.2 (1 bull). The naturally infected bulls and 3 of the artificially infected bulls had been infected for more than a year. The 3 remaining artificially infected bulls had been infected for 1 month before the start of the experiment. For both parts of the study, all bulls remained infected throughout the study period because each bull had at least 1 positive test on the last sample day. All bulls were housed in an outdoor pen at a facility designed for housing bulls.

The sensitivities of 2 diagnostic tests (TF-CK and TF-DMT) for T. foetus were evaluated using the same experimental design and methods for both study groups. Preputial samples were collected from the bulls twice a week. For the first part of the study, samples were collected for 4 weeks during the summer of 1998. The second part of the study was conducted from January to May 2001. Samples were collected a minimum of 3–4 days apart for both sampling periods. On each sampling day, 2 samples were collected from each bull, and each sample was tested with one of the 2 respective diagnostic tests. The experiment was a complete randomized block design, with 2 blocking factors and 1 treatment factor (Table 1). The 2 blocking factors, the side of the bull from which the sample was collected (right or left) and the sequence in which the sample was taken (first or second), had been identified in earlier studies as likely having an effect on test sensitivity. The treatment factor was the diagnostic test used (TF-CK or TF-DMT). In the initial
week of the study, the first bull sampled was randomly assigned to 2 combinations of side, sequence, and diagnostic test (Table 1). All other bulls were then allocated, as they entered into the chute, to other sampling combinations, such that all possible combinations were equivalently performed during each sampling day. Because all factors had 2 levels, there were 8 possible combinations for sampling each bull. The bulls were sampled twice during 1 sampling period with different mutually exclusive combinations, and during the study period, each bull was sampled twice with each combination of factors (Table 1).

Sample collection was accomplished using a 53-cm artificial insemination (AI) pipette and a 20-ml syringe. The pipette was inserted halfway into the prepuce, the syringe was attached, and the pipette was scraped back and forth rhythmically 10 times while 15 ml of suction was applied. Each scrape traversed the prepuce from the fornix to a point just caudal to the preputial orifice. The scrapes were performed at consistent pressure, angle, and site in the prepuce. When the sample was collected from the bull’s right side, the pipette was inserted into the right side of the prepuce and to the left side of the prepuce when the bull was sampled from the left side. If suction was lost during the process, it was reestablished by removing the syringe, carefully depressing the plunger, reattaching the syringe to the pipette, and creating the suction again.

The first test (Tf-CK) was a commercially available culture kit that was used for transport and culture of samples as per the manufacturer’s instructions. The sample was deposited from the AI pipette into the upper chamber of the container, and the pipette was then rinsed with medium present in the upper chamber of the container. The pouches were closed and transported at room temperature in a Styrofoam cooler, which arrived at the laboratory within 3 hours of collection. Samples were maintained at room temperature for 1 day and incubated at 37 C for 7 days. Day 0 was defined as the day when samples were placed in the incubator. The pouches were placed in the holder provided by the manufacturer and examined for approximately 3–5 minutes under a compound microscope at 100× magnification on days 0, 3, and 7. A positive result was recorded if T. foetus were seen on any of the 3 reading days.

For the second test (Tf-DMT), TFTM<sup>3</sup> was used along with MDM<sup>7,10</sup> for culture. Samples were deposited from the AI pipette into tubes containing TFTM. The samples were transported at room temperature in a Styrofoam cooler, which arrived at the laboratory within 3 hours of collection. Samples were maintained at room temperature for 1 day and then processed as follows. Samples were centrifuged at 850 × g for 10 minutes, and the supernatant was removed to leave approximately 0.5 ml or twice the pellet size, whichever was greater. A wet-mount slide was prepared from the resuspended sediment and examined for the presence of T. foetus using a compound microscope at 100× magnification. The remainder of the suspension was inoculated into a tube containing 5 ml of MDM by layering it on the top of the medium. The MDM contained 0.05% agar, gentamicin<sup>3</sup> 50 µg/ml, streptomycin<sup>3</sup> 1.0 mg/ml, and amphotericin B<sup>4</sup> 10 µg/ml. The initial microscope reading constituted the day 0 reading. On days 3 and 7, wet-mount slides were prepared from media at or near the bottom of each tube containing MDM and examined microscopically. All these described steps comprise the CFIA’s T. foetus diagnostic test incorporating TFTM and MDM, which has been referred to in the manuscript as Tf-DMT. A positive result was recorded if T. foetus were seen on any of the 3 reading days.

To determine whether results from the 2 parts of the study could be combined, it was necessary to determine that relationships between factors and outcome were similar in both parts. The following analysis was performed for that purpose. Unadjusted odds ratios were calculated to determine the effect of each factor on the result of the test. The factors examined included the diagnostic test, left or right side collection, sequence in which the sample was obtained, the date the sample was collected, and the infection source of the bull. Results from each part of the study were examined separately to determine whether there was any change in the relationships between factors and outcome. Unadjusted odds ratios were compared from both study parts. Also, a multivariate modeling approach, generalized estimating equations (GEE), was used to estimate whether the part of the study from which a result was obtained would have a significant effect on the outcome of the test. Because no changes in relationships were found and the part of the study did not have a significant effect, results from the first and second parts of the study are presented together in this article.

Odds ratios and associated chi-square values were calculated to compare test performance. The experiment was designed such that 2 factors that may have an effect on the sensitivity of the diagnostic tests being evaluated (the side of the bull from which the sample was collected and the sequence in which the sample was collected)<sup>12</sup> were accounted for by blocking. This meant that an overall odds ratio could be used for evaluating the effect of the type of diagnostic test used on the outcome of the test. Because no interaction effects between side, sequence, diagnostic test, and result were detected, an overall odds ratio comparing diagnostic tests was appropriate.

However, repeated samples from the same bulls can-
found that a diagnostic test using a Diamond’s medium was more sensitive than the commercial kit. In the study where no difference was found, a group of 150 clinically suspect bulls was tested once.1 The commercial kit and the Diamond’s medium detected a similar number of infections among the bulls (62/63 vs. 61/63, respectively). Because the infection status of the remaining 87 bulls was not determined, an absolute estimate of sensitivity cannot be calculated. It is likely that the study would have overestimated the sensitivity of both tests. Because the ease of detecting T. foetus infection varies among individual bulls,11 the fact that some difficult to detect bulls were excluded from the comparison (because they were negative on the single test) may not have allowed adequate comparison of the tests. The current study included bulls that had been tested 3 times to determine their infection status.

In the study in which the commercial kit was reported to be less sensitive than the test using Diamond’s culture medium,14 the 2 tests were not evaluated concurrently. Because factors influencing diagnostic sensitivity may have changed between the 2 sampling intervals, the authors noted that these results should be interpreted with caution.14 In repeated samplings from infected bulls, it appears that before clearing an infection, the proportion of positive samples from a bull tends to decrease (Parker, unpublished data) and the probability of getting positive samples from individual bulls may have decreased in the course of that study.11 Another factor that should be considered from that study is that samples collected for culture in the commercial kit5 were divided before inoculation into the kit. This may have decreased the sensitivity of the test through decreasing the number of organisms inoculated into the kit. The sensitivity estimate that they developed from repeated tests of 14 bulls was 88.0%,14 which is lower than the estimate reported in this study, although not significantly different given the likely CI for both estimates.

It is likely that the design of the 2 published studies1,14 was not suitable for an adequate comparison of the performance of diagnostic tests using the commercial kit or Diamond’s medium. Other work5 suggested that lower concentrations of T. foetus in samples can be detected in the commercial kit compared with culture medium in a tube where small aliquots are removed onto a microscope slide and examined. The present results suggest that the commercial kit also has an increased diagnostic sensitivity over diagnostic tests using a Diamond’s culture medium. The relative magnitude of this increase may depend on the population of bulls being sampled. In this study, differences in relative test performance were noted for different bulls. This indicates that the inclusion of such
bulls should have a significant impact on observed differences in test performances.

The relative difference observed in this study compared with that reported in the previous 2 studies comparing tests using the commercial kit and Diamond’s medium may have arisen from differences in the prepared diagnostic media (TFTM and MDM) in the various studies. This is difficult to evaluate completely because the composition of the media is not adequately described in the previous studies. Assuming that the media used in the other studies were prepared as described, then they differ by an increased volume (10 vs. 5 ml) and in the use of a different antibiotic combination. The choice of antibiotics may be a critical factor because overgrowth of contaminants in the cultured sample may hinder the growth of the *T. foetus*.2,10 Another difference between this study and the other 2 studies is the choice of transport media for the samples cultured in Diamond’s medium. In one study,1 the sample was layered directly onto the Diamond’s medium. In the other study,14 the sample was rinsed with phosphate-buffered saline and then layered onto Diamond’s medium. Both these studies had a higher proportion of positive samples for samples cultured in Diamond’s medium than this study, so it is possible that the method of handling the sample before inoculation into the Diamond’s culture medium had an effect on test sensitivity.

The commercial kit had some other advantages as compared with the use of the prepared transport and culture media. The kit was easy to use in the field, did not involve media production, and required minimum preparatory work before microscopic examination of inoculated pouches. It would, however, be more difficult to remove organisms from the commercial kit for procedures such as phase microscopy, preparation of stained slides, or polymerase chain reaction amplification used to confirm identification of *T. foetus*.

In clinical practice, the diagnostic sensitivity of a diagnostic test depends on a number of factors. The sampling for this study was carried out under ideal conditions that would be difficult to duplicate consistently in clinical settings. Bull restraint was optimal, conditions for transport of samples to the laboratory were optimized and consistent, and sample preparation, processing, and microscopic examination were performed by skilled laboratory personnel experienced in detecting *T. foetus*. The commercial kits were newly purchased, and the MDM was freshly prepared biweekly in a laboratory in accordance with quality-assurance principles. Diamond’s medium has been described as losing efficacy during prolonged storage,2 possibly because of deterioration of the antibiotics included in the formulation. Although the commercial kit has an advertised shelf life of 1 year, we were unable to find documentation in the published literature to support the validity of this claim under field conditions.

In this study, the specificity of these 2 diagnostic tests was not evaluated. Although specificity has always been assumed to be close to 100% for culture-based diagnostic tests for *T. foetus*, recent reports have suggested that organisms very similar to *T. foetus* may be collected in preputial samples and multiply in some media.3 The specificity of the diagnostic test can be increased by carrying out confirmatory steps such as more detailed morphological examination using phase microscopy or stained organisms or with alternate techniques such as a DNA-based assay.

From the results of this study, it appears that the commercial kit, if used properly, is a useful tool for diagnosing *T. foetus* infection in bulls. Further work needs to be done to compare the specificity of these 2 diagnostic culture methods and to determine whether the field performance of the commercial kits is satisfactory throughout the currently stated shelf life.

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**16S ribosomal RNA sequence–based identification of veterinary clinical bacteria**

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**Abstract.** This study evaluated 16S rRNA gene sequence analysis methods as tools for identification of 22 phenotypically difficult to identify veterinary clinical bacterial isolates in a veterinary diagnostic laboratory. The study compared 16S rRNA gene sequencing and conventional phenotypic identification methods. Using 16S rRNA full-gene sequencing, 95% (21/22) of the isolates were identified to the genus level and 86% (19/22) to the species level. The conventional or commercially available manual identification phenotypic characterization methods presumptively identified 91% (20/22) of the isolates to the genus level and 1 isolate to the species level. However, only 55% (12/22) or 4.5% (1/22) of the phenotypic identifications were correct at the genus or species level when they were compared with the 16S rRNA full-gene sequencing. This study also compared 16S rRNA full-gene and partial-gene sequencing. The results demonstrated that the best 16S rRNA gene–sequencing approach is full-gene sequencing because it gives the most precise species identification. Sequencing of the variable regions 1, 2, and 3 of the 16S rRNA gene could be used for tentative identification because the ability of this sequencing to identify bacteria to the genus level is similar to that of the 16S rRNA full-gene sequencing. This method identified only 14% (3/22) isolates differently to the species level compared with the 16S rRNA full gene sequence. Sequencing of the variable regions 7, 8, and 9 is not recommended because it gives more ambiguous identifications. The cost of a 16S RNA full-gene–sequencing analysis was Can $160 and Can $60 for a partial 16S rRNA gene sequence, i.e., sequencing of variable regions 1, 2, and 3 or variable regions 7, 8 and 9.

Since the 1980s, sequencing of the 16S rRNA gene has been used as an important tool for phylogenetic analysis and classification of bacteria. The 16S rRNA gene contains regions well conserved in all organisms that are ideal for primer design, polymerase chain reaction (PCR) or sequencing, and sequence alignment. It is possible to design universal primers for most of the bacteria. It also contains species-specific variable regions that allow species identification. Therefore, sequence analysis of the 16S rRNA gene is becoming a powerful technology for identification of bacterial isolates in the human clinical diagnostic laboratory. This method has also been used to identify or analyze veterinary clinical isolates, including *Mycobacterium* strains recovered from cats, *spirorchetes* associating with a late-term abortion in a mare, nonporcine isolates of *Actinobacillus suis*, and a new biotype of *Actinomyces hyovaginalis* in pig tissues.

The size of a 16S rRNA gene is about 1,540 base pairs (bp) (Fig. 1), and current sequencing technology can read over 500–700 bp per reaction. Thus, it requires about 6 reactions in each direction to generate an accurate sequence. To reduce cost and labor intensity, methods using partial–16S rRNA sequence analysis for bacterial identification have been demonstrated. Sequencing of 3 consecutive divergent regions, V7, V8, and V9 (hereafter defined as V7-8-9 sequencing)