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Diagnosis of bovine trichomoniasis

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Abstract

Bovine trichomoniasis was first identified in the US in 1930s as a cause of reproductive failure in cattle and its impact continues. Because bovine trichomoniasis transmission is strictly venereal under natural breeding conditions, its control and eradication are feasible. However, failure to consistently and accurately identify infected animals and culling them have been major challenges. Generally, errors in the 3 diagnostic phases (preanalytical, analytical, and postanalytical) prevent correct classification of *Tritrichomonas foetus* status of tested animals. The purpose of this review is to define these phases of diagnostic testing and discuss potential errors in each phase..

Keywords: *Tritrichomonas foetus*, cattle, trichomoniasis

Introduction

Bovine trichomoniasis was first reported in the US in 1932[1] and continues to cause reproductive failure in US herds. Because this disease transmission under natural conditions is strictly venereal, it should be amenable to control and even eradication. Although eradication has occurred in some cattle populations, the causative organism, *Tritrichomonas foetu*s, continues to circulate through cattle herds across the US. The most substantial challenge to successfully eliminate this organism from a cattle population is the ability to consistently and accurately identify infected animals for their removal from the breeding herd.

The diagnostic process consists of 3 basic phases: preanalytical (sample collection and handling), analytical (performance of the diagnostic test), and postanalytical (test result reporting and interpretation). Examples of preanalytical errors include inappropriate test request, misidentification of the sample, sample collection and handling, and storage and transportation of the sample. Examples of analytical errors include equipment malfunction, sample mix-ups, and undetected quality control failure. Excessive turnaround period, improper data entry, incorrect interpretation, and inappropriate follow up plan are examples of postanalytical errors. Most errors that occur during the entire diagnostic process occur in the pre- and postanalytical phases.[2] The purpose of this discussion is to examine the 3 phases of diagnostic testing as they relate specifically to bovine trichomoniasis, with a focus on pre- and postanalytical phases.

Preanalytical considerations

Sampling location

Distribution of *T. foetus* in the preputial cavity of bulls was described in detail in 1943.[3] Highest number of organisms were at the midshaft and caudal portion of the free penis, followed by the prepuce surrounding penis and then the galea glandis, and the prepuce near the preputial opening.[3] This information has been used as the basis for recommending the collection of preputial samples from the fornix of the prepuce for trichomoniasis testing. Although this recommendation is still supported4 further work is necessary to unequivocally confirm that fornix is the optimal site for collecting samples.

Sampling devices

A variety of sampling devices and sampling techniques have been used to collect preputial samples for *T. foetus* testing including preputial lavage, cotton swabs, washing of artificial vaginas, specialized collection devices, and uterine infusion pipettes.[5] However, without strong evidence supporting 1 method over another, the technique of utilizing a uterine infusion pipette and syringe has become the most widely accepted method for *T. foetus* sample collection in the US.

The Pizzle Stick (Lane Manufacturing, Inc., Denver CO) is a recently marketed product that is based on a *T. foetus* sampling device developed in the Soviet Union and described in 1969.[6] The current device is a long plastic hollow rod with circular grooves around the diameter of the rod at the end that appears to provide an atraumatic sample collection option. Although the original device was superior to pipette aspiration for sample collection[6,7] the diagnostic sensitivity between these 2 methods was not different.[8]

A recently developed *T. foetus* sampling device (TRICHIT, Morris

Livestock Products, Delavan, WI) has a uterine infusion pipette with a small plastic collection cup affixed to 1 end. The cup is designed to increase the scraping circumference of the pipette while also allowing the sample to be aspirated into the pipette. The purpose of the collection cup is to increase the volume of sample collected while doing so in a less traumatic manner. Efficacy of TRICHIT over other devices remains unknown.

Another more recent technique described as a direct swabbing of the extended penis and prepuce with a gauze sponge has been proposed as a means for collecting a sample from a wider area of the penis and prepuce while allowing the collector the opportunity to avoid areas of gross contamination.[9]

Because there is limited evidence to support any technique or device over another, the best approach is to determine a procedure that works best for the operator and is approved by the laboratory that tests collected samples.

Sample quality

Determining what constitutes a quality preputial sample for *T. foetus* testing is a challenge considering the variety of potential contaminates present in the prepuce including soil, feces, urine, semen, blood, and other organisms. Recent reports[10-13] emphasized the need to better understand this preanalytical aspect of trichomoniasis testing.

An investigation into vaginitis in a group of cows in Switzerland involved testing for *T. foetus*.[10] Although polymerase chain reaction (PCR) testing of vaginal samples indicated a substantial number of *T. foetus* positive cows, further investigation revealed these to be false-positive tests due to a cross reaction with Simplicimonas-like organisms. An earlier report also identified false-positive PCR results due to this trichomonad in samples from bulls.[11]

Bacteria in samples may also provide diagnostic challenges to correctly identify *T. foetus* infected bulls. Bacterial contamination, if not inhibited in medium, had a negative impact on diagnostic sensitivity.[12] Among biological materials (blood, semen, and urine) urine had a negative effect on the correct classification of the *T. foetus* status of the sample.[13]

These reports not only highlighted the need to understand the limitations of the tests used to analyze *T. foetus* samples but also recognized the importance of reducing and eliminating contamination.

Sample handling

Once an adequate sample has been collected, the conditions under which it is transported to a diagnostic laboratory for testing can have a substantial impact on the results of the test. Despite several studies examining the influence of cold temperatures on both culture and PCR testing, not much is known regarding the effects of higher temperatures on *T. foetus* survival and its relationship to test accuracy.

Although temperatures above 98.6°F affected the ability to culture *T. foetus* positive samples, they had no negative influence on the ability of PCR to identify the organism, even though the growth rate of the organism was affected.[14] Exposure of inoculated pouches (with fewer organisms) to higher temperatures (39, 68, 98.6, and 107.6°F for 24, 48, and 72 hours, respectively) were positive to culture and PCR tests except for 107.6°F.[15] Authors attributed this discrepancy to a difference in the number of organisms inoculated and the cutoff values used for the PCR. However, both studies supported the recommendation to protect all *T. foetus* samples from temperatures above 98.6°F.

Analytical considerations

Testing samples for PCR was described in 1997 that provided a substantial improvement over traditional culture-based testing protocols.[16] Despite advances made in PCR techniques (improved workload management for laboratories, decreased turnaround times for reporting results, and potentially improved analytical sensitivity and specificity versus the traditional culture techniques), it has not necessarily improved the overall diagnostic process for bovine trichomoniasis.[17-19]

The apparent lack of diagnostic improvement for trichomoniasis may have more to do with our expectations of a diagnostic test than the assay itself. An excellent review[20] of diagnostic test terminology summarized the following. Analytical sensitivity is an assay's ability to detect a very low concentration of a given substance or organism in a biological sample, whereas diagnostic sensitivity is the assay's ability to detect an individual with the condition in the population. For trichomoniasis, the PCR technique has been recognized to have extremely high analytical sensitivity with the theoretical ability to detect as few as 2 *T. foetus* organisms per ml sample.[18] However, the ability of a test to detect a *T. foetus*-infected animal in a population is our priority and that is an example of diagnostic sensitivity. The diagnostic sensitivity of PCR is ~ 98%.[21]

Analytical specificity is an assay's ability to exclusively identify a target substance or organism in a sample, whereas diagnostic specificity is the ability of the assay to correctly identify an individual in a population who does not have the disease in question. The diagnostic specificity of PCR for trichomoniasis testing is ~ 98%.[17,18]

Postanalytical considerations

While a variety of errors may occur in the postanalytical phase, the focus of the discussion for this phase of the diagnostic process will be on interpretation of results. Diagnostic test sensitivity and specificity receive much attention, but they are characteristics of the test itself. Positive and negative predictive values are clinically relevant information related to the

diagnostic process. Positive predictive value is the probability that an individual that tests positive has the disease of interest. Negative predictive value is the probability that an individual that tests negative does not have the disease of interest.[20] In other words, predictive values give some indication as to how strongly the results of the test can be trusted to represent the true infection status of an animal.

Predictive values are greatly influenced by the prevalence of the disease in a population tested, as described in the following examples using these assumptions: the prevalence of trichomoniasis in the test population is 1%, the diagnostic sensitivity of the test is 98%, and the diagnostic specificity of the test is 98%.

If 10,000 bulls fit the description of a test population, then we would expect to have 100 (10,000 x 0.01) infected bulls in the 10,000 bulls tested. With a diagnostic sensitivity of 98%, 98 (100 x 0.98) of the 100 infected bulls would result in a positive test and 2 (100 minus 98) of the 100 infected bulls would result in a negative test. Because 100 of the 10,000 bulls are infected, then 9,900 (10,000 minus100) of the bulls would be uninfected. With a diagnostic sensitivity of 98%, 9,702 (9,900 x 0.98) of 9,900 uninfected bulls would result in a negative test and 198 (9,900 minus 9702) of the 9,900 uninfected bulls would result in a positive test.

The positive predictive value is calculated by dividing the number of infected bulls which tested positive by the total number of test positive bulls. In this example, the positive predictive value is 0.331 (98/296) or the probability of a test positive bull being infected with *T. foetus* is 33%. The negative predictive value is calculated by dividing the number of uninfected bulls that tested negative by the total number of test negative bulls. For this example, the negative predictive value is rounded to 1.00 (9702/9704), which means that the probability of a test negative bull not being infected with *T. foetus* is very close to 100%.

If the same assumptions are made regarding diagnostic sensitivity and diagnostic specificity, but the prevalence is changed to 20% as might be expected in trichomoniasis infected herds, then the positive predictive value is 0.925 and the negative predictive value is 0.995. In other words, the level of confidence in a positive test is lower in low prevalence situations, but much higher in high prevalence situations. In both scenarios the confidence in a negative test was high. These examples highlight the need for carefully interpreting trichomoniasis test results, regardless of the purported diagnostic sensitivity and diagnostic specificity.

Conclusion

Eradication of *T. foetus* from the US cattle population has proven elusive for a variety of reasons with consistent, accurate identification of infected animals as 1 of these contributing factors. Although the process of diagnosing trichomoniasis may be imperfect, paying close attention to details in the steps of diagnostic process reduces preanalytical, analytical, and postanalytical errors and ultimately will enhance the prospects for control and perhaps eradication of this disease in the future.

Conflict of interest

None to report.

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