Side of chute and handedness does not affect the sensitivity of *Tritrichomonas foetus* sample collection in bulls

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Abstract

Tritrichomonas foetus, a sexually transmitted pathogen of cattle, continues to plague the cattle industry despite control efforts. Pre-analytical diagnostic considerations such as sample collection and sample handling continue to be a shortcoming in the diagnosis of *T. foetus* in bulls. Preputial scraping is the most common means of sample collection. Previous studies have shown an effect of handedness of the sample collector and the side of the chute that collection was made from as factors that affected the diagnostic sensitivity of the test. The aim of our study was to determine if being right or left-handed and the side of the chute the collection occurred on impacted the diagnostic sensitivity or specificity. Eleven sexually mature bulls naturally infected with *T. foetus* were enrolled in a 2-factor cross-over study design. Samples were taken once a week for 8 weeks by either a right-handed or left-handed veterinarian from either the left then the right side of the chute or right then left side of the chute, only using their dominant hand to make the scraping motion used to obtain the sample. Utilizing a 2-way ANOVA, we found no significant difference in the chance of acquiring a positive sample per bull ($P = 0.8708$) regardless of whether the sample was taken from the right or left side of the chute and regardless of dominant hand. In conclusion, using current RT-qPCR methods, the authors found no effect of handedness or side of chute on diagnostic sensitivity.

Key words: bull; *Tritrichomonas foetus,* trich, sampling, bovine

Introduction

Tritrichomonas foetus, a sexually transmitted pathogen in cattle, continues to plague the United States and worldwide cattle industries despite years of control programs. Bulls infected with *T. foetus* are asymptomatic carriers. Bulls maintain their carrier status for life, and act as the main transmitter of the disease, spreading the protozoa during coitus to the female.[1,2] Following transmission of the disease to the female, the protozoa traverses the cranial vagina and cervix causing inflammation of the uterus and oviducts often resulting in embryonic death and abortion ultimately resulting in open cows.[3-5] The loss of calves, prolonged calving period, and ultimate culling of positive animals are economically devastating for those who experience *T. foetus* infections within their herds.

There are currently no approved treatments for *T. foetus* in the United States. Consequently, it is recommended that known positive animals are culled from the herd along with any open females. A vaccine is available, but is only labeled for control of disease in females and not prevention or treatment of the disease.

Bulls are tested for *T. foetus* by obtaining a sample of preputial smegma. Commonly used sampling techniques for diagnosing *T. foetus* in bulls is scraping of the preputial and penile mucosa with either a mare artificial insemination pipette, a specially designed *T. foetus* testing device such as the Pizzle Stick Trich testing device[a] or the Trichit™ [b] testing device to obtain an adequate sample of preputial smegma that harbors the protozoa. The area of collection should be focused on the location of where the distal portion of the free penis is when it is retracted in the preputial cavity. This area has been previously described as the area of greatest organisms by Hammond et al.[6]

One reason for the continued prevalence of *T. foetus* throughout the United States and the world is that accurate diagnosis is complicated in the fact that retrieval of organisms for correct diagnosis and subsequent culling of true positive animals may be compromised by multiple factors including, but not limited to, how samples are collected.[7] Sample collection and shipping are often classified as preanalytical conditions.[8] A previous study by Parker et al. noted that right-hand-dominant practitioners were more commonly successful in retrieving *T. foetus* in known positive bulls when collecting from the right side of the bull compared to the left; however, the opposite was not investigated for left-hand-dominant practitioners and only cultures were used to declare whether a sample was positive or not.[9] While it appears that simple variables can influence test outcomes further research is needed to assist practitioners in making deliberate decisions about how they approach sample collection. Current testing with RT-qPCR allows the detection of positive samples with as little as two trichomonads per ml of sample.[10,11]

The focus of this study was to determine if certain factors such as handedness of the sample collector and side of the chute used for collection are preanalytical conditions that should be of primary concern for practitioners when collecting *T. foetus* samples. A crossover study was performed to evaluate the ability of both right-hand and left-hand-dominant practitioners to successfully retrieve organisms as determined by a positive sample using RT-qPCR when samples were collected from the right side or left side of the bull.

Materials and methods

Eleven sexually mature bulls previously diagnosed as naturally infected with *T. foetus* as determined by a preputial smegma sample submitted for RT-qPCR to a state diagnostic laboratory were purchased. Bulls ranged in age from 2-6 years old, and all were of English or Continental breeding. All bulls were reconfirmed positive for *T. foetus* by RT-qPCR upon arrival at the research facility. They were housed in a paddock and fed

a balanced ration at the Bushland Research Facility in Bushland, Texas. This project was approved by IACUC 2022-1177. All animals remained infected throughout the study.

The project was designed as a 2-factor cross-over design. A single right-hand-dominant veterinarian and single left-hand-dominant veterinarian experienced in the collection of preputial samples for the purpose of testing for *T. foetus* collected all samples with the testing device held in their dominant hand regardless of the side of the chute the sample was collected from. The first bull into the chute was randomly assigned to either the right-hand or left-handed veterinarian based on a flip of a coin. The initial side of collection was also randomly assigned via coin flip regarding whether the left or right side of the chute being chosen. All successive bulls were collected by alternating veterinarians and alternating starting sides (Figure 1). This trial was designed such that, on each sampling day, samples were collected from both sides of the chute for the veterinarian, and in subsequent weeks, the starting side of the chute was switched for that veterinarian and bull allowing us to determine if sampling side or sequence of collection had any effect on the ability to obtain a positive sample. Bulls were sampled again 7 days following the first with the alternate veterinarian and the alternate side being sampled first for a total of 8 weeks (Table 1).

Samples were obtained by scraping the preputial epithelium 10 times with the Pizzle Stick Trich testing device attached to a sterile 20 mL syringe. The Pizzle Stick was inserted into the sheath and directed caudal-to-just cranial to the preputial fornix. Negative pressure was maintained on the syringe and 10 back and forth searching motions focused on the approximate location of the midshaft and caudal portion of the free penis where the highest number of organisms have been reported.[6] Once obtained, samples were transferred to a sterile cryovial containing 2mL of sterile phosphate buffered saline (PBS) and submitted the same day of collection to our in-house Infectious Disease Diagnostic Lab for RT-qPCR testing. This diagnostic testing included automated nucleic acid extraction and purification and RT-qPCR following all procedures and controls indicated by Ginter Summarell et al., which is currently employed as the preferred diagnostic method at the Texas Veterinary Medical Diagnostic Laboratory.[10]

Results were plotted and analyzed with GraphPad Prism Version 10.0.0 (GraphPad Software, Boston, MA) utilizing a 2-way ANOVA. A standard Cq value of < 40 was used as the cutoff in accordance with Summarell et al. This value does exceed the Cq cutoff used by some state diagnostic labs.

Results

All samples regardless of veterinarian, side of the chute, or bull were found to be positive with a Cq value of < 40.0 for the entire 8 weeks (Figure 1). There was no significant difference in the chance of obtaining a positive sample per bull ($P = 0.8708$) regardless of whether the sample was taken from the right or left

Figure 1: Average weekly Cq values for *T. foetus* RT-qPCR testing for each of the 11 bulls across 8 weeks of sampling. The traditional *T. foetus* Cq cut-off of 35.0 is indicated by red lines, and samples with a Cq > 35.0, are indicated by red data points.

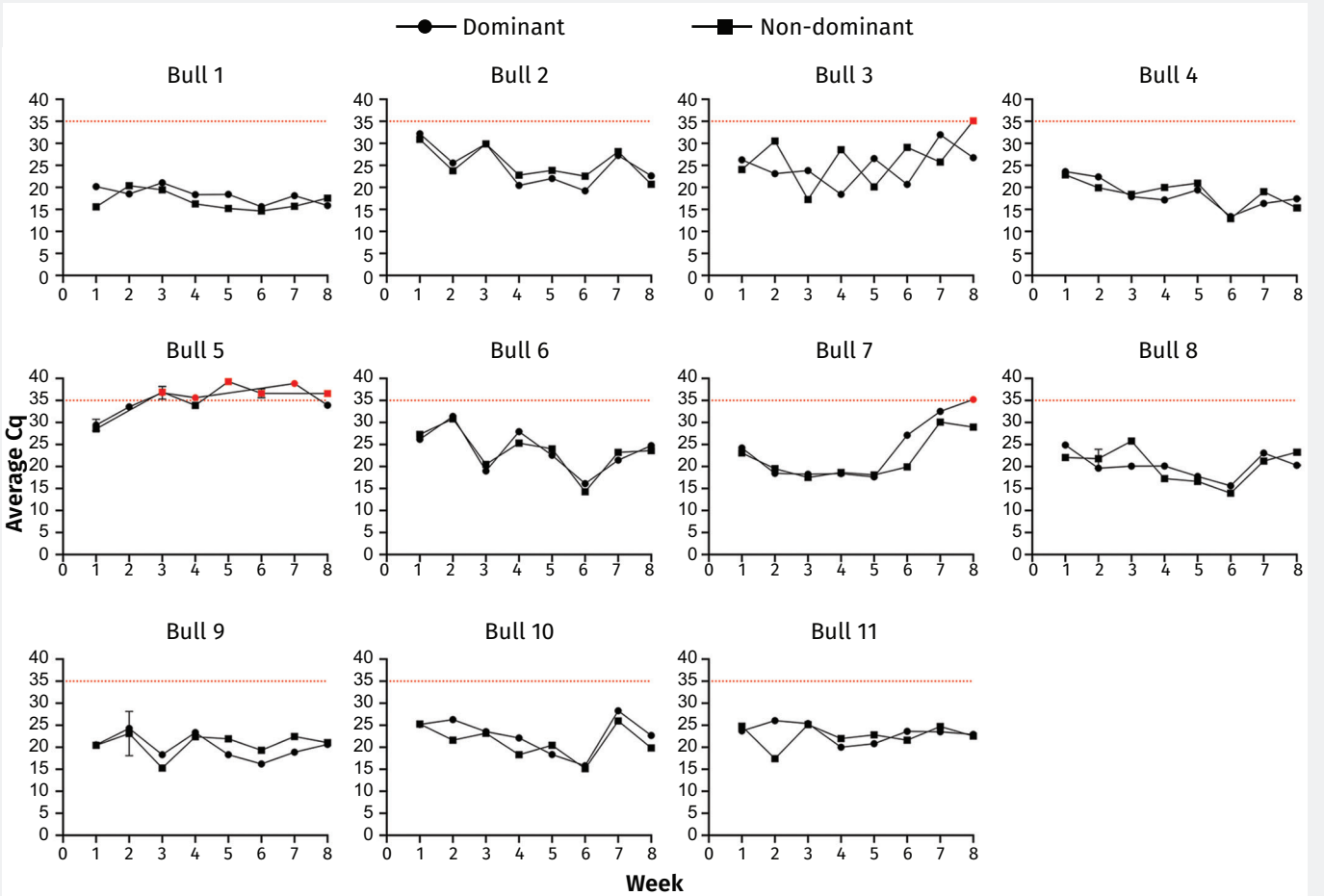

Table 1: Example sample scheduling for 2 of the 11 bulls showing the alternating of veterinarians and alternating initial side of the chute for testing of *T. foetus*: For example, for Bull A on Week 1 the LH- left-hand-dominant veterinarian, R → L the left-handed veterinarian would take T. foetus sample first from the right side of chute then the left side of the chute.

Bull	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Bull A	LH R → L	RH L → R	LH L → R	RH R → L	LH R → L	RH L → R	LH L→ R	RH R → L
Bull B	RH L → R	LH R → L	RH R → L	LH L → R	RH L → R	LH R → L	RH R → L	LH L → R

side of the chute and regardless of the veterinarian's handedness. Similarly, we found no significant difference in acquiring a positive sample across the eight-week study ($P = 0.0962$), also regardless of chute side or veterinarian's handedness.

Discussion

The collection of quality preputial samples is of paramount importance in correctly identifying *T. foetus*-positive bulls. Preanalytical considerations such as sampling location, sampling device, quality of sample recovered, and shipping considerations are all factors that should be considered when testing bulls. Previous data by Parker et al. suggested that differences observed in *T. foetus* test sensitivity were due to the side of the chute from which the sample was taken.[9] This was attributed to handedness and the ease of access to each side of the chute in their study. Our results show that neither handedness nor side of the chute impacted the sensitivity of *T. foetus* RT-qPCR. Furthermore, the sequence of side that the sample was taken from was also not a factor. There are multiple reasons that this difference between studies could have occurred including the differences in testing methodology, differences in collection devices, and variability environmental factors such as ease of access to both sides of the chute in our study.

The use of PCR for testing *T. foetus* samples was first described in 1997 and has since proved to be a significant improvement regarding improved specificity over traditional culture-based testing protocols.[10,12] The use of RT-qPCR has continued to offer a significant improvement in detecting positive samples as compared to previous culture-based PCR sampling due to the ability to use phosphate buffered saline instead of the traditional culture media used for *T. foetus* sampling. The need for a culture medium as indicated with PCR and qPCR posed significant impediments to accurate testing due to the overgrowth of smegma-derived bacteria. These bacteria can result in acidic pH and conditions that adversely affect the growth of *T. foetus*. The low pH supports *T. foetus* DNase activity that results in damage and leads to lack of or limited detection of *T. foetus* DNA.[13]

Another difference beside the use of laboratory tests between this study and Parker et al. was the collection device used. In the current study, the Pizzle Stick was used to obtain samples whereas an artificial insemination (AI) pipette was used in the previous study.[9] This device is a 24.5-inch long, hollow rod with a 2-inch sample collection area that is grooved around the diameter with a blunt end. This blunt end allows for minimization of trauma to prepuce and penis. This design is similar to one that was designed in the Soviet Union and described in 1969.[14] The original device was superior to the AI pipette for sample collection.[15,16] Previous work however found no difference in diagnostic sensitivity between the 2 sampling methods.[17]

Handedness is defined as the strong preference for using one hand over the other for manual tasks.[18] While handedness did not influence the ability to obtain a positive *T. foetus* sample in this study, ergonomic considerations should be considered by the veterinarian. Making the back-and-forth searching motion with the hand and arm when the dominant hand is used on the opposite side of the chute (e.g., collection of samples with the right hand on the left side of the chute) may put unnecessary strain on the individual's shoulder, elbow and wrist due to the movement necessary to make the searching motion required. Musculoskeletal discomfort of the upper extremities (including the neck, shoulder, upper back, arms, elbows, wrists and hands) has been demonstrated to occur in high prevalence among large animal veterinarians.[19-22] In a study by Zeng et al., breeding soundness evaluations were considered one of the top 3 most strenuous task categories as reported by bovine practitioners.[22] While *T. foetus* testing was not directly named in this survey, it is considered to be one component of routine breeding soundness evaluations by some veterinarians. *T. foetus* testing requires similar movements to those performed during the collection of semen that occurs during breeding soundness exams which allow for the reasoning that ergonomic strain would be of concern in such situations.

While handedness is usually associated with increased strength and dexterity, this may not always be the case and could potentially not be true, especially for large animal veterinarians.[18] Ambidextrousness of the veterinarians was not assessed in this study. However, many food animal veterinarians likely have some degree of ambidextrousness secondary to transrectal palpation skills. In a report by Reist et al., 58% of survey participants declared that they use their non-dominant hand for transrectal palpation.[23] Consequently, those individuals may have dexterity in both hands regardless of handedness which would negate the need to use one hand over the other to improve the chances of achieving a positive sample. A limitation of this study was the number of veterinarians that were included in the study as only 1 left-hand-dominant and 1 right-hand-dominant veterinarian was included in the study.

A salient clinical observation that has been noted by the authors along with other researchers is that bulls infected with *T. foetus* may not produce positive results when tested multiple times,[8] or as in the case of this project, Cq values are consistent across the individual animal, collector and collection method, but can be variable from week-to-week and animal-to-animal. One bull (Bull 5 – see Figure 1) was consistently at or above the traditional Cq cutoff value of 35, while other bulls had consistently lower Cq values. While undoubtedly positive due to repeated testing, this particular bull could possibly be considered negative by some diagnostic labs that use a Cq value of 35 as their diagnostic cutoff between positive and negative. Data from the current study indicates that similarly to

the findings of Ondrak et al., laboratory or test factors are not the likely driver of the phenomenon.[8] Repeated positive samples over 8 weeks from both sides of the animal from 2 different veterinarians negate the possibility of influences such as nonoptimal preputial samples being a repeatable concern in this clinical observation. While sample holding and transport issues cannot be directly ruled out due to the conditions of this study, they are likely not the entire reason for variations in Cq values or false-negative tests. Inconsistent *T. foetus* mucosal spatial distributions are also less likely due to the factors tested in this study. Further investigation of fluctuations in mucosal protozoal populations or differences in strains of *T. foetus* warrants further investigation.

In summary, the results of this study suggest that handedness and side of the chute used for collection have no impact on the ability to obtain a positive *T. foetus* sample when utilizing the Pizzle Stick and RT-qPCR.

Endnotes

[a] Pizzle Stick Trich Testing Device, Lane Manufacturing, Denver, CO

[b] Trichit™, Morris Livestock Products, Delavan, WI

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Conflict of interest

The authors have no competing interests to report.

Author Contributions

All authors on this manuscript contributed to conception of design; acquisition, analysis and interpretation of data; drafting and submission of the manuscript.

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