Collection of preputial material by scraping and aspiration for the diagnosis of *Tritrichomonas foetus* in bulls

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ABSTRACT

Two trials were done to assess the diagnostic sensitivity and practicality of preputial scraping as a method of collecting preputial material from bulls infected with *Tritrichomonas foetus*. In the first, preputial material was collected by simultaneous scraping and aspiration from three infected and one uninfected bull ten times over a five week period. In the second trial, samples from five infected bulls were collected by both sheath washing and scraping on six occasions, while eight uninfected animals were sampled three times. Samples were cultured using a modified Trichomonas culture medium (Oxoid).

In the first trial, 29 out of 30 samples from infected bulls were found to be positive. In the second trial, 83 per cent of samples collected by both methods tested positive. In neither trial were any samples from the control bulls found to be positive.

Scraping was found to be quick, safe, and offered the advantages over preputial washing that urine contamination was easily avoided, samples were smaller and more concentrated and contamination by environmental contaminants was reduced. It may be subject to greater operator variability than sheath washing.

It is concluded that preputial scraping is equal in sensitivity to washing and represents a suitable alternative to preputial washing for the collection of material for direct examination and culture of *Tritrichomonas foetus*.

**Key words:** *Tritrichomonas foetus*, venereal disease, bull, diagnosis, preputial scraping, preputial wash
INTRODUCTION

Tritrichomonosis is a major source of economic losses for the beef industry in South Africa. The disease is characterised by embryonal and foetal death, resulting in lowered calving percentages, prolonged intercalving periods, heifers failing to conceive, sporadic abortions, aberrant oestrous cycles, and the presence of post-coital pyometra in some animals. Prevalence of the disease for various regions in southern Africa range from 0 to 46 per cent of herds (Bloemfontein Veterinary Laboratory, pers. comm., 1999) (Louis Trichardt Veterinary Laboratory, pers. comm., 1999) (S M Pefanis, Vrede Veterinary Laboratory, pers. comm., 1999).

Despite awareness of the disease and proven control programmes based on well-researched epidemiological principles, the disease remains problematic. One factor contributing to this situation is the lack of a highly sensitive and specific test for carrier animals. Culture of preputial material from bulls is the most commonly-used test. While this technique yields sensitivities of almost 100 per cent in some hands, diagnostic rates in the 70 to 90 per cent range are more commonly reported. Factors contributing to reduced sensitivities include remoteness of farms, fractious animals, sample and animal identification errors, collection of large numbers of samples at once, sample contamination and overgrowth, and inconsistent laboratory techniques. It is therefore necessary to test a bull repeatedly to obtain a reliable result, a requirement which is not universally adhered to due to the associated cost and inconvenience.

Sheath washing and scraping are the two most widely utilised methods for the collection of preputial material. Material may also be collected by rinsing the liner of an artificial vagina after semen collection. Scraping is most commonly performed with a dry Perspex artificial insemination (AI) pipette attached to a rubber bulb or syringe, which enables aspiration of preputial smegma as the preputial lining is scraped. Custom-made instruments for the collection of preputial scrapings have not shown any advantage. Despite the fact that both sheath washing and scraping have been well described and do not differ in sensitivity, sheath washing remains the predominant technique used by veterinary practitioners and animal health technicians in South Africa.

The aim of this trial was to show that scraping and aspiration is a practical method of collecting preputial material for testing bulls for the presence of *Tritrichomonas foetus* infection, and that samples collected by this method achieve a high diagnostic sensitivity when subjected to culture.
MATERIALS AND METHODS

In Trial 1, three adult Bonsmara bulls, which were known to be *Trichomonas foetus* carriers and one uninfected two-year-old Jersey bull were used for the study. Preputial material was collected on ten occasions over a five-week period, with a mean interval of 3.2 days between collections. Collection was by means of scraping and simultaneous aspiration using a dry perspex AI pipette (AI pipettes, Kyron Laboratories) connected to a sterile disposable 20 ml hypodermic syringe by means of a silicon-rubber tube. For collection bulls were restrained in a sturdy crush with a neck clamp and by means of an assistant applying a tail-grip. Additional restraint consisted of tying one back leg or application of low level electrical stimulation delivered via an electro-ejaculator probe placed in the rectum. This was only necessary in a minority of collections where reaction of the bull was such that safety of the operator was deemed to be at risk.

The technique of collection was as follows: The collection apparatus was held in one hand by grasping the syringe. The tip of the pipette was guided into the caudal reaches of the preputial cavity and manipulated vigorously with an in-and-out movement while suction was applied with the syringe. The tip of the pipette was guided to different areas of the preputial membrane and glans penis using the other hand (Fig. 1). After an average of approximately twenty strokes the pipette was withdrawn and the contents inspected. If insufficient cloudy material was present the procedure was repeated for a longer period.

![Collection apparatus in the preputial cavity of a bull.](image)

**Figure 1.** Collection apparatus in the preputial cavity of a bull.
The material was then transferred to a plastic tube containing approximately 4ml of phosphate-buffered saline (PBS Dulbeco, Onderstepoort Biological Products). The material in the pipette and syringe was flushed into the PBS by aspirating the medium up into the pipette repeatedly (Fig. 2).

![Figure 2. Transferring the content of the pipette and syringe to a tube containing buffered saline.](image)

The collection apparatus and disposable latex gloves were changed for each bull.

Tubes were marked with a sample number only, placed in a polystyrene cool box with a frozen ice pack and transported to the Agricultural Research Council - Onderstepoort Veterinary Institute within two hours of collection. Laboratory staff were unaware of the origin of each sample. Samples were cultured in modified Oxoid's consisting of Trichomonas medium (CM161, Oxoid Limited), horse serum, distilled water and antibiotics. Cultures were examined by direct microscopy.
In Trial 2, five known positive bulls on two extensive commercial farms were sampled by sheath washing and scraping on six occasions over a period of 18 days. The washing was done first by instilling 50 ml PBS into the preputial cavity through a sterile latex tube, massaging the preputium vigorously for approximately 100 strokes, and then siphoning the fluid back into the sample bottle through the tube. This was followed by sheath scraping samples, which were collected as described in Trial 1. Eight uninfected bulls were sampled three or more times each. Four of the control bulls were on one of the farms described above, while four were housed semi-intensively in a university teaching animal unit. Samples were collected by one of two operators on each occasion. Samples were chilled and transported to the laboratory within 6 or 24 hours. Only a small fraction of each sample was utilised for culture, the remainder being used for other purposes.

The two-tailed Chi squared test was used to test for differences between the samples collected by the two sampling methods.

RESULTS

Besides some mild discomfort during collection no bull showed any adverse reaction to the repeated collection of material at short intervals.

In all cases sufficient opaque whitish or bloody, mucoid material could be obtained using scraping. Collection of the sample could generally be accomplished on the first attempt without additional restraint of the bull. Good samples could usually be obtained without the use of the free hand to guide the tip of the pipette, which enhanced operator safety. As the trial progressed the time required for the collection of sheath scrapings reduced.

In Trial 1, 29 of 30 samples collected from the infected bulls were found to be positive on laboratory examination giving a sensitivity of 0.96 with a 95 per cent confidence interval of 0.83 to 0.99. The one negative result was obtained on the eighth collection. None of the 10 samples from the uninfected control bull were positive.

The results of tests on infected bulls in Trial 2 are summarised in Table 1. Twenty four of 29 samples collected by both methods tested positive (0.83). There was agreement on 21 of the 29 samples. One bull was unavailable for testing on one occasion. No positive results were obtained from the control animals.
There was a significant difference in sensitivity between operators in the sheath scraping samples in Trial 2 (9/14 compared to 15/15, P<0.05).

**Table 1:** Culture results from five infected bulls collected by sheath washing and scraping on six successive occasions.

| Bull  | Sample Operator | Transport time | 1 | 2 | 3 | 4 | 5 | 6 |
|-------|-----------------|----------------|---|---|---|---|---|---|
|       |                 | 6 hours 24 hours | A | A | B | A | B | B |
| 9763  | Wash            | 6 hours 24 hours | - | - | + | - | + | - |
|       | Scrape          |                | + | + | + | + | + | - |
| 9924  | Wash            |                | + | + | + | + | + | + |
|       | Scrape          |                | + | + | - | + | + | + |
| 9968  | Wash            |                | - | + | n/a | + | + | + |
|       | Scrape          |                | + | + | n/a | + | + | - |
| 99001 | Wash            |                | + | + | + | + | + | + |
|       | Scrape          |                | + | + | + | + | + | + |
| BIG   | Wash            |                | + | + | + | + | + | + |
|       | Scrape          |                | + | + | + | + | + | + |

n/a: Not available for sampling

**DISCUSSION**

The collection of preputial material by scraping with simultaneous aspiration has several practical advantages over preputial washing. Speed of collection, the ability to collect the sample without an assistant, and the fact that contamination of the sample by urine is easily avoided are significant advantages. Although *Trichomonas foetus* organisms do survive in urine\(^{16}\), dilution of the cellular content of the sample is undesirable. The use of disposable collection apparatus eliminates the possibility of cross-contamination of samples or of the transmission of organisms between successive animals. Mechanical transmission of *Trichomonas foetus* is a potential hazard whenever infected animals are examined\(^{10}\). Special receptacles containing the larger volume of PBS do not have to be ordered beforehand, and the smaller volume of the sample obtained by scraping facilitates sample transport and laboratory processing. Lastly, as the sample is
collected primarily from the caudal reaches of the preputial cavity there is less likelihood of contamination from the environment, particularly in bulls which have gross contamination of the cranial portion of the preputial cavity caused by habitual eversion of the lamina interna. This is in agreement with findings of other workers sampling for *Campylobacter fetus*.

The presence of blood in the sample did not constitute a problem, which confirms the findings of other authors. Roughening the tip of the pipette, as is commonly advocated, was found to be unnecessary to obtain a satisfactory sample. The fact that scraping was done after washing on all occasions may have biased the results in Trial 2 by reducing the number of organisms in the preputial cavity.

The high diagnostic sensitivity attained in Trial 1 is ascribed to animal factors, sampling technique, proximity to the laboratory facilitating rapid delivery of samples, optimal handling facilities, experienced staff and the small number of bulls sampled on any one occasion.

The lower diagnostic sensitivity attained in Trial 2 for both methods is ascribed to the less optimal collection conditions and to the fact that only a small volume of each sample was available for direct examination and culture. While some authors have found a decreased sensitivity with a 24 hour delay in processing of preputial samples, we could not demonstrate any effect.

There was a tendency for more false negative tests towards the end of the sampling period in Trial 2 but not in Trial 1. This phenomenon has been seen by other authors, who ascribed it to an increase in bacterial contamination in the sheath after repeated sampling. It is known that more false negative cultures are obtained from bulls during periods of active breeding, presumably due to a reduction in numbers of organisms in the preputial cavity. A similar reduction in the number of organisms due to frequent sheath washing is one plausible explanation for our observation. Alternative explanations include the increased exposure of organisms to blood containing antibodies and other serum factors by virtue of repeated scrapings, and an increase in bacterial contamination of the preputial cavity.

The difference between operators in the sensitivity obtained on sheath scraping suggests that this technique is more prone to operator variability than sheath washing, although larger sample sizes may have demonstrated differences in the latter method as well. This warrants further investigation. If this is the case, thorough training of operators would be a necessity to attain consistent diagnostic accuracy.
Whether sheath scraping or washing are equally suited to the collection of samples for molecular diagnostic techniques requires further work.

It is concluded that preputial scraping is a suitable alternative to preputial washing for the collection of material for culture of *Tritrichomonas foetus*, offering several important practical advantages over that method. Routine use of this technique by competent operators can be expected to render at least equal diagnostic rates to preputial washing.

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