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

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ABSTRACT

Two trials were carried out to assess the diagnostic sensitivity and practicability of preputial scraping as a method of collecting preputial material from bulls infected with *Tritrichomonas foetus*. In the 1st trial, preputial material was collected by simultaneous scraping and aspiration from 3 infected and 1 uninfected bull 10 times over a 5-week period. In the 2nd trial, samples from 5 infected bulls were collected by both sheath washing and scraping on 6 occasions, while 8 uninfected animals were sampled 3 times. Samples were cultured using a modified Trichomonas culture medium (Oxoid). In the first trial, 29 of 30 samples from infected bulls were found to be positive. In the second trial, 83 % 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 and safe, and offered advantages over preputial washing in that urine contamination was easily avoided, samples were smaller and more concentrated and contamination was reduced. It may, however, be subject to greater operator variability than sheath washing. It is concluded that preputial scraping is as effective as washing and represents a suitable alternative for the collection of material for direct examination and culture of *Tritrichomonas foetus*.

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

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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 oestrus cycles, and the presence of post-coital pyometra in some animals. Prevalence of the disease in herds in various regions of southern Africa range from 0 to 46 %[6,13,17,22] (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 % in some instances, diagnostic rates in the 70–90 % range are more commonly reported[1,3,5,9,12,14,18–21,23,25,27,28]. Factors contributing to reduced sensitivity include remoteness of farms, fractious animals, sample and animal identification errors, collection of large numbers of samples at the same time, sample contamination and overgrowth, and inconsistent laboratory techniques[7,20]. It is therefore necessary to test a bull repeatedly to obtain a reliable result, a requirement which is not universally adhered to due to cost and inconvenience.

Sheath washing and scraping are the 2 most widely used methods for the collection of preputial material. Material may also be collected by rinsing the liner of an artificial vagina after semen collection[11]. 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 to have any advantage[15]. Although both sheath washing and sheath scraping have been well described[2,3,24,27] and do not differ in effectiveness[23], 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 high diagnostic sensitivity when subjected to culture.

MATERIALS AND METHODS

In Trial 1, 3 adult Bonsmara bulls, which were known to be *Tritrichomonas foetus* carriers, and 1 uninfected 2-year-old Jersey bull were included. Preputial material was collected on 10 occasions over a 5-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 with a silicon-rubber tube. For collection, bulls were restrained in a sturdy crush with a neck clamp while an assistant applied a tail-grip. Additional restraint consisted of tying one back leg or the application of low-level electrical stimulation delivered by an electroejaculator probe placed in the rectum. This was only necessary in a few instances when the reaction of the bull placed the operator 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

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Fig. 1: **Collection apparatus in the preputial cavity of a bull.**

the pipette was withdrawn and the contents inspected. If insufficient cloudy material was present the procedure was repeated for a longer period.

The material was then transferred to a plastic tube containing approximately 4 mℓ phosphate-buffered saline (PBS Dulbeco, Onderstepoort Biological Products) (Fig. 2). The material in the pipette and syringe was flushed into the PBS by repeatedly aspirating the medium into the pipette. New collection equipment and disposable latex gloves were used 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 ARC-Onderstepoort Veterinary Institute within 2 hours of collection. Laboratory staff were unaware of the origin of each sample. Samples were cultured in a medium consisting of Trichomonas medium (CM161, Oxoid Limited), horse serum, distilled water and antibiotics. Cultures were examined by direct microscopy.

In Trial 2, 5 positive bulls on 2 large commercial farms were sampled by sheath washing and scraping on 6 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

Fig. 2: **Transferring the contents of the pipette and syringe to a tube containing phosphate-buffered saline.**

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 3 or more times each. Four of the control bulls were on one of the farms mentioned above, while 4 were housed semi-intensively in the Faculty of Veterinary Science's teaching animal unit. Samples were collected by 1 of 2 operators on each occasion. Samples were chilled and transported to the laboratory within 6 or 24 hours. Only a small portion of each sample was used for culturing and the remainder for molecular diagnostic techniques.

A two-tailed Chi-square test was used to test for differences between the samples collected using the 2 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 1st attempt without additional restraint of the bull. Adequate 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 % confidence interval of 0.83– 0.99. The 1 negative result was obtained on the 8th collection. None of the 10 samples from the uninfected control bull was positive.

The results of tests on infected bulls in Trial 2 are summarised in Table 1. Twenty-four of the 29 samples collected by both methods tested positive (0.83). Twenty-one of the 29 samples were in agreement. One bull was unavailable for testing on one occasion. No positive results were obtained for 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, $P < 0.05$).

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 *T. foetus* organisms do survive in urine[16], dilution of the cellular content of the sample is undesirable. The use of disposable collection equipment eliminates the possibility of cross-contamination of samples or of the transmission of

Table 1: **Culture results for 5 infected bulls collected by sheath washing and sheath scraping on 6 successive occasions.**

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

n/a: not available for sampling.

organisms between successive animals. Mechanical transmission of *T. foetus* is a potential hazard whenever infected animals are examined[10]. Special receptacles for the larger volume of PBS need not 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 that have gross contamination of the anterior 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*[26].

The presence of blood in the sample did not constitute a problem, which confirms the findings of other authors[2]. Roughening the tip of the pipette, as is commonly advocated, is not necessary 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 sampling technique, close 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 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 decreased sensitivity with a 24 h delay in processing of preputial samples[15,25], 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 has also been observed by other authors, who ascribed it to an increase in bacterial contamination in the sheath after repeated sampling[8]. 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[4]. 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 by 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 necessary to attain consistent diagnostic accuracy.

Whether sheath scraping or washing are equally suited to the collection of samples for molecular diagnostic techniques requires further investigation.

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 the latter method. Routine use of this technique by competent operators is expected to render at least diagnostic rates equal to preputial washing.

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