COMPARISON OF THREE SAMPLING METHODS FOR THE DIAGNOSIS OF GENITAL VIBRIOSIS IN THE BULL*

L. F. TEDESCO, F. ERRICO AND L. P. DEL BAGLIVI

“Miguel C. Rubino” Veterinary Research Centre, Castilla de Correo 177, Montevideo, Uruguay

SUMMARY: Three different methods of collecting preputial material for bacteriological examination were compared using 3 bulls infected with Campylobacter fetus subsp. fetus. The first method utilised a specially designed instrument to scrape the preputial and penile mucosa, in the second method plastic pipettes were used to aspirate material and the third method involved washing the preputial cavity with sterile peptone water. Bacteriological examination of the samples showed conclusively that scraping was the method of choice because more C. fetus positive samples were identified and there was less interference from contaminating organisms.

Introduction

Many epidemiological studies have shown the important role played by the bull in the transmission of bovine venereal vibriosis (Schutte 1969; Clark 1971) but it was not until the development of selective media for the isolation of Campylobacter fetus subsp. fetus (Smibert 1974) from preputial fluids that the bull became the animal of choice for the herd diagnosis of the disease (Florent 1953; Plastridge and Koths 1961). Workers have had varying success using selective media to improve the isolation rates from infected bulls (Plumer et al 1962; Shepler et al 1963; Winter et al 1965; Dufty and McEntee 1969) and different techniques have also been used to collect samples (Hughes 1956; Murnane et al 1959; Dufty and McEntee 1969; Sutka and Katai 1969; Gibson et al 1970). Since the first diagnosis of bovine venereal vibriosis in Uruguay, preputial washing has been routinely used.

The purpose of the work now reported was to compare the efficiency of 3 methods for the collection of preputial material for diagnosis in the bull.

Materials and Methods

Three Holstein bulls, known to be infected with C. fetus subsp. fetus, were not permitted natural service. Preputial samples were collected by 1 or other of 3 methods, decided at random, up to 28 times over a period of 30 weeks usually at weekly intervals. The preputial orifice was cleaned with a cloth well moistened with 1/1000 p-choro-m-xylene and guided to the anterior and dorsal aspect of the penis. The preputial and penile mucosa was scraped with 15 to 20 backward and forward movements, the instrument withdrawn and the scraping surface washed in 8 ml sterile peptone water in a test tube. The process was repeated a second time using the same instrument and again washed in the original 8 ml peptone water. The instrument was sterilised by submerging in boiling water for 5 minutes and cooled in sterile peptone water between collections from each bull.

Scraping Method

A scraping instrument was made following the work of Sutka and Katai (1969). It consisted of a metal handle 70 cm long and 3 mm diameter on to which was soldered a solid metal cylinder 13 cm long and 8 mm diameter. The cylinder was engraved with 31 circular grooves to permit a scraping action (Figure 1). The instrument was introduced into the prepuce and guided to the anterior and dorsal aspect of the penis. The preputial and penile mucosa was scraped with

Aspiration Method

A sterile flexible plastic tube 60 to 70 cm long with external and internal diameters of 10 mm and 8 mm respectively and bevelled at one end was used. The bevelled end was introduced into the preputial sac and the external end connected to a finer flexible tube of convenient length, usually 45 cm, fitted with a glass tube mouth-piece plugged with cotton wool. Material was aspirated into the first tube by the scraping action and mouth suction of the operator. Collected material was flushed into a sterile container with 8 ml of sterile peptone water. A fresh sterile plastic tube was used to collect each sample.

Preputial Washing

Twenty-five ml of sterile peptone water was introduced into the preputial cavity using a sterile 200 ml metal syringe connected to a rubber tube 60 cm long with bore of 10 mm. The preputial orifice was closed by hand pressure and the prepuce vigorously massaged for 1 minute. The fluid was then withdrawn and transferred to a sterile container. The equipment was sterilised by immersion in boiling water for 5 minutes between collection from different bulls.

All samples were placed immediately after collection in an insulated box containing refrigerant capable of maintaining a temperature of 4° to 8°C and processed in the laboratory within 60 minutes.

Laboratory Procedure

Samples were centrifuged at 2000g for 10 minutes. Approximately 0.1 ml of the top layer of the supernatant fluid was seeded on to each of 4 plates of the

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selective medium "B" described by Dufty and McEntee (1969) and incubated in an atmosphere of 10% CO₂ at 37°C. Plates were examined on the seventh day and the number of C. fetus colonies noted. Representative colonies from each plate were identified on the basis of characteristic morphology, production of catalase and failure to produce H₂S. The degree of contamination was recorded as high, medium, low or nil according to the recommendations of Dufty (1967). Plates with low or nil contamination were recorded as "low" to simplify statistical examination.

Results

No difficulties were encountered in collecting samples by any of the 3 methods. The bevelled plastic tube caused slight bleeding on some occasions but no blood was ever seen in samples collected by the scraping method. The number of samples from which C. fetus was isolated on 1 or more of the 4 plates using the 3 methods of collecting samples is recorded in Table 1. There was a significant difference (p < 0.001) in favour of the scraping technique when compared with the remaining 2 methods. There was no statistical difference in isolation rates between the washing and aspiration methods.

The degree and frequency of contamination encountered is recorded in Table 2. The scraping method produced a higher proportion of plates with low contamination than the other 2 methods (p < 0.001). There was also a significant difference between the aspiration and washing methods in the proportions of plates with low contamination (p < 0.01). The relationship between the average number of countable C. fetus colonies per plate and the degree of contamination is shown for each sampling method in Table 3.

Discussion

It has long been recognized that bovine vibriosis is a true venereal disease and that infected bulls are a key factor in diagnosis and control (Dufty 1967; Schutte 1969). Although Dufty and McEntee (1969) mention that collection of preputial samples with a plastic pipette is superior to preputial washing for bacterial examination our results show that there was no statistical difference in the 2 methods. Our results do show however that the number of contaminating colonies was reduced and the number of identifiable C. fetus colonies enhanced when samples were collected by the aspiration rather than by the washing method. Nevertheless it is clearly demonstrated that the new method of scraping the preputial and penile mucosa with the instrument evolved by Sutka and Katai (1969) for their work on Trichomonas foetus is superior to both the aspiration and washing method.

**Figure 1.** General features of the entire instrument (left) and enlargement of metal cylinder showing details of the scraping surface (centre) and shadow image of grooves (right).

**Table 1**

Recovery of Campylobacter fetus Using 3 Sampling Methods

<table>
<thead>
<tr>
<th>Sampling Method</th>
<th>Samples Positive</th>
<th>Samples Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scraping</td>
<td>20</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td>Aspiration tube</td>
<td>9</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Washing</td>
<td>8</td>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37</strong></td>
<td><strong>47</strong></td>
<td><strong>84</strong></td>
</tr>
</tbody>
</table>

**Table 2**

Degree and Frequency of Contamination Using 3 Sampling Methods

<table>
<thead>
<tr>
<th>Sampling Method</th>
<th>Degree of Contamination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Scraping</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>Aspiration tube</td>
<td>63</td>
<td>14</td>
</tr>
<tr>
<td>Washing</td>
<td>104</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>205</strong></td>
<td><strong>36</strong></td>
</tr>
</tbody>
</table>
TABLE 3
Relationship Between the Average of Number of Campylobacter fetus Colonies per Plate and Contamination

<table>
<thead>
<tr>
<th>Sampling Method</th>
<th>Average Number of Colonies per Plate</th>
<th>Contamination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapping</td>
<td>189</td>
<td>High 34 Medium 11 Low 55</td>
</tr>
<tr>
<td>aspiration tube</td>
<td>92</td>
<td>High 63 Medium 14 Low 23</td>
</tr>
<tr>
<td>washing</td>
<td>59</td>
<td>High 84 Medium 8 Low 8</td>
</tr>
</tbody>
</table>

methods previously used. Not only was the scraping method superior in relation to isolation rate, C. fetus being isolated from nearly 3 of every 4 samples, but the number of contaminants was markedly reduced facilitating examination of the culture plates.

The scraping method has been used on over 500 occasions with numerous bulls without any discomfort or secondary effect and is practical and simple to perform. It can be recommended to replace both the aspiration and washing methods, especially in view of Sutka and Katai’s (1969) statement that it was an efficient method for collecting samples for the diagnosis of T. foetus infection.

As both trichomoniasis and vibriosis are often found concurrently in areas where bovine veneral infertility exists, trials are under way to confirm the utility of the scraping method in the diagnosis of T. foetus infection.

Acknowledgments
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References

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BOOK REVIEW

THAT WE MAY EAT

The Yearbook of Agriculture published by the United States Department of Agriculture is always a valuable document; the Volume for 1975* is no exception to this rule and presents an historical resumé of the first 100 years’ work of the State Agricultural Experiment Stations. The influence of these Stations and the associated State Colleges of Agriculture on plant and animal production in the United States of America can only be described as profound; but this influence has spread to many other countries — many Australian agriculturalists and veterinarians have been privileged to study in these institutions and have benefited from their philosophies.

This book presents some of the highlights of research and its application to the major crops and farm animals; it is a veritable mine of information on the historical developments but, regrettably, is virtually devoid of references to original sources. Specialist chapters are devoted to beef cattle, chickens, pigs, dairy cows and fish; the Australian reader will note that the sheep does not merit attention! Contributions to the nutrition of animals and man are also prominent.

Although, naturally, the emphasis is on success stories, there are penetrating comments on problems associated with scientific and technological developments — for example, the epidemic of a fungal disease in the corn crops in 1970, and the potential dangers of excessive use of antibiotics and agricultural chemicals. Nor is there any complacency; the book ends with the statement: “We think that teams of agricultural scientists and farmers have done quite well, thank you. Our people are not only fed, but fed well with the world’s most plentiful supply of nutritious, healthful food for the smallest part of their incomes anywhere in the world. But that’s in the past. The tougher job lies ahead.”

I. W. McDonald


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