Campylobacter fetus in Artificial Insemination Unit and Slaughterhouse Bulls in Ontario

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ABSTRACT

Preputial fluid samples were collected from 90 bulls in two Ontario artificial insemination units using a penial glove swab technique previously developed by one of us for use in donor bulls. No *Campylobacter fetus* organisms were identified from the prepuce or from samples of semen collected at the same time from these bulls. The distal genitalia of 200 bulls were collected at a slaughter house. One isolation of a *Campylobacter fetus* subspecies *venerealis* was obtained on a culture from the fornix area of the prepuce of one of these bulls.

Key words: *Campylobacter fetus*, swab technique, prepuce, culture, fluorescent antibody, artificial insemination unit.

RÉSUMÉ

Cette expérience visait à récolter des échantillons de liquide préputial, chez 90 taureaux, dans deux centres d'insémination artificielle de l'Ontario, à l'aide de la technique du gant à écouvillonnage pénien, préalablement mise au point par l'un des auteurs. La culture de ces écouvillons et d'échantillons simultanés du sperme des mêmes taureaux ne se solda pas par l'isolement de *Campylobacter fetus*. Le deuxième volet de l'expérience consistait à recueillir les organes génitaux distaux de 200 taureaux, à l'abattoir, et à y rechercher le même microorganisme. Les auteurs réussirent à isoler *C. fetus*, sous-espèce *venerealis*, du cul-de-sac du prépuce d'un de ces taureaux.

Mots clés: *Campylobacter fetus*, technique d'écouvillonnage, prépuce, culture, anticorps fluorescents, centre d'insémination artificielle.

Since 1918, *Vibrio*-like organisms have been isolated from bovine fetuses and reproductive tracts in herds experiencing temporary infertility and early embryonic deaths (1,2). *Campylobacter fetus* infection in both beef and dairy cattle has been shown to cause infertility due to fetal death with abortions occasionally detectable (1,2,3).

The prepuce of the bull serves as a natural reservoir for *C. fetus* (3). Bulls, especially those over four years of age, appear to become chronic carriers (1,3).

Improvements in management practices, such as a reduction in bull sharing, increased use of artificial insemination and effective vaccination and antibiotic therapy programs have reduced the prevalence of infection in some countries (3). However, *C. fetus* has been responsible for serious reproductive problems in several countries (2,4,5).

Campylobacter fetus has been isolated from the prepuce of bulls in Canadian artificial insemination (AI) units in recent years. The infection has been eliminated by use of a vaccine and an antibiotic (6).

This paper describes an attempt to isolate *C. fetus* from samples collected from bulls in two AI units and from bulls at an Ontario slaughter house. Transport enrichment medium (TEM) (6) was utilized to enhance recovery of the organisms. Both culture and fluorescent antibody techniques were used as diagnostic tests (7).

Preputial fluid samples were obtained from 45 bulls in each of two AI units (Units A and B). In unit A, thirty-eight dairy and seven beef bulls ranging in age from three to eleven years were sampled. They had been vaccinated subcutaneously in July 1980 with 2 mL of vibrin (Norden Laboratories, Lincoln, Nebraska) and injected with 22 mg dihydrostreptomycin per kg body weight eleven months previous to the 1982 collection. In unit B, thirty-six dairy and nine beef bulls ranging in age from four to ten years were sampled in August 1982. They had been injected with 22 mg per kg dihydrostreptomycin in October 1981 and vaccinated with 2 mL of vibrin in April 1982.

The penial glove swab (PGS) method of sample collection from AI bulls was selected because it precludes the necessity of restraining the bulls and does not interfere with semen quality and quantity in the regular collection program. In addition, a greater area of preputial and penal mucosa can be sampled. Our experience (Stovell — unpublished comparative study) has demonstrated that this collection method is effective.

In preparation for sample collection, a 10 cm x 8 cm section of J cloth club towel (Johnson & Johnson Ltd., Montreal, Toronto, Vancouver, Canada) was attached to a sterile surgical glove by means of double-sided masking tape placed across the palm and around the glove

Upon false mount, the penis was directed toward the collector with one hand. The erect penis was then grasped by the other hand in a manner to contact the most mucosal surface possible with the swab. Considerable pressure was exerted while moving the swab

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Submitted February 8, 1984.

towards the glans of the penis. By covering a large mucosal surface, we collected most of the cellular debris and preputial fluids. When the glove was removed, the swab was inverted inside it for protection.

The swab was removed from the glove within a few minutes of collection and placed in the barrel of a 10 mL disposable syringe. Four mL of supplemented saline (SS) (1 L distilled water, 8.5 g NaCl, 2.9 g Albimi Brucella broth medium) was drawn into the syringe from a screw capped vial, and after flushing through the swab, the SS was expressed back into the vial. One mL of this swab extract was injected into each of two TEM vials.

Immediately following preputial sample collection, semen was collected from each bull using an artificial vagina. One mL of this semen was placed in each of two TEM vials.

The TEM vials were transported to the laboratory at ambient temperature and placed in a 37°C incubator. The balance of the swab extract and semen samples was carried to the laboratory in a refrigerated box (4°C) within three hours of collection. Using a cotton tipped applicator, an aliquot of each was plated directly on cystine heart agar (CH) and cystine heart agar with antibiotics (CHAB). These plates were incubated at 32°C for six days in a microaerophilic atmosphere of 4% O_2, 10% CO_2 and 86% N_2 and examined at four and six days postinoculation. In addition, a drop of the swab extract was examined by darkfield microscopy (400x) to evaluate bacterial motility and morphology.

Following four days incubation, samples from each TEM vial were plated on CH and CHAB, incubated for six days at 32°C and observed at four and six days.

A suspension was prepared for direct fluorescent antibody (FA) examination by sweeping a loop across the surface of the CHAB plate culture or in the case of no growth, the initial inoculation site and mixing with 2 mL of distilled water. One drop of this suspension was placed on a microscope slide, air dried and fixed with absolute ethyl alcohol. *Campylobacter fetus* fluorescein isothiocyanate (FITC) conjugate was used to stain the slide.

During the period June to December 1982, the prepuce and penis of 200 mature bulls were obtained at an Ontario slaughter house. The breeding history of these bulls, 60% beef and 40% dairy, was unknown. The mucosa of the prepuce and penis in the area of the fornix and toward the glans was scraped with a scalpel blade and the scrapings (approximately 1 g) were washed into 4 mL SS. To filter, this suspension was poured into the barrel of a 10 mL disposable syringe containing a pledget of glass wool and expressed through the glass wool into a sterile vial. One mL of the filtrate was injected into each of two TEM vials. Procedures used in the transportation, incubation, culture and FA examination of these TEM and remaining filtrate samples were identical with the samples from the AI bulls.

No *C. fetus* organisms were isolated from the perputial samples or semen of the AI unit bulls, and there was no evidence of *C. fetus* on FA examination of these samples. *Campylobacter fetus* subsp. *venerealis* was isolated on one CHAB culture plate from a TEM vial from a slaughter house Hereford bull. Fluorescence indicative of *C. fetus* was observed on routinely prepared slides from this plate. No evidence of *C. fetus* was noted on direct plates from the penial scrapings of this bull.

The PGS method of sample collection in AI units is well accepted by AI unit personnel because stressful restraint of the bull is avoided and very little interference with the regular semen production program of the bull is experienced. The area of mucosa swabbed is much greater than during pipet scraping and the application of pressure with the swab should result in the recovery of more cellular debris and preputial fluids than by preputial washing. It was noted, however, that in grasping the penis close to the preputial orifice, the opportunity for contamination of the sample by foreign matter was increased.

Contamination in both preputial and semen samples posed a problem on some isolation culture plates which neither antibiotics nor filtering eliminated entirely. Regardless of contamination, the plates were examined by FA.

Our failure to demonstrate *C. fetus* in bulls in AI units may be taken as evidence of the success of their programs of vaccination and prophylactic antibiotic treatment.

The isolation of *C. fetus* from one slaughter house bull indicates that the organism is present and, therefore, good breeding practices must be maintained.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. Kris Rahn whose technical expertise and dedication allowed this project to be completed. The cooperation of the AI industry and J.M. Schneider Co. Ltd., Kitchener, Ontario is appreciated.

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