

BACTERIOLOGICAL STUDY OF SEMEN FROM INTENSIVELY HOUSED BULLS

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SUMMARY

Three hundred and forty-nine first ejaculates of semen collected from 56 bulls, using an artificial vagina, were cultured to determine the relative incidence of bacteria present. Micrococci, *Proteus* spp., coliforms, haemophilic Gram-negative bacilli, *Bacillus* spp. and *Corynebacterium* spp. were the bacteria most frequently isolated. These organisms were repeatedly isolated in the semen of four bulls sampled routinely over 4 years and were considered to constitute the normal bacterial flora in semen. Alpha-haemolytic streptococci were frequently isolated from one of the four bulls sampled for this period but were infrequently found in other samples.

Organisms less frequently encountered were *Klebsiella* spp., *Pseudomonas* spp., unidentified Gram-negative bacilli, *C. pyogenes*, *Staphylococcus aureus*, non-haemolytic streptococci and *Serratia* spp. These organisms occurred only sporadically and were considered as abnormal flora in the semen.

I. INTRODUCTION

Spallanzani (1785) was probably the first to report the presence of a varied bacterial flora in bovine semen. Most of the early information on the bacteriology of bovine semen has resulted from sterility investigations in bulls. Gilman (1920), Williams and Kingsbury (1920) and Webster (1932) studied bacteria in semen recovered from cows' vaginas immediately after service. Hatziolos (1937), Gunsalus, Salisbury, and Willett (1941), a 1942 report of the United States Department of Agriculture cited by Almquist, Prince and Reid (1949) and Prince, Almquist, and Reid (1949) gave results of bacteriological examination of semen collected by the artificial vagina. The variable nature of the bacterial flora of bovine semen is evident from these reports and it is considered likely that this was because the studies were conducted in different geographical areas on different bull populations. As no reports of the bacteria

in bovine semen have been published in Australia, the results of routine examination of samples from bulls housed under intensive conditions were compiled to determine the types of bacteria present and their relative frequency.

II. MATERIALS AND METHODS

The bulls were held at the Department of Primary Industries Animal Husbandry Research Farm at Rocklea, Brisbane, tethered in individual stalls in a covered building with a concrete floor. Wood shavings used for bedding were changed completely every morning and any fouled with excreta every afternoon. The breeds represented were Jersey, Friesian and Australian Illawarra Shorthorn. All bulls were negative to serological tests for brucellosis, *Leptospira pomona*, *L. hyos* and bovine contagious pleuropneumonia and to the intradermal test for tuberculosis.

Over 4 years, 349 semen samples were examined from 56 animals. Bulls were introduced at 4–6 months of age and sampling commenced at about 18 months. As bulls were purchased annually, the number of individual bull samples tested varied. The bull sample distribution is shown in Table 1. Semen was collected using an artificial vagina. A clean artificial vagina with a sterile glass collection tube was used for each bull to avoid cross contamination of samples. Liners and cones were washed with soap and water, rinsed, then boiled in distilled water for 15 min prior to each collection.

TABLE 1
BULL SAMPLE DISTRIBUTION

No. of Samples	No. of Bulls	No. of Samples	No. of Bulls	No. of Samples	No. of Bulls
1	7	7	4	16	1
2	7	8	4	17	1
3	5	9	4	18	1
4	8	10	1	19	1
5	4	11	1		
6	3	14	4		

First ejaculates were cultured at intervals of approximately 3 months. A loopful of the semen was plated onto 10% sheep's blood agar plates, which were incubated at least 3 days in 10% carbon dioxide—90% air atmosphere at 37°C. Media for the isolation of *Vibrio fetus* and *Tritrichomonas foetus* were also inoculated with semen, but as no organisms of these two types were isolated, they are not included in the results. Colonial morphology and Gram stains were used to indicate the type of organism present. Further characterization was done as follows:

Pseudomonas spp. and *Serratia* spp.: pigment production. Coliforms: Gram-negative rods resembling *Escherichia coli* in colonial and staining reactions

and producing acid from lactose. *C. pyogenes*: liquefaction of Loeffler's slopes, acid from glucose, lactose, sucrose and maltose, no acid from mannitol, urease and H_2S negative. *Corynebacterium* spp.: coryneform morphology but not having the characteristics of *C. pyogenes*, *C. renale* or *C. bovis*. *Staph. aureus*: haemolysis. *Klebsiella* spp.: indole and methyl red negative, Voges-Proskauer positive, acid and gas production from glucose, non-motile. *Proteus* spp.: urease test positive within 24 hr. Haemophilic Gram-negative bacilli: similar to organisms described by Crawley, Wills, and Macgregor (1950).

III. RESULTS

A variety of bacteria was cultured from semen samples and the relative incidence of various organisms in ejaculates is shown in Figure 1. In 57 samples, representing 13.5% of first ejaculates collected, no bacteria were isolated. The bacteria most frequently isolated were *Proteus* spp. (28.1%), micrococci (25.8%), coliforms (19.2%), haemophilic Gram-negative bacilli (18.3%), *Bacillus* spp.

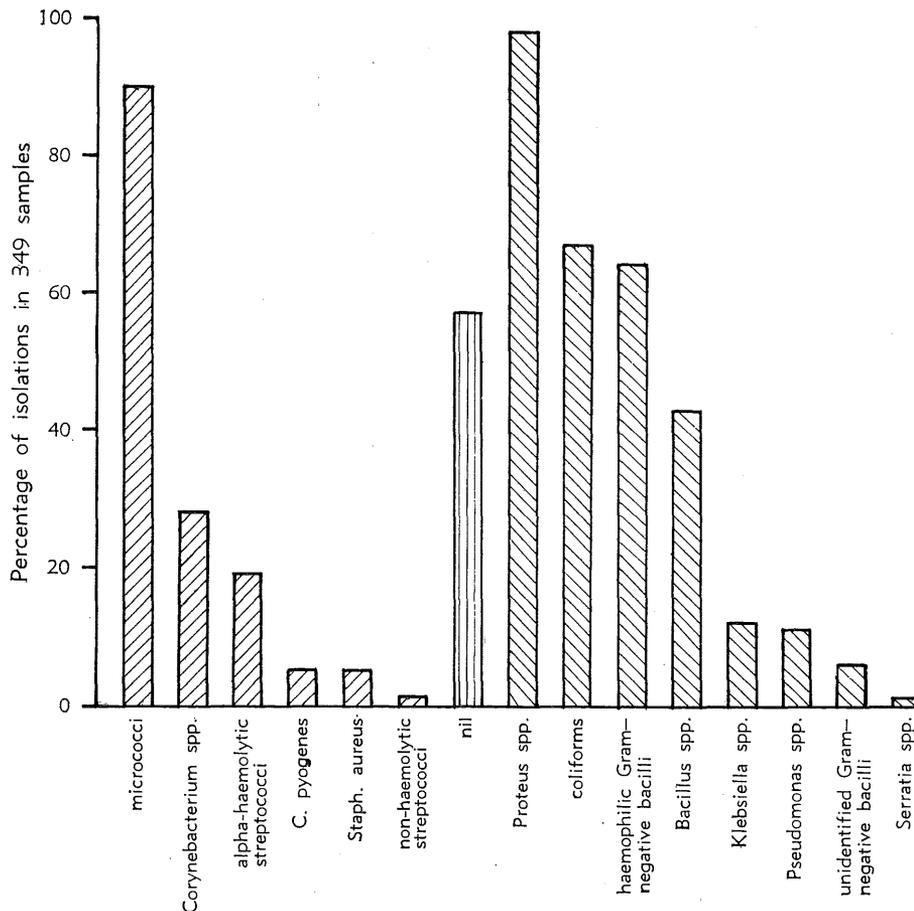


Fig. 1.—Organism incidence in semen samples.

(12.3%), *Corynebacterium* spp. (9.0%) and alpha-haemolytic streptococci (6.0%). Other organisms isolated were *Klebsiella* spp. (3.4%), *Pseudomonas* spp. (3.1%), unidentified Gram-negative bacilli (1.7%), *C. pyogenes* (1.4%), *Staph. aureus* (1.4%), non-haemolytic streptococci (0.3%) and *Serratia* spp. (0.3%).

The bull/organism incidence is shown in Figure 2. Bacteria were cultured from one or more first ejaculates from all of the 56 bulls sampled. No one organism was found to be common to the semen of all bulls. Bacteria isolated from the semen of a large percentage of the bulls sampled were micrococci

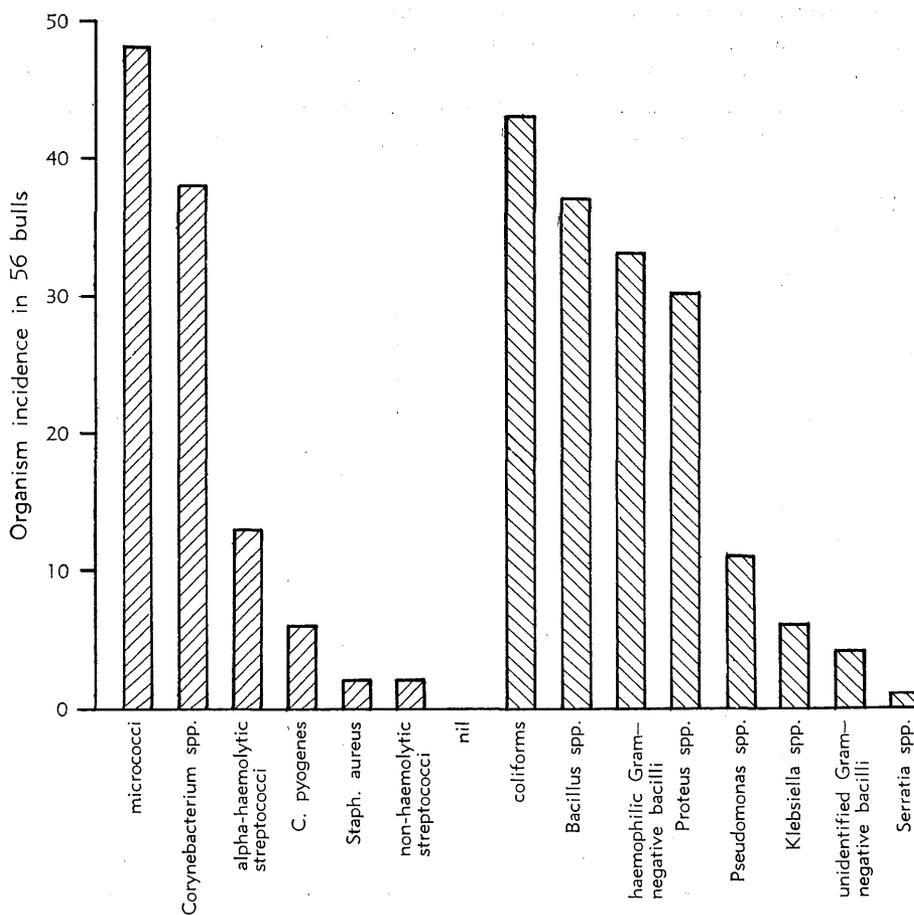
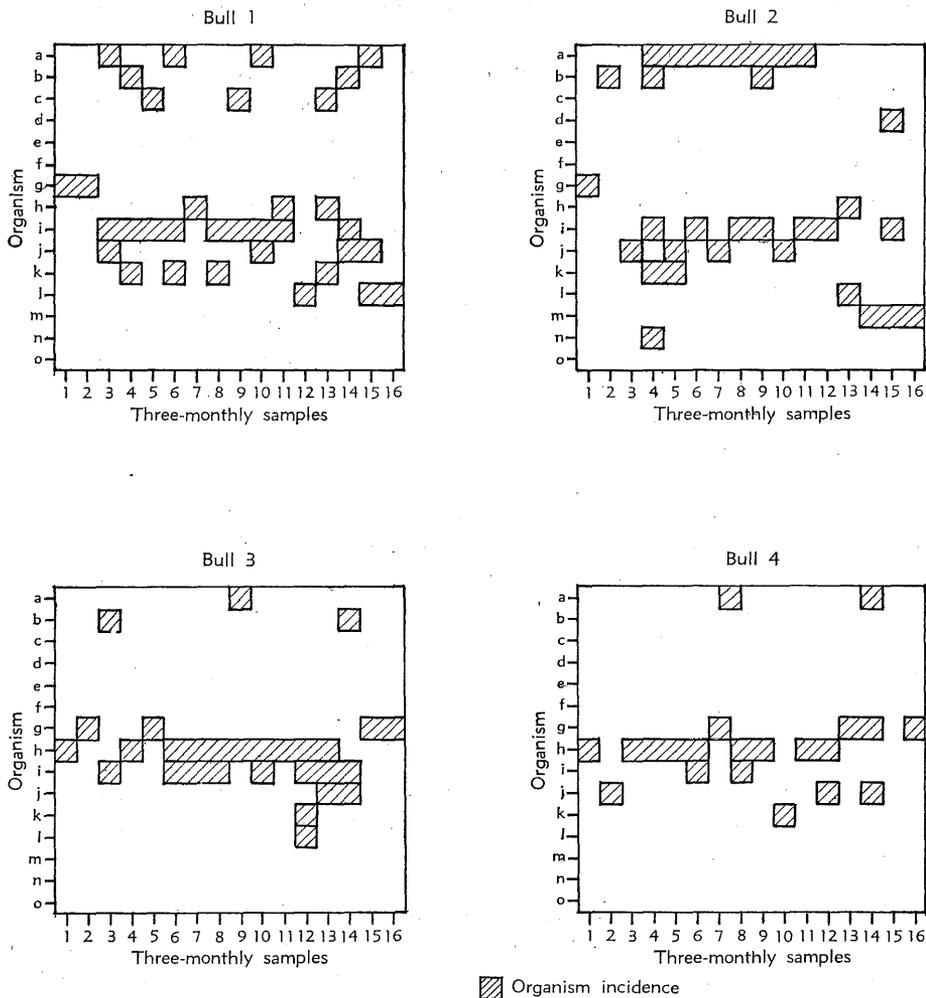


Fig. 2.—Organism incidence in bulls.

(85.7%), coliforms (76.8%), *Corynebacterium* spp. (67.8%), *Bacillus* spp. (66.1%), haemophilic Gram-negative bacilli (59.1%) and *Proteus* spp. (53.6%). Alpha-haemolytic streptococci (23.0%), *Pseudomonas* spp. (19.8%), *C. pyogenes* (10.7%), *Klebsiella* spp. (10.7%), unidentified Gram-negative bacilli

(7.1%), *Staph. aureus* (3.6%), non-haemolytic streptococci (3.7%) and *Serratia* spp. (1.8%) had a lower incidence in the semen of the bull population studied.

The persistence of organisms in the semen of four bulls sampled every 3 months for 4 years is illustrated in Figure 3. In three of the four bulls, coliforms, *Proteus* spp. and haemophilic Gram-negative bacilli were frequently isolated throughout the 4 years under review. In the fourth bull, in each instance, the organisms though less frequently isolated were all demonstrated at least once.



(a) micrococci (b) *Corynebacterium* spp. (c) alpha-haemolytic streptococci (d) *C. pyogenes* (e) *Staph. aureus* (f) non-haemolytic streptococci (g) nil (h) *Proteus* spp. (i) coliforms (j) haemophilic Gram-negative bacilli (k) *Bacillus* spp. (l) *Klebsiella* spp. (m) *Pseudomonas* spp. (n) unidentified Gram-negative bacilli (o) *Serratia* spp.

Fig. 3.—Persistence of bacteria in semen samples.

during the 4 years. The same pattern of organism persistence was found with micrococci and *Corynebacterium* spp. in two of the four bulls and with alpha-haemolytic streptococci and *Bacillus* spp. in one of the four bulls.

IV. DISCUSSION

The types of organisms cultured from the semen of bulls used in this study were generally in agreement with those reported by other workers. The relative incidence, however, was not entirely similar. The bacteria most frequently isolated were micrococci, *Proteus* spp., coliforms, haemophilic Gram-negative bacilli, *Bacillus* spp. and *Corynebacterium* spp. (Figure 1). Coliforms have been claimed (Prince, Almquist, and Reid 1949, and a 1942 report of the United States Department of Agriculture cited by Almquist, Prince, and Reid 1949) to occur only occasionally in bovine semen and their presence has been attributed to faecal contamination. The bulls used in this study were housed in stalls and the high incidence of coliforms in their semen is likely to be due to a higher level of faecal contamination of the prepuce operating under this system of management than would be expected under less intensive conditions. This observation is supported by the high persistence rate demonstrated for coliforms (Figure 3).

Staphylococci and *Pseudomonas* spp. have been found by Gunsalus, Salisbury, and Willett (1941), Prince, Almquist, and Reid (1949), and the 1942 report referred to above to predominate in bull semen. In this study *Pseudomonas* spp. were found to have a moderately high bull incidence (Figure 2) but the incidence in individual ejaculates (Figure 1) was low. *Staph. aureus* was isolated only rarely and bull incidence was low.

The isolation of both haemolytic and non-haemolytic streptococci is in agreement with the work of Edmondson, Tallman, and Hersman (1948) and the low incidence agrees with the findings of Prince, Almquist, and Reid (1949), who reported that streptococci were not characteristic in bovine semen. *Serratia* spp. and unidentified Gram-negative bacilli occurred only rarely in the semen of the bulls studied and their presence cannot be considered part of the normal microflora of semen.

Klebsiella spp. and *C. pyogenes* were each isolated from the semen of six bulls out of the 56 examined during the study. The incidence in ejaculates was low. In no case was the occurrence of either of these bacteria accompanied by any other sign of genital infection in the carrier bulls.

Of the organisms most frequently isolated from the bulls studied, micrococci, *Proteus* spp., coliforms, haemophilic Gram-negative bacilli, *Corynebacterium* spp. and *Bacillus* spp. were found to appear repeatedly in the semen of four bulls sampled regularly over a period of 4 years (Figure 3). This evidence further suggests that these organisms might be considered as the normal bacterial flora in semen of the population studied. In addition to these organisms, alpha-haemolytic streptococci were found repeatedly in one of the four bulls studied

but on no occasion during the 4 years was this organism isolated from the semen of the other three bulls. Accordingly, alpha-haemolytic streptococci cannot be considered as a normal inhabitant of semen of all bulls. In the four bulls sampled at three-monthly intervals for 4 years, all of the other organisms appeared only sporadically and were considered to be abnormal in their semen.

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