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Objekttyp: **Article**

Zeitschrift: **Schweizer Archiv für Tierheilkunde SAT : die Fachzeitschrift für Tierärztinnen und Tierärzte = Archives Suisses de Médecine Vétérinaire ASMV : la revue professionnelle des vétérinaires**

Band (Jahr): **124 (1982)**

PDF erstellt am: **21.06.2024**

Persistenter Link: https://doi.org/10.5169/seals-588007

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Schweiz. Arch. Tierheilk. *124*, 273–295, 1982

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Experimental Genital Ureaplasmosis in the Bull*

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1. Introduction

1.1 Isolations of Ureaplasmas from the Bovine Genital Tract

The first isolations of ureaplasmas from the bovine were made in 1967 by *Taylor-Robinson et al.* Since then, the organisms, often associated with other mycoplasmas[3], have been recovered from the genital tract of cows (*Langford*, 1975a, 1975b; *Panangala et al.*, 1978; *Ruhnke et al.*, 1978a; *Ball and McCaughey*, 1979), preputial swabbings or semen of bulls (*Taylor-Robinson et al.*, 1969; *Anderson*, 1974; *Langford*, 1975b; *Onoviran et al.*, 1975; *Jurmanova and Sterbova*, 1977; *Jurmanova et al.*, 1977) and the upper genital tract of bulls (*Blom*, 1979).

Reports on the pathogenicity of ureaplasmas for the reproductive tract of the bull have been controversial. In the study by British workers (*Taylor-Robinson et al.*, 1969) ureaplasmas were recovered from all preputial washings of ten bulls, two out of six preputial washings from steers and from raw semen of 27 out of 32 bulls reported to have normal fertility. Based on the high recovery rate from apparently normal bulls ureaplasmas were considered commensals in the bovine preputial cavity. *Onoviran et al.* (1975) examined clinically normal bulls from two artificial breeding units and isolated ureaplasmas from 46 of 132 penile-preputial swabbings, 34 of 140 raw and six of 42 processed semen samples. In a study involving bulls from artificial breeding units and a group of range bulls, ureaplasmas were recovered from 79 out of 267 pre-

* This material is taken from a thesis submitted by Rudolf O. Waelchli-Suter to the Faculty of Graduate Studies of the University of Guelph in partial fulfillment of the requirements for the Master of Science degree.

This study was supported by the Swiss National Foundation for Scientific Research and by the Ontario Ministry of Agriculture and Food.

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[3] In this paper the following terminology will be used according to Tully and Razin (1977) with respect to the classification of agents of the mycoplasma group: The general trivial term 'mycoplasmas' refers to organisms of the two families Mycoplasmataceae comprising the genera *Mycoplasma* and *Ureaplasma*, and Acholeplasmataceae with the genus *Acholeplasma*. Both families belong to the order of the Mycoplasmatales. The terms '*Mycoplasma* spp.' and '*Acholeplasma* spp.' refer to agents belonging to the genera *Mycoplasma* and *Acholeplasma*, respectively. Agents of the genus *Ureaplasma* are referred to as 'ureaplasmas'.

putial washings and 39 out of 168 raw semen samples (*Langford*, 1975b). *Jurmanova and Sterbova* (1977) found a correlation between contamination of semen with mycoplasmas (mainly *M. bovigenitalium* and ureaplasmas) and lowered sperm motility. In one study involving 100 processed semen samples, 80% were found to yield ureaplasmas (*Jurmanova and Mazurova*, 1978) while another found 75% of 80 raw semen samples positive (*Truscott*, 1981). *Blom* (1979) cultivated ureaplasmas in large numbers from inflamed vesicular glands of two bulls one of which had a mixed infection with *C. pyogenes*.

Ureaplasma isolations from cows were first reported by *Taylor-Robinson et al.* (1967) following recovery of the organism from scrapings from vagina, urethra, and bladder of eight out of 21 animals examined. Anderson (1974) recovered ureaplasmas from vulvar swabs of nine out of 11 cows developing a purulent discharge shortly after breeding and from the frozen semen which had been used to inseminate one of the cows. *Langford* (1975a) recovered ureaplasmas from cervico-vaginal mucus of 88 out of 633 beef cows without signs of reproductive disease. *Panangala et al.* (1978) found a higher recovery rate from cervico-vaginal mucus of repeat breeder cows as compared to normal fertile cows but the difference was not statistically significant. Infection with ureaplasmas has been associated with bovine granular vulvitis (*Ruhnke et al.*, 1978a, 1978b; *Doig et al.*, 1979). In an outbreak of this disease higher recovery rates from vulvar swabs were found in cows with moderate or acute vulvitis as compared to animals without clinically apparent lesions. It was suggested that ureaplasmas be viewed as one of the causes of bovine granular vulvitis. Ureaplasmas have also been implicated in bovine abortion (*Ball et al.*, 1978; *Ruhnke*, 1981).

1.2 Experiments on the Pathogenicity of Microorganisms for the Male Bovine Genital Tract

The inoculation of microorganisms directly into seminal vesicles of the bull has been used as a model to examine the potential pathogenicity of the organisms for the male bovine reproductive tract. In recent years such experiments were conducted with *Mycoplasma bovigenitalium* and *Mycoplasma bovis* (*La Faunce*, 1972; *Parsonson et al.*, 1974; *Holzmann et al.*, 1979).

1.3 Purpose of Research

The purpose of this study was to investigate the potential pathogenicity of a ureaplasma strain for the seminal vesicle of the bull following direct inoculation.

2. Materials and Methods

2.1 Experimental Design

An experiment was designed to study the effect of ureaplasma inoculation directly into seminal vesicles of bulls. The right seminal vesicle of 11 bulls (experimental bulls) was inoculated with 5 ml of ureaplasma broth culture containing a field isolate (approximately 10^9 colony forming units [c.f.u.]) from a case of bovine granular vulvitis, and the right seminal vesicle of ten other bulls (con-

trol bulls) with 5 ml of ureaplasma culture medium. Two experimental and two control bulls each were slaughtered after inoculation at periods of one day, three days, one week, and eight weeks. Three experimental and two control bulls were killed four weeks after inoculation.

Semen and semino-urethral fluid (i.e., fluid collected directly from the prolapsed penis by means of manual massage of the seminal vesicles, thus avoiding preputial contamination) (*Parsonson et al.*, 1971) were collected during the pre- and postinoculation periods and cultured for mycoplasmas and bacteria. At necropsy the reproductive organs were removed and microbiologic and pathologic evaluation performed. The experiment was carried out between May, 1979 and September, 1980.

2.2 Animals

Twenty-one Holstein bulls between one and three years of age were used. All bulls were purchased at a local stockyard sale. A thorough physical examination was performed on each bull on admission including examination of the reproductive tract by rectal palpation and external palpation of the testes, epididymides, and spermatic cords. Blood for complete blood count was drawn from either jugular vein or from coccygeal vessels twice weekly. Serum antibody titers against Infectious Bovine Rhinotracheitis (IBR), Bovine Virus Diarrhea (BVD), *Brucella abortus*, and *Leptospira pomona* and *hardjo* were evaluated on admission, a few days before inoculation, and again shortly before or at slaughter.

2.3 Sampling Procedures

2.3.1 Preputial Swabs

Preputial swabs taken on admission and shortly before or on the day of slaughter. A disposable sterile 'guarded culture instrument'[4] consisting of an inner and an outer protective tube was used to obtain a sample from the preputial fornix. The swab was immediately transferred to a cepti-seal Culturette[5] tube containing an ampule with 0.5 ml of modified Stuart's Transport Medium[6] and immediately submitted to the laboratory for culture of mycoplasmas and bacteria.

2.3.2 Semen

At least two ejaculates were collected from each bull prior to inoculation using a sterile artificial vagina. Postinoculation collections were made once or twice during the first week and once weekly thereafter until slaughter. Approximately 1 ml of semen was transferred into a sterile tube and submitted for culture of mycoplasmas. Culture for bacteria was performed on one preinoculation and one postinoculation semen sample. All semen samples were evaluated for volume, color, consistency, odor, concentration, pH, total solids, dead-live ratio, catalase value, mass and individual motility, and morphology. Air dried Wright's stained smears were used to identify inflammatory cells.

2.3.3 Semino-Urethral Fluid

At least two samples were collected from each bull prior to inoculation. After the first week postinoculation collections were made once weekly. The technique used was similar to the method of *Parsonson et al.* (1971) with the modification that the collection tube was shorter and only introduced into the urethra for approximately 7 cm. One ml of the fluid was submitted to the laboratory and cultured for mycoplasmas and bacteria. Air dried Wright's stained smears were used to identify inflammatory cells.

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[4] Kalayjian Industries, Inc., Long Beach, California, U.S.A.

[5] Scientific Products, Marion Scientific Corp., Rockford, Illinois, U.S.A.

[6] Sodium Glycerophosphate 1%, Sodium Thioglycolate 0.1%, Calcium Chloride Dihydrate 0.01%.

2.3.4 Necropsy Specimens

The bulls were either shipped to a local slaughterhouse or necropsied in the postmortem room at the Ontario Veterinary College. Specimens for microbiologic and histologic examination were collected not longer than two hours after the animal had been killed. The reproductive tract was examined for gross abnormalities and tissue specimens were obtained for microbiologic examination from both seminal vesicles, both bulbourethral glands, tail and head of both epididymides, and both testes. Swabs were obtained from both ampullae ductus deferentis, the proximal (between the dorsal arch of the sigmoid flexure and the insertion site of the ischiocavernosus muscle) and distal urethra (between the ventral arch of the sigmoid flexure and the glans penis) and bladder.

Tissue specimens for histologic examination were taken from the same organs as for microbiologic examination and additionally from the body of both epididymides and from the prostate. Tissues were fixed in Bouin's solution (Luna, 1968) for 24 hours and stored in 70% ethyl alcohol. The *Hematoxylin-Eosin* stain was employed routinely on all tissues. Sections from seminal vesicles of experimental bulls were stained with *Masson's Trichrome* and sections from seminal vesicles of all bulls with *Lendrum's Chromotrope 2R Method for Eosinophils* (Anon., 1966).

2.4 Culture Techniques

2.4.1 Culture Media for Ureaplasmas

For direct culture of ureaplasmas on plates Shepard's A–3 medium (*Shepard*, 1969) was used with the addition of yeast extract, urea, phenolred, and manganese sulfate (*Shepard and Lunceford*, 1976). The complete agar medium (pH 6 adjusted with 1N HCl) had the following composition: sterile A-3 agar base, 70 ml; unheated horse serum[7], 20 ml; yeast extract (25% solution) (*Hayflick*, 1965), 10 ml; urea[8] (10% solution), 1 ml; phenolred[9] (1% solution), 0.2 ml; penicillin G potassium, 10^5 units, 0.5 ml; $MnSO_4$[10] (3% solution), 0.5 ml. Complete ureaplasma agar plates were prepared weekly. Incubation was at 37 °C in a mixture of 95% hydrogen and 5% carbon dioxide using the anaerobic GasPak system[11]. Negative plates were examined every 48 hours for seven to ten days.

For indirect culture of ureaplasmas the broth medium described by *Taylor-Robinson et al.* (1968) was used, without thallium acetate. The complete ureaplasma broth was dispensed in 3 ml volumes and stored at -20 °C until required but was generally used within two weeks. Incubation was aerobically at 37 °C. The presence of ureaplasmas in broth cultures showing a color change from yellow to pink was always confirmed by subculture to agar medium.

2.4.2 Culture Media for *Mycoplasma* and *Acholeplasma* spp.

The medium for direct culture on plates was slightly modified from that described by Hayflick (1965) and was composed of 21 g Difco PPLO[12] broth and 8 g Oxoid Ionagar # 2[13] in 1,000 ml double distilled deionized water supplemented with 20% inactivated porcine serum[14], 10% yeast extract (*Hayflick*, 1965), 1,000 units penicillin G potassium per ml, and 1:5,000 thallium acetate[15]. The pH was approximately 7.8. Complete agar plates were prepared weekly. Incubation was at

[7] Gibco Laboratories, Grand Island, New York, U.S.A.

[8] Calbiochem, Los Angeles, California, U.S.A.

[9] Canlab, Toronto, Ontario, Canada.

[10] Fisher Scientific Company, Toronto, Ontario, Canada.

[11] Baltimore Biological Laboratories, supplied by Canlab, Toronto, Ontario, Canada; or Oxoid Ltd., London, England.

[12] Difco Laboratories, Detroit, Michigan, U.S.A.

[13] Oxoid Ltd., London, England. Canadian distributor: Med-Ox Chemicals Ltd., Ottawa, Canada.

[14] Gibco Laboratories, Grand Island, New York, U.S.A.

[15] Fisher Scientific Company, Toronto, Ontario, Canada.

37 °C in an atmosphere of 7 to 10% carbon dioxide. Plates were kept for one week before the culture was identified as negative.

For indirect culture of *Mycoplasma* and *Acholeplasma* spp. in broth the same medium was used with the addition of glucose 1:1,000 and phenolred[16] 1:5,000. Incubation was aerobic. Subculture from the broth was made onto plates if no growth was evident on the primary plate 48 hours after inoculation.

2.4.3 Culture Media for Bacteria

Blood agar (bovine, 5%), incubated in 10% carbon dioxide, and MacConkey agar plates, incubated aerobically were used for bacteriologic culture. Incubation was at 37 °C for 24 to 48 hours.

2.4.4 Culture of Preputial Swabs

When only one swab was available, blood agar and MacConkey agar plates were inoculated before the mycoplasma media which contained antibiotics.

For indirect culture of ureaplasmas broth was inoculated and a ten-fold dilution was made to a second tube of the medium. No dilution was made from the Hayflick's medium.

2.4.5 Culture of Semen and Semino-Urethral Fluid

For indirect culture 0.3 ml semen was inoculated into 3 ml of the complete broth media. From the ureaplasma broth, but not from the Hayflick's medium, four serial ten-fold dilutions were made. The same procedure was followed for semino-urethral fluid.

For direct culture three drops of 0.01 ml from each semen dilution and from undiluted and ten-fold diluted semino-urethral fluid were placed on mycoplasma plates at three sites. Incubation was as described for preputial swabs. The plates were examined for growth every 48 hours for one week. If colonies were present they were counted, and the concentration was expressed as number of c.f.u. per ml of fluid.

For bacterial culture the same procedure as for preputial swabs was applied.

2.4.6 Culture of Necropsy Specimens

Culture of swabs taken at necropsy was done as described for preputial swabs. From the remaining organs, tissue pieces approximately 1 cm^3 in size were dipped in ethyl alcohol and ignited for surface sterilization. Fresh surfaces were cut and swabs taken for bacterial culture before impression smears for direct culture were made onto plates for mycoplasmas.

For indirect culture the tissue samples were cut in half and minced with scissors and suspended in 3 ml of broth for mycoplasmas. The broth was removed from the minced tissue to a tube and incubated. Four serial ten-fold dilutions were made from the ureaplasma broth.

2.5 *Identification of Mycoplasmas*

Ureaplasmas were identified by the color change in broth due to the metabolism of urea, and the typical brown colonies on agar plates containing manganese sulfate. Hyperimmune sera were prepared in rabbits against eight reference ureaplasma strains (A417, Bu2, Mmb167, D48, T44, T95, T288, T315) received from *Dr. C. Howard*, Institute for Research on Animal Diseases, Compton, U.K. and against strain 2312 used for inoculation of the bulls (*Howard et al.*, 1978). The antisera were used in typing some of the ureaplasmas isolated by the indirect immunofluorescent antibody test (*Rosendal and Black*, 1972).

Mycoplasma and *Acholeplasma* spp. were differentiated using the indirect immunofluorescent antibody test on unfixed colonies as described by *Rosendal and Black* (1972). Antisera used are listed in Table 1.

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[16] Canlab, Toronto, Ontario, Canada.

2.6 Inoculation

2.6.1 Inoculum for Experimental Bulls

A culture of ureaplasma strain 2312 isolated from a cow with clinically acute granular vulvitis was used for the inoculum which was prepared as previously described (*Doig et al.*, 1980). A 5 ml volume containing between 4.2×10^8 and 2×10^9 c.f.u. was used for each experimental bull.

2.6.2 Inoculum for Control Bulls

Control bulls received the same 5 ml volume, with the culture suspension (1 ml) being replaced by 1 ml of buffered ureaplasma broth.

2.6.3 Technique

Inoculations were made through the right pararectal fossa using the technique described by *Al-Aubaidi et al.* (1972) and *Parsonson et al.* (1974) (Figure 1). One third of the inoculum was injected into the cranial, middle, and caudal part, respectively, of the right seminal vesicle.

3. Results

3.1 Clinical Examination and Serology

Enlargement of the right seminal vesicle to approximately one and a half times normal diameter was rectally palpable within 24 hours of inoculation in nine experimental and eight control bulls. All bulls exhibited signs of pain to a variable extent on rectal palpation of the right seminal vesicle which usually subsided within two weeks of inoculation. Two experimental bulls had signs resembling those seen in traumatic reticuloperitonitis lasting for a few days after inoculation.

There was no difference between experimental and control bulls with regard to IBR and BVD titers. All bulls were negative for *Brucella* and *Leptospira* antibodies.

The mean number of circulating leukocytes, mainly segmented neutrophils, was slightly higher in experimental than in control bulls during the first ten days postinoculation, but the difference was not statistically significant.

3.2 Microbiologic Examination

The results of the mycoplasma cultures from prepuce, semen, and semino-urethral fluid are listed in Tables 2 and 3.

3.2.1 Isolations of Ureaplasmas

3.2.1.1 Preputial Swabs

Prior to inoculation nine of 11 experimental and nine of ten control bulls had positive preputial cultures. After inoculation swabs were obtained from eight experimental and ten control bulls and all were positive.

3.2.1.2 Semen

Isolations were made from preinoculation semen samples of ten of the 11 experimental and of all ten control bulls. Twenty-four (92%) of 26 samples collected from

11 experimental and 20 (91%) of 22 samples collected from ten control bulls were positive.

After inoculation 35 semen samples were collected from nine experimental bulls and all were positive. Numbers of c.f.u. in postinoculation ureaplasma positive semen samples of experimental bulls ranged between 4,000 and 55 million per ml with a mean of approximately 4.5 million. After inoculation 30 semen samples were collected from seven control bulls and all were positive. Numbers of c.f.u. in ureaplasma positive samples of controls ranged between 200 and 610,000 per ml with a mean of approximately 75,000. The number of ureaplasmas in postinoculation semen samples of experimental bulls was approximately 60 times higher than in samples from controls.

3.2.1.3 Semino-Urethral Fluid

Prior to inoculation isolations were made from ten of the 11 experimental and nine of the ten control bulls. Twenty-one (91%) of 23 samples collected from 11 experimental and 18 (90%) of 20 samples collected from ten control bulls were positive.

After inoculation five experimental bulls were collected and one or more positive cultures were obtained from all five with eighteen (78%) of 23 samples being positive. Four control bulls were collected postinoculation and all had at least one positive culture. Seventeen (85%) out of 20 samples were positive.

3.2.1.4 Necropsy Specimens

Results of ureaplasma cultures of the urogenital tract of the experimental and control bulls are listed in Tables 4 and 5, respectively. Ureaplasmas were isolated from the right seminal vesicle of seven experimental bulls necropsied one day, one week, and four weeks after inoculation. Isolates were made from the left seminal vesicle, right ampulla ductus deferentis, or bulbourethral glands from three bulls of the one week and four week groups. These three animals also had positive cultures from the bladder. The urethra was positive for ureaplasmas in ten of the 11 experimental bulls. In control bulls culture of the bladder yielded low numbers of ureaplasmas in three animals. The urethra was positive in six of the ten control bulls.

3.2.1.5 Serotyping of Ureaplasmas

Most isolates among those serotyped from prepuce, semen, semino-urethral fluid, and necropsy specimens of experimental bulls were confirmed by fluorescent antibody technique to be the same strain as used for inoculation; however, a few colonies of different serotypes were occasionally isolated from prepuce, semen, semino-urethral fluid, and urethra. Isolates from control bulls were serotypes different from the one used for the inoculation of the experimental bulls.

3.2.2 Isolation of *Mycoplasma* and *Acholeplasma* spp.

3.2.2.1 Preputial Swabs

Prior to inoculation all 11 experimental and five of ten control bulls had positive preputial cultures. After inoculation swabs were obtained from eight experimental

and ten control bulls. All bulls were positive except for one control which was negative throughout the pre- and postinoculation periods. All positive cultures yielded *M. bovigenitalium*. Other isolates in order of frequency were *M. canadense, A. laidlawii,* and *Mycoplasma* sp. (California calf).

3.2.2.2 Semen

Isolations were made from preinoculation semen samples of all 11 experimental and of seven out of ten control bulls. Twenty-two (85%) of 26 samples collected from 11 experimental and 13 (59%) of 22 samples collected from ten control bulls were positive.

After inoculation nine experimental bulls were collected and one or more positive cultures were obtained from eight with thirty-three (94%) of 35 samples being positive. Seven control bulls were collected postinoculation and five had at least one positive culture. Twenty-four (80%) out of 30 samples were positive.

Ninety-two (81%) of a total of 113 samples from both experimental and control bulls pre- and postinoculation were positive. Species recovered included *M. bovigenitalium, A. laidlawii, M. canadense*, and *Mycoplasma* sp. (California calf). Positive semen samples contained less than 30 to more than two million c.f.u. per ml with a mean of approximately 150,000.

3.2.2.3 Semino-Urethral Fluid

Prior to inoculation isolations were made from seven of the 11 experimental and five of the ten control bulls. Eleven (48%) out of 23 samples collected from 11 experimental bulls and nine (45%) out of 20 samples collected from ten control bulls were positive.

After inoculation five experimental bulls were collected and one ore more positive cultures were obtained from four animals. Nine (39%) out of 23 samples were positive. Four control bulls were collected postinoculation and three had at least one positive culture. Seven (35%) out of 20 samples were positive. Species recovered included *M. bovigenitalium* and *A. laidlawii*.

3.2.2.4 Necropsy Specimens

Low numbers of *M. canadense* and *M. bovigenitalium* were isolated from the bladder of an experimental and a control bull, respectively.

3.2.3 Isolation of Bacteria

3.2.3.1 Preputial Swabs

All preputial swabs obtained from 17 of the 21 bulls were positive. Cultures were not attempted from four bulls. Multiple isolates were common. *Haemophilus somnus, Corynebacterium pyogenes,* and *Escherichia coli* were isolated from ten bulls, *Proteus* and α hemolytic streptococci from seven, and *Pseudomonas* spp., *Pasteurella* spp. or *Pasteurella*-like bacilli from six animals on at least one occasion. Other isolates were staphylococci, diphtheroids, *Bacillus* and *Neisseria* spp. There was no significant dif-

ference between experimental and control bulls or between pre- and postinoculation periods with respect to bacterial isolations. The same was true for semen and semino-urethral fluid.

3.2.3.2 Semen

Semen samples from 18 bulls were examined for bacteria on at least one occasion and positive cultures were obtained from 15 animals. All 26 semen cultures from these 15 bulls were positive. Three animals were negative on a single culture. Multiple isolates were common. The organisms most frequently isolated were *H. somnus* (seven bulls) and *C. pyogenes*, diphtheroids, and *Pasteurella*-like bacilli (six bulls each). Other isolates included *E. coli* (four bulls), α hemolytic streptococci (three bulls), *Flavobacterium* (two bulls), and *Pasteurella, Pseudomonas aeruginosa*, non hemolytic staphylococci, and *Proteus* (one bull each).

3.2.3.3 Semino-Urethral Fluid

Semino-urethral fluid from 20 bulls was examined for bacteria on at least one occasion and positive cultures were obtained from nine animals. Fifteen (21%) of 72 specimens cultured contained bacteria. Species isolated included *Pasteurella* spp. or *Pasteurella*-like bacilli (four bulls), *C. pyogenes* and diphtheroids (three bulls each), α hemolytic streptococci (two bulls), and *E. coli, H. somnus, Pseudomonas aeruginosa*, and non hemolytic staphylococci (one bull each).

3.2.3.4 Necropsy Specimens

Low or moderate numbers of streptococci were recovered from one testis and from both epididymides and bladder of an experimental bull. Low numbers of *P. aeruginosa* and/or *Pasteurella*-like bacilli were isolated from both seminal vesicles and ampullae and from the bladder of a control bull. In three other bulls, two experimental and one control, low numbers of streptococci and/or *Pseudomonas* spp. were recovered from the bladder, from one animal also from one epididymis.

3.3 *Gross and Microscopic Examination of Semen*

In postinoculation ejaculates of experimental bulls the means of volume, sperm concentration, sperm motility, and catalase value were slightly lower and the means of pH and morphologic sperm abnormalities slightly higher as compared to control bulls; however, the differences were not statistically significant. Color changes to red or brown due to admixture of erythrocytes were observed in postinoculation semen samples of experimental and control bulls. An admixture of white blood cells, mainly neutrophils, in postinoculation semen samples was the only parameter among those examined to be significantly different in experimental and control bulls. Neutrophils were present in postinoculation semen samples of eight out of nine experimental bulls appearing as early as two days postinoculation. One control bull shed large numbers and two others occasional neutrophils in one sample. Nineteen (53%) out of 36 samples from experimental and four (14%) out of 29 samples from control bulls contained neutrophils.

3.4 Morphologic Postmortem Examination

The histopathologic findings pertaining to the right seminal vesicles of experimental and control bulls are summarized in Table 6. Inflammatory signs were present in the seminal vesicle of ten of the 11 experimental bulls. The lesion was acute with multifocal distribution in those necropsied one and three days postinoculation (Figure 2), and subacute with multifocal or diffuse distribution in bulls necropsied one week postinoculation. Moderate or severe diffuse chronic seminal vesiculitis dextra with moderate interstitial fibrosis and variable epithelial degeneration (Figure 3) was diagnosed in all but one experimental bull of the four and eight week groups. One experimental bull had degenerative epithelial lesions in the absence of inflammation. A control bull of the four week group had a seminal vesiculitis with negative culture. In control bulls necropsied within a week of inoculation, seminal vesicular hemorrhage (Figure 4) was the only significant lesion. Low or moderate numbers of eosinophils were present in the right seminal vesicle of nine of the 11 experimental bulls and in the seminal vesicle of the control bull with the chronic seminal vesiculitis.

Acute, subacute, or chronic inflammatory lesions of varying severity consisting of neutrophils, eosinophils, plasma cells, or lymphocytes alone or in combination were present in the proximal and/or distal urethra of eight experimental and six control bulls. In seven experimental animals and in the six control bulls the inflammation was accompanied by a positive ureaplasma culture.

4. Discussion

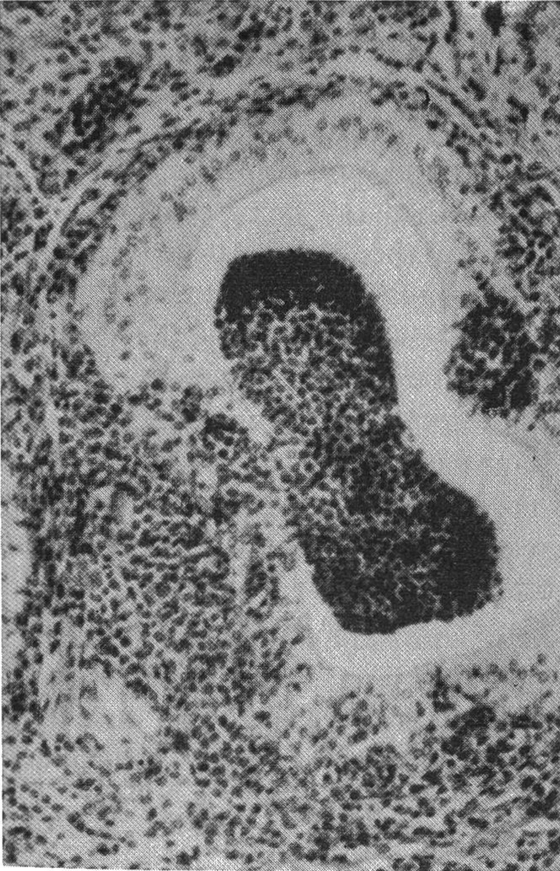
The inoculation of microorganisms into seminal vesicles of bulls has been used as a model to examine the pathogenicity of organisms for the male bovine reproductive tract. Owing to the location of the glands within the genital tract it is possible to observe the migration of the infection and/or inflammation, if any, in an ascending or descending direction. Examples of such experiments are those done by *Christensen* (1948), *Blom and Dam* (1967), *Blom and Ernø* (1967), *Boryczka* (1970), *La Faunce*

Figure 1: Inoculation of the right seminal vesicle. The left hand was introduced into the rectum of the bull and a 17 gauge, 7 inch needle with stylet was inserted through the skin and driven toward the brim of the pelvis near the midline. One third of the inoculum was injected into the cranial, middle, and caudal portion of the seminal vesicle, respectively.

Figure 2: Acute seminal vesiculitis in an experimental bull necropsied 24 hours after inoculation of ureaplasma culture into the right seminal vesicle. Neutrophils and monocytes are migrating from the interstitial tissue into a glandular acinus. (H. & E. stain, x320)

Figure 3: Chronic seminal vesiculitis with epithelial degeneration in an experimental bull necropsied 4 weeks after inoculation of ureaplasma culture into the right seminal vesicle. The epithelial degeneration in this bull was more severe than in the two other experimental animals necropsied 4 weeks postinoculation. (H. & E. stain, × 200)

Figure 4: Seminal vesicular hemorrhage in a control bull necropsied 24 hours after inoculation of ureaplasma broth into the right seminal vesicle. The acini are distended with red homogeneous material. (H. & E. stain, × 50)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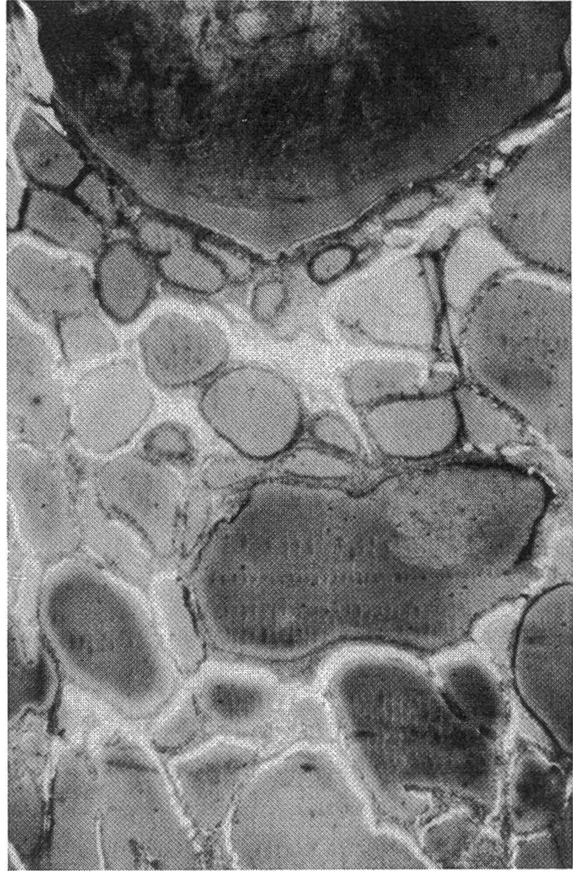
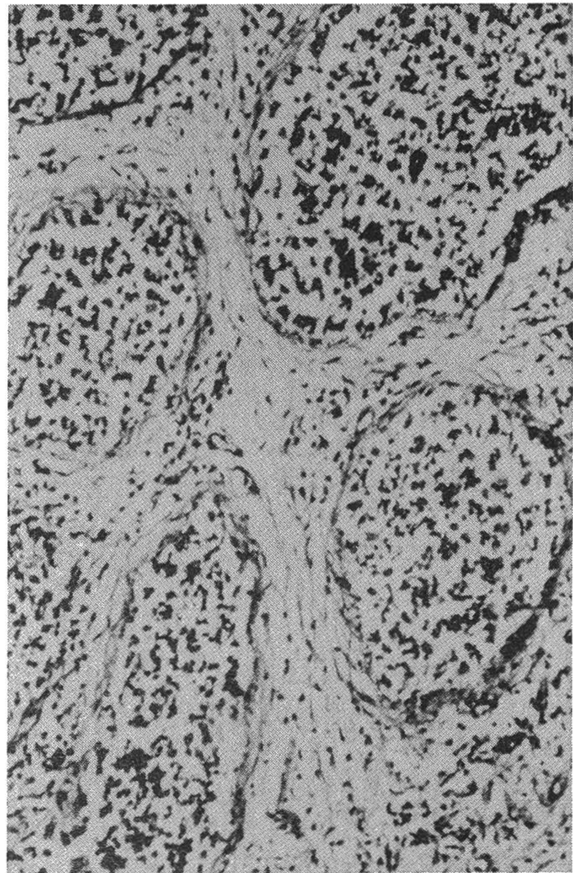

4

1


3

(1972), *Parsonson et al.* (1974), and *Holzmann et al.* (1979). The mode of infection, however, is highly artificial. The origin of seminal vesiculitis may be ascending from the prepuce or the distal urethra, descending from the testes, or by the hematogenous route. With direct intravesicular inoculation acinar and interstitial tissue will become infected simultaneously which may not occur under natural conditions. In fact, two distinct types of seminal vesiculitis have been described, one being characterized predominantly by interstitial inflammatory lesions, and the other by degenerative changes in the lumen and in the epithelial tissue (*Galloway*, 1964). Furthermore, direct inoculation causes a local trauma representing a locus minoris resistentiae which may impair local defense mechanisms.

The use of bulls already positive for ureaplasmas in the prepuce and semen could not be avoided in this study. Twenty of the 21 purchased bulls shed ureaplasmas and there was no practical way to obtain more negative animals. In order to lessen the effect of this experimental limitation an increased number of control bulls was used to accurately evaluate the consequences of additional artificial infection.

Except for the admixture of leukocytes no significant differences in semen parameters were found between experimental and control bulls. *Galloway* (1964) postulated that there are no changes in semen morphology to be expected in bulls with seminal vesiculitis as long as normal conditions exist in the testes and epididymides. In most of the bulls investigated by *Galloway* (1964) semen morphology was normal. In this experiment no infection or signs of inflammation were found in the testes or epididymides of those bulls from which semen collections were made during the postinoculation period. *Parsonson and co-workers* (1974) did not observe any changes in the morphology of the spermatozoa although infection of the epididymides, uni- or bilaterally, had occurred in all the bulls which were inoculated with *M. bovigenitalium*. On the other hand, *Holzmann et al.* (1979) found a significant increase in sperm abnormalities, particularly spermatozoa with detached head piece and loop-shaped types, following inoculation of *M. bovigenitalium* into the right seminal vesicle of bulls.

In the experiment done by *Parsonson et al.* (1974) shedding of white blood cells persisted in all bulls up to the time of slaughter ranging from two to 17 weeks postinoculation. In this experiment four of the five bulls of the four and eight week groups had discontinued shedding by day 17 to 25. One bull of the four week group shed neutrophils up to the time of necropsy.

As confirmed on histologic examination, the discontinuation of the shedding of leukocytes in the semen did not reflect the disappearance of inflammatory signs in the right seminal vesicle. Rather, it is suspected that swelling and inflammatory exudates within the diseased seminal vesicle had led to an occlusion of the excretory duct with subsequent retention of the secretion within that gland. The presence of leukocytes in the semen of bulls with seminal vesiculitis has been described by numerous workers. However, some reports also indicated that leukocytes may be absent in semen of affected bulls (*Lagerlöf et al.*, 1942; *Teunissen*, 1946; *Galloway*, 1964; *Blom*, 1979).

Positive preinoculation cultures for ureaplasmas were obtained from the prepuce of 18 (86%) of 21 bulls and from 38 (93%) of 41 preputial swabbings. Preputial recovery rates between 30% and 100% have been reported (*Langford*, 1975b; *Onoviran et al.*, 1975; *Taylor-Robinson et al.*, 1969; *Truscott*, 1981). The significance of the preputial colonization with ureaplasmas is unclear. In none of the bulls of this experiment could any clinical signs of disease be detected. It therefore appears that the role of this agent in the prepuce is usually that of a commensal. However, it is unknown whether or under what conditions ureaplasmas may spread from the preputial cavity to the upper reproductive tract to cause infection and disease.

Ureaplasmas were recovered from 44 (92%) of 48 preinoculation semen samples of 20 (95%) of 21 bulls. These percentages are considerably higher than those reported by *Langford* (1975b) and *Onoviran et al.* (1975) (23% of 168 bulls and 24% of 140 raw semen samples from 74 bulls, respectively). Positive ureaplasma cultures form 80% of 100 and from 75% of 80 semen samples were reported by *Jurmanova and Mazurova* (1978) and by *Truscott* (1981), respectively. The factors accounting for differences in recovery rates of ureaplasmas from the preputial cavity or from semen of bulls are not known, although variables such as culture techniques, antibiotic treatments, type of housing, and possibly others should be considered. It can be assumed that the ureaplasmas in the semen originated mainly from the penile and preputial mucous membranes. However, ureaplasmas from the urethra may also contribute to the seminal contamination. Supporting this would be the fact that the majority of the semino-urethral samples, which were collected by avoiding contamination from the penile and preputial mucous membranes, were also heavily contaminated with ureaplasmas.

The review of the literature had indicated a high incidence of mycoplasmas in the prepuce of healthy bulls, a fact also observed in this experiment. Semino-urethral fluid was collected in order to obtain secretions from accessory sexual glands, mainly seminal vesicles, free of preputial contamination. It was hoped that the fluid could be used to identify infection of the upper urogenital tract. The technique was based on a report by *Parsonson et al.* (1971) who found 154 of 158 samples from 22 bulls to be free of bacterial contamination. The specimens were cultured for *Mycoplasma* and *Acholeplasma* spp., but not for ureaplasmas. In the present experiment positive culture results of semino-urethral fluid were not related to positive cultures of organs of the upper genital tract. At necropsy all accessory sexual glands of control bulls were free of ureaplasmas and other microorganisms, yet all four control bulls which were collected postinoculation yielded positive cultures for ureaplasmas in semino-urethral fluid. It therefore appears that the ureaplasmas in the fluid originated from the urethra.

Mycoplasma and/or *Acholeplasma* spp. were recovered from 36 (42%) of 86 samples from the 21 bulls. *Parsonson et al.* (1971) failed to recover mycoplasmas from 158 samples collected from 22 bulls. In this experiment the collecting tube was introduced into the urethra only about 7 cm as compared to 20 to 25 cm as proposed by *Parsonson et al.* (1971). This modification was done in an attempt to reduce possible contamination of proximal parts of the urethra with microorganisms from the ure-

thral opening. Large colony mycoplasmas were not recovered at necropsy from the urethra at a level approximately 20 to 25 cm from the glans penis, and cultures distal to that site were not done. It is therefore assumed that the contaminants of the semino-urethral fluid originated from the distal urethra close to the urethral orifice.

The isolation of *H. somnus* from the prepuce and from semen of ten and seven bulls, respectively, is of interest. In a recent investigation (*Humphrey*, 1981) *H. somnus* was found to be a common inhabitant of the bovine preputial cavity. The agent was also present in a significant proportion of 31 urogenital tracts of bulls in bladder, accessory sexual glands, and ampulla ductus deferentis. *H. somnus* was recovered from two purulent semen samples of two bulls in pure culture (*Corboz and Nicolet*, 1975). At present the importance of *H. somnus* for the genital tract of the bull awaits clarification.

Ureaplasmas were not recovered from the right seminal vesicle of experimental bulls of the three day and eight week groups. With respect to the three day group it is assumed that a sampling error may have accounted for the negative culture since the inflammatory lesion and possibly the infection were multifocal rather than diffuse.

Throughout the experimental period, the two bulls of the eight week group shed considerably lower numbers of ureaplasmas in their semen than the bulls of the four week group. Since no signs of inflammation were observed in the right seminal vesicle of one experimental bull of the eight week group it was suspected that an infection failed to establish. In this experiment the immune status of the bulls with regard to mycoplasmas was not examined and no antibody titers against ureaplasmas were measured. Therefore, it is a matter of speculation if certain bulls might have had an acquired resistance to ureaplasma infection as a result of a previous natural exposure.

In none of the bulls could isolates be made from sites proximal to the pelvic accessory sexual organs. *Parsonson et al.* (1974) inoculated *M. bovigenitalium* into the right seminal vesicle of 13 bulls and recovered the organism from the right seminal vesicle of all bulls killed at various intervals up to 17 weeks after inoculation. The left seminal vesicle of several bulls necropsied between three and 12 weeks postinoculation was also positive. The right epididymis was also positive in all 13 experimental bulls, and the left in five. In four cases cultures of testes, uni- or bilaterally, were positive. It appears that the strain of ureaplasmas used in this experiment, when inoculated into seminal vesicles, is not as persistent and does not have the same tendency to invade other organs as compared to the *M. bovigenitalium* strain used by *Parsonson and co-workers*.

It is of interest that in this experiment the urethra at the sigmoid flexure was negative for bacteria and large colony mycoplasmas in all 21 bulls whereas it was positive for ureaplasmas in six out of ten control bulls.

Enlargement of the right seminal vesicle occurred in bulls in both experimental and control groups and differences could not be detected by rectal palpation of the two groups. The reason for the enlargement was thought to be mainly the hemorrhage due to the trauma of inoculation.

Histologic evidence of inflammation in the right seminal vesicle was observed in 10 of the 11 experimental bulls. The distribution of the lesion was multifocal in bulls

Table 1 Antisera used for Identification of *Mycoplasma* and *Acholeplasma* spp.

Species	Strain	Supplier of antiserum[a]
M. bovigenitalium	PG 11	VSL[b]
M. bovis	227	VSL
M. bovirhinis	NCTC[c] 10118	VSL
M. arginini	108	VSL
M. canadense	466	VSL
A. laidlawii	NCTC 10116	VSL
M. alkalescens	Leach group 8	VSL
M. species Leach group 7	NCTC 10133	VSL
A. modicum	NCTC 10134	VSL
A. axanthum	NCTC 10138	NIH[d]
M. species	California calf	NIH
M. verecundum	107	NIH

[a] Rabbits were used for production of antisera. The conjugate[1] (fluorescein labelled anti-rabbit globulin) was of caprine origin.
[b] Veterinary Services Laboratory, Ontario Ministry of Agriculture and Food, Guelph, Ontario, Canada.
[c] National Collection of Type Cultures, Colindale, England.
[d] National Institute of Health, Bethesda, Maryland, U.S.A. Courtesy of Dr. J.G. Tully.

[1] Baltimore Biological Laboratories, Baltimore, Maryland, U.S.A.

necropsied one and three days postinoculation and it was diffuse in bulls of the four and eight week groups. A mixture of the two patterns was observed in the two bulls of the one week group. Since the inoculation of ureaplasmas was done in a 'multifocal' pattern, it can be concluded that between one and four weeks were required for the infection and the inflammation to spread through the gland. *Parsonson et al.* (1974) also observed diffuse inflammatory lesions in the seminal vesicle of bulls two weeks after inoculation with *M. bovigenitalium*. It is suspected that the artificial method of infection applied in this experiment accounted for the fact that in most experimental bulls necropsied four and eight weeks after inoculation, chronic inflammatory changes had occurred simultaneously with degenerative epithelial lesions. In one bull necropsied eight weeks postinoculation, chronic interstitial inflammatory lesions were present without a positive culture. It is suspected that the ureaplasmas had been eliminated by that time. Cases of seminal vesiculitis lacking an etiologic diagnosis have been described in the past (*Blom and Christensen*, 1947; *McEntee*, 1962; *König*, 1962; *Galloway*, 1964; *Blom*, 1979). The question as to the etiology and the possible involvement of ureaplasmas in those cases remains a matter of speculation.

The seminal vesiculitis in the one control bull could have been caused by contamination at the time of inoculation or by organisms which had been present in the upper reproductive tract and subsequently invaded the hemorrhagic gland.

Inflammatory lesions in the urethra accompanied by positive ureaplasma isolation were found in seven experimental and in six control bulls, but in other animals urethritic lesions occurred without positive ureaplasma culture, and isolations from

Table 2 Isolations of Ureaplasmas from Prepuce, Semen, and Semino-Urethral Fluid

	Experimental Bulls (bulls or samples positive/bulls or samples cultured)		Control Bulls	
	pre[a]	post[b]	pre	post
Prepuce (bulls)	9/11	8/8	9/10	10/10
Semen (bulls)	10/11	9/9	10/10	7/7
Semen (samples)	24/26	35/35*	20/22	30/30*
S.-U. fluid[c] (bulls)	10/11	5/5	9/10	4/4
S.-U. fluid (samples)	21/23	18/23	18/20	17/20

[a]preinoculation, [b]postinoculation, [c]semino-urethral fluid.
* experimental bulls shed numbers approximately 60 times higher than did controls.

Table 3 Isolations of *Mycoplasma* and *Acholeplasma* spp. from Prepuce, Semen, and Semino-Urethral Fluid

	Experimental Bulls (bulls or samples positive/bulls or samples cultured)		Control Bulls	
	pre[a]	post[b]	pre	post
Prepuce (bulls)	11/11	8/8	5/10	9/10
Semen (bulls)	11/11	8/9	7/10	5/7
Semen (samples)	22/26	33/35	13/22	24/30
S.-U. fluid[c] (bulls)	7/11	4/5	5/10	3/4
S.-U. fluid (samples)	11/23	9/23	9/20	7/20

[a]preinoculation, [b]postinoculation, [c]semino-urethral fluid.

the urethra were also made in the absence of inflammatory lesions. However, the fact that no other mycoplasmas and no bacteria were isolated from the proximal and distal urethra may suggest a positive relationship between urethritis and ureaplasma infection observed in this experiment. In an experiment involving humans, *Ureaplasma urealyticum*, isolated from men suffering from nonspecific urethritis, produced urethritis when introduced intraurethrally into healthy men (*Taylor-Robinson et al.*, 1977).

The presence of eosinophilic granulocytes was a striking feature observed in the right seminal vesicle of nine of eleven experimental bulls and one of the controls. Eosinophils were found as early as 24 hours after inoculation. An eosinophilic response was also observed in mastitis (*Karbe*, 1967; *Mosher et al.*, 1968), in endometritis (*Hartman et al.*, 1964) and in salpingitis (*Hirth et al.*, 1966) experimentally caused by *M. bovis*. The same phenomenon was also reported in mastitis (*Ernø*, 1967) and arthritis (*Ernø*, 1969) caused by *M. bovigenitalium*. Similarly, *Blom and Ernø*, inoculating a broth culture of the same agent into seminal vesicles of one and into testes of three bulls, observed inflammatory changes characterized by the presence of eosino-

Table 4 Recovery of Ureaplasmas from the Urogenital Tract[1] of Experimental Bulls

Bull	Group	Right seminal vesicle	Left seminal vesicle	Right ampulla	Right bulbo-urethral gland	Left bulbo-urethral gland	Bladder	Proximal urethra	Distal urethra	Prepuce
17	1 d. exp.	+						+	+	ND
452	1 d. exp.	++						+		++
482	3 d. exp.							++		+++
488	3 d. exp.	+/−							1 col.	+++
457	1 w. exp.	+++	+		+		+++		+++	+++
487	1 w. exp.	+++		+	+		+	+	+	+++
18	4 w. exp.	+++						+		ND
19	4 w. exp.	+						+++	+	ND
453	4 w. exp.	+++	+	+	+	+	+++	+++	+++	+++
451	8 w. exp.									+++
460	8 w. exp.							+++	+++	+++

[1] Cultures from organs not listed were negative.
+/− = change in pH, subculture negative; + = low numbers; ++ = moderate numbers; +++ = large numbers; col. = colony; ND = not done; d = day; w = week; exp. = experimental.

Table 5 Recovery of Ureaplasmas from the Urogenital Tract[1] of Control Bulls

Bull	Group	Right ampulla	Bladder	Proximal urethra	Distal urethra	Prepuce
480	1 d. contr.	+/−	1 col.			+++
483	1 d. contr.					+++
462	3 d. contr.		+	+++	++	+++
486	3 d. contr.		+	+++	+++	+++
459	1 w. contr.			+	++	+++
484	1 w. contr.					+++
454	4 w. contr.					+++
481	4 w. contr.			+++	+++	+++
416	8 w. contr.			++		+++
478	8 w. contr.		+/−	+++	+++	+++

[1] Cultures from organs not listed were negative.
+/− = change in pH, subculture negative; + = low numbers; ++ = moderate numbers; +++ = large numbers; col. = colony; d = day; w = week; contr. = control.

Table 6 Lesions in the Right Seminal Vesicle of Experimental and Control Bulls

Bull	Group	Inflammation[1]	Distribution[2]	Periadenitis[1]	Hemorrhage[1]	Eosinophils[3]	Fibrosis[1]	Increase in size[1]	Epithelial Degeneration[1]
17	1 d. exp.	+ + +[a]	m	+	+	+ +		+	
452	1 d. exp.	+ + +[a]	m	+	+	+		+	
480	1 d. contr.			+	+ +			+	
483	1 d. contr.			+	+ + +			+	
482	3 d. exp.	+ +[a]	m	+	+ +	+ +		+	
488	3 d. exp.	+[a]	m		+ +	+			
462	3 d. contr.				+ + +			+ +	
486	3 d. contr.				+			+ +	
457	1 w. exp.	+ + +[s]	d	+	+ +	+		+ +	
487	1 w. exp.	+ + +[s]	m	+	+ +	+		+	
459	1 w. contr.				+ + +			+ +	
484	1 w. contr.								
18	4 w. exp.	+ + +[c]	d	+		+ +	+ +	+ +	+ +
19	4 w. exp.	+ + +[c]	d	+		+	+ +	+ +	+
453	4 w. exp.	+ + +[c]	d	+			+ +	+ +	+ + +
454	4 w. contr.	+ + +[c]	d			+	+ + +	+ +	+ +
481	4 w. contr.							+	
451	8 w. exp.	+ +[c]	d			+	+ +		+ +
460	8 w. exp.							+	+ + +
416	8 w. contr.								
478	8 w. contr.							+	

[1] + = mild, + + = moderate, + + + = severe.
[2] d = diffuse, m = multifocal.
[3] + = low numbers, + + = moderate numbers.
a = acute, s = subacute, c = chronic, d = day, w = week, contr. = control, exp. = experimental.

philic granulocytes (*Blom and Ernø*, 1967; *Ernø and Blom*, 1972). *Parsonson et al.* (1974) observed that eosinophils were commonly present in seminal vesicles inoculated with *M. bovigenitalium*. It was thought that the mycoplasmas provided a constant source of antigen which combined with locally produced antibodies to form antigen-antibody complexes that attracted eosinophils. The fact that immune complexes attract eosinophils and that this cell typ accumulates at the site of immune complex formation was demonstrated by *Litt* (1961, 1962). *Ernø and Blom* (1973) concluded from an experiment with bulls artificially infected with *M. bovigenitalium* into seminal vesicles that antibodies may be present locally at the site of inoculation. In the

absence of immunologic studies no definitive conclusions can be drawn as to the significance of the eosinophilic response observed in this experiment.

The possibility that the artificial mode of infection used in this experiment contributed to the developement of morphologic changes in the inoculated seminal vesicles cannot be dismissed. However, in all but one control bull the lesion was limited to hemorrhage, in most cases associated with an increase in size, and to periadenitis in two bulls. The mere inoculation of broth did not result in seminal vesiculitis, thus the inflammatory lesions observed in the experimental bulls can be regarded as being associated with ureaplasma infection.

Histopathologic lesions were also observed in organs of the urogenital tract other than the right seminal vesicle in experimental and control bulls. The inflammatory lesions were most often chronic in nature and were frequently found in the absence of positive cultures. In a study involving 7,359 bulls, *Ball and co-workers* (1964) found histopathologic lesions in reproductive organs of bulls with or without seminal vesiculitis. Since mononuclear cell types were predominant, it was suggested that a viral etiologic agent might have been involved in some cases.

Based on the findings of this study it can be concluded that the ureaplasma strain used has pathogenic potential for the bovine seminal vesicle. It can cause seminal vesiculitis if inoculated directly into the gland at a dose of approximately 10^9 c.f.u. Based on this study no conclusions can be made as to the role of ureaplasmas in field cases of bovine seminal vesiculitis.

The findings also support the previous observation that the isolation of ureaplasmas from the prepuce, urethra, or raw semen does not necessarily indicate disease of any portion of the reproductive tract. Apparently normal bulls can shed ureaplasmas in semen, the pathogenicity of which under natural conditions remains to be determined.

Acknowledgement

The authors acknowledge the technical assistance of Mrs. Lois Parker and Mr. Jim Rahn.

5. Summary

An experiment was designed to study the effect of a ureaplasma strain inoculated directly into seminal vesicles of one to three year old Holstein bulls. The right seminal vesicle of 11 bulls was inoculated with 5 ml of ureaplasma broth culture containing approximately 10^9 c.f.u. of a field isolate from a case of bovine granular vulvitis, and the right seminal vesicle of ten control bulls with 5 ml of ureaplasma culture medium. The animals were necropsied one day, three days, one week, four weeks, or eight weeks postinoculation. Semen and semino-urethral fluid free of preputial contamination were collected during the pre- and postinoculation periods and cultured for ureaplasmas and other microorganisms. At necropsy microbiologic and pathologic examinations were performed on the urogenital tract. Ureaplasmas were recovered from preinoculation semen samples and preputial swabs of 20 and 18 bulls, respectively. The mean number of c.f.u. in postinoculation semen samples of experimental bulls was approximately 60 times the number in semen of control bulls. Neutrophils were present in postinoculation semen samples of eight of nine experimental bulls. One control bull shed large numbers, and two others occasional neutrophils in one sample.

At necropsy ureaplasmas were recovered from the right seminal vesicle of the experimental bulls necropsied one, seven, and 28 days postinoculation. They were confirmed by fluorescent anti-

body technique to be the same strain as used for inoculation. Ureaplasmas were not recovered from accessory sexual glands of control bulls.

On histologic examination of the right seminal vesicle, inflammatory signs were found in 10 of 11 experimental bulls. One experimental bull had degenerative epithelial lesions in the absence of inflammation. A control bull of the four week group had a chronic seminal vesiculitis whereas in the controls necropsied within a week of inoculation hemorrhage in the right seminal vesicle was the only significant lesion.

Experimental limitations and microbiologic and pathologic findings are discussed. It was concluded that the strain of ureaplasma used has a pathogenic potential for the bovine seminal vesicle.

Zusammenfassung

In einem Experiment sollte untersucht werden, welche Auswirkungen die direkte Inokulation von Ureaplasmen in die rechte Samenblase von ein- bis dreijährigen Holstein Stieren hat. Der zur Inokulation verwendete Ureaplasmenstamm wurde vom Geschlechtsapparat einer Kuh isoliert, welche an granulärer Vulvovaginitis ('Knötchenseuche') erkrankt war. Das Inokulum für 11 experimentelle Stiere bestand aus 5 ml einer Ureaplasmenkultur (ca. 10^9 koloniebildende Einheiten), dasjenige für zehn Kontrollstiere aus 5 ml des Nährmediums. Die Stiere wurden nach einem Tag, drei Tagen, einer Woche, vier oder acht Wochen geschlachtet und ihr Urogenitalapparat histologisch und mikrobiologisch untersucht. Sowohl vor als nach der Inokulation wurden Samenproben und Sekrete aus Samenblasen und Harnröhre, gewonnen unter Ausschluss präputialer Kontamination, mikrobiologisch untersucht.

Vor der Inokulation gelang die Isolierung von Ureaplasmen aus Ejakulaten und präputialen Tupferproben von 20 beziehungsweise 18 Stieren. Nach der Inokulation war die durchschnittliche Anzahl von Ureaplasmen in Samenproben von experimentellen Stieren rund 60mal so gross wie in solchen von Kontrollstieren. Ejakulate von acht der neun experimentellen Stiere, welche nach der Inokulation abgesamt wurden, enthielten Leukozyten, während ein Kontrollstier ein Ejakulat mit sehr zahlreichen und zwei weitere Kontrollen je eine Samenprobe mit einigen wenigen Eiterzellen zeigten.

Die Isolierung von Ureaplasmen aus der rechten Samenblase gelang bei denjenigen experimentellen Stieren, welche nach einem, sieben und 28 Tagen geschlachtet wurden. Mittels Immunofluoreszenz-Technik konnte nachgewiesen werden, dass es sich bei den Isolaten um denselben Stamm handelte, der zur Inokulation verwendet worden war. Aus akzessorischen Geschlechtsdrüsen von Kontrollstieren konnten keine Ureaplasmen isoliert werden.

Mittels histologischer Untersuchung von Gewebeschnitten wurde bei zehn der elf experimentellen Stiere eine Vesiculitis seminalis dextra diagnostiziert, während bei einem Stier Epitheldegeneration ohne Entzündung beobachtet wurde. Bei einem Kontrollstier wurde eine Samenblasenentzündung diagnostiziert. Bei den Kontrollen, die innerhalb einer Woche nach der Inokulation seziert wurden, konnten lediglich Blutungen in der rechten Samenblase beobachtet werden.

Aufgrund der mikrobiologischen und histologischen Befunde wurde die Schlussfolgerung gezogen, dass der zur Inokulation verwendete Ureaplasmenstamm unter experimentellen Bedingungen für die Samenblase des Rindes pathogen ist.

Résumé

Lors d'une expérience, on a voulu démontrer quelles conséquences pouvait avoir l'inoculation directe d'uréaplasmes dans la vésicule séminale droite de taureaux de la race Holstein, âgés de 1 à 3 ans.

La souche d'uréaplasmes utilisée pour l'inoculation fut isolée à partir de l'appareil génital d'une vache atteinte d'une vulvovaginite granuleuse. Onze taureaux reçurent un inoculum de 5 ml d'une culture d'uréaplasmes correspondant à 10^9 c.f.u. (colony forming units), alors que les 10 animaux de contrôle reçurent 5 ml du milieu de culture pour uréaplasmes.

Les taureaux furent abattus à l'intervalle de 1 ou 3 jours, 1, 4 ou 8 semaines après inoculation. A l'autopsie, des prélèvements de l'appareil urogénital furent effectués pour l'analyse bactériologique et histologique.

Des échantillons de sperme et de sécrétion sémino-urethrale, exempts de contamination praeputiale, furent prélevés aussi bien avant qu'après l'inoculation et soumis à un examen bactériologique.

Avant l'inoculation, on réussit à isoler des uréaplasmes à partir de l'éjaculat et de prélèvements au niveau du prépuce chez 20, respectivement 18 taureaux. Après l'inoculation les uréaplasmes dans le sperme des taureaux servant à l'expérience étaient en moyenne 60 fois plus nombreux que chez les taureaux de contrôle. Le sperme prélevé après l'inoculation contenait des leucocytes chez 8 des 9 taureaux choisis pour l'expérience, alors que chez les taureaux de contrôle, on dénotait une fois un nombre élevé de leucocytes, et deux fois la présence de quelques neutrophiles.

La mise en évidence d'uréaplasmes à partir de la vésicule séminale droite réussit chez les taureaux choisis pour l'expérience abattus après 1, 7 et 28 jours. A l'aide de la technique d'immunofluorescence, on a pu démontrer qu'il s'agissait de la même souche que celle utilisée pour l'inoculation. On n'a pas pu isoler d'uréaplasmes à partir des glandes sexuelles accessoires chez les taureaux de contrôle.

A l'examen histologique de la vésicule séminale droite, on a pu établir le diagnostic de vésiculitis seminalis dextra chez 10 des 11 taureaux servant à l'expérience. Chez les taureaux de contrôle, dont l'autopsie eut lieu durant la semaine qui suivait l'inoculation, on ne peut observer que des hémorrhagies dans la vésicule séminale droite.

Sur la base des résultats microbiologiques et histologiques on a tiré les conclusions suivantes: la souche d'uréaplasmes utilisée pour l'inoculation est, dans des conditions expérimentales, pathogène pour la vésicule séminale du bovin.

Riassunto

In un esperimento è stato indagato l'effetto della diretta inoculazione di ureaplasmi nella vescicola seminale destra di tori Holstein di età variante da uno a tre anni. Il ceppo usato per l'inoculazione è stato isolato dall'apparato genitale di una vacca, affetta da vulvovaginite granulare (la cosiddetta «Knötchenseuche»). L'inoculo per undici tori da esperimento consisteva in 5 ml di una coltura di ureaplasmi (ca. 10^9 unità formanti colonie), quello per dieci tori di controllo era costituito da 5 ml di terreno di coltura. I tori sono stati macellati ad intervalli di 1 giorno, tre giorni, una settimana, quattro o otto settimane e gli apparati urogenitali sono stati analizzati da un punto di vista istologico e microbiologico.

Prima della inoculazione si era riusciti ad isolare ureaplasmi dall'eiaculato di 20 tori e dai tamponi prepuziali di altri 18 animali. Dopo l'inoculazione il numero medio di ureaplasmi nei campioni di sperma degli animali da esperimento era 60 volte superiore a quello presente nei campioni dei tori di controllo. Gli eiaculati di otto dei nove animali da esperimento, prelevati dopo l'inoculazione, contenevano leucociti, mentre un toro di controllo presentava un eiaculato con un numero superiore di leucociti e due altri animali di controllo hanno presentato ognuno un campione di sperma con alcuni granulociti.

L'isolamento di ureaplasmi dalla vescicola seminale destra ha avuto successo in quei tori da esperimento che sono stati macellati dopo uno, sette e ventotto giorni. Per mezzo dell'immunofluorescenza si è potuto dimostrare che gli isolati provenivano dallo stesso ceppo, e cioè da quello usato per l'inoculazione. Non è stato possibile isolare ureaplasmi dalle ghiandole sessuali accessorie degli animali di controllo.

Per mezzo delle indagini istologiche è stata diagnosticata una vescicolite seminale destra in 10 tori da esperimento su 11, mentre in un animale è stata osservata una degenerazione epiteliale senza infiammazione. In un toro di controllo è stata diagnosticata una vescicolite. Negli animali di controllo, che sono stati sottoposti ad autopsia entro una settimana dalla inoculazione, si sono potuto constatare solo emorragie nella vescicola seminale destra.

Sulla base dei reperti microbiologici ed istologici si è potuto concludere che il ceppo di ureaplasmi usato per la inoculazione è patogeno, in condizioni sperimentali, per la vescicola seminale del bovino.

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Registration of the manuscript: April 1st, 1982