Reproduction Session

Dr. Neil Anderson, *Presiding*

Ureaplasma (T Strain Mycoplasma) Infection of the Bovine Reproductive Tract.

P. A. Doig, *D.V.M., M. Sc.,*
A. L. MacKay, *D.V.M., and*
H. L. Ruhnke, *B.S.A., M. Sc.*
Department of Clinical Studies,
University of Guelph,
Guelph, Ontario, Canada,
(Doig); Listowel Veterinary Clinic,
Listowel, Ontario (MacKay) and
Veterinary Services Branch,
Ontario Ministry of Agriculture and Food,
Guelph, Ontario (Ruhnke).

Introduction

The mycoplasmas or more correctly the "Mycoplasmatales", have been recognized as disease causing agents for over eighty years. The organisms were first isolated from cases of contagious bovine pleuropneumonia and for this reason, subsequent isolates were initially termed pleuropneumonia-like organisms (PPLO). In more recent years numerous additional mycoplasma species have been isolated from animals, birds, man, plants and insects. The group has now been divided into three major families based on morphological and biochemical criteria (16).

Taxonomy
Class - Mollicutes (bacteria with no cell wall)
Order - Mycoplasmatales
Family 1 Mycoplasmataceae sterol required for growth
Genus 1 - Mycoplasma

Commonly referred to as "large colony mycoplasmas". Do not hydrolyze urea. Have been divided into over fifty (50) species by biochemical and serological criteria. Species designation should be made when referring to these organisms.

Genus II - Ureaplasma

Formerly called T strains (T for tiny) but now ureaplasma because of ability to hydrolyze urea. Consists of a single species with a number of serotypes.

Family II Acholeplasmataceae - do not require sterol for growth.

Genus I - Acholeplasma
Seven species identified.

Family III Spiroplasmataceae - primarily plant and insect isolates.

General Characteristics of Mycoplasmas

The mycoplasmas are the smallest free living organisms, being smaller than some of the large viruses. Like bacteria but unlike viruses and chlamydia, they can live and reproduce on synthetic media.

An important characteristic of the organisms is that they do not have a cell wall and are therefore resistant to penicillin. Both pathogenic and non-pathogenic strains have been identified and with few exceptions, infections tend to be species specific in animals.

The organisms generally cause chronic rather than acute disease. Infections most often involve the lung, joints, urogenital tract, mammary gland and eyes.

From the practitioners viewpoint an important feature of the mycoplasmas is that only a limited number of laboratories, in a given area, may be equipped or capable of isolating the organisms. This is particularly true of ureaplasmas. Samples must be submitted in transport media or as frozen tissue and should reach the laboratory within twenty-four hours. It has been well established that ureaplasmas may not survive transport through the mail.

Bovine Reproductive Disease Associated With Mycoplasmas and Acholeplasmas.

A. *Mycoplasma Species*

Since the first isolation in 1947 (13) a number of Mycoplasma sp. have been recovered from the bovine urogenital tract or aborted fetuses. In most cases experimental studies have not been carried out to establish their exact role in bovine reproductive disease.

Mycoplasmas in this group include ***M. bovirhinis, M. canadense, M. sp. Group 7, M. alkalescens, M. alvi, M. araginini*** and ***M. verecundum*** (16).

Two Mycoplasma species have received more attention as possible bovine reproductive pathogens.

a) *M. bovigenitalium*

M. bovigenitalium has been identified in a number of studies as being a common bovine urogenital isolate (9, 14, 28, 33, 35). Although there has been serological evidence of an association between *M. bovigenitalium* infection and infertility in the cow (44), culture studies have been inconclusive. Isolation rates from cervicovaginal mucus of healthy and infertile cows have been reported to be similar (28, 35, 38). As well, the organisms were rarely recovered from uterine samples taken from infertile cows or aborted fetuses (6, 28, 29, 40). Pathogenicity in one study could not be demonstrated following uterine inoculation (14). Granular vulvitis was produced experimentally but only if the vulva was lightly scraped prior to inoculation (2). The failure to recover the organism from a high percentage of field cases precluded any cause effect relationship.

In bulls the situation is also controversial in spite of more experimental inoculation studies. *M. bovigenitalium* has been isolated from field cases of seminal vesiculitis (3, 8, 15) and the disease reproduced following direct re-inoculation (3, 15, 36). However, although cultures from the prepuce and semen of bulls in artificial insemination units have demonstrated a high level of contamination, there has been little evidence of associated disease or a detrimental effect on fertility (4, 8, 29, 33).

Additional studies are needed to clarify the role of *M. bovigenitalium* in bovine reproductive disease. At present there is little to indicate that isolates from the lower genital tract are associated with disease. The organism may be viewed as significant if recovered in pure culture from diseased portions of the upper reproductive tract, especially in bulls.

b) *Mycoplasma bovis*

Mycoplasma bovis is well known to most practitioners as a cause of bovine mastitis, polyarthritis and pneumonia. Most of the evidence incriminating the organism in bovine reproductive disease has come from experimental inoculation studies. Varying degrees of endometritis and salpingitis of sufficient severity to cause infertility were produced by direct uterine inoculation and following insemination with contaminated frozen semen (17, 18). The organisms were found to remain viable in frozen semen for as long as eighteen months when added prior to extension and freezing in liquid nitrogen (19).

Although abortion has been experimentally produced following direct inoculation into the amniotic fluid (41) and an association made serologically with infertility (44) there has been little supportive field evidence that *M. bovis* at present, plays an important role in bovine reproductive disease. Isolations from aborted fetuses have been infrequent and the organism has rarely been recovered from the uterus, cervicovaginal mucus or vulva of infertile cows (29, 35, 40).

B. *The Acholeplasmas*

The most common member of this family in genital infections is *Acholeplasma laidlawii* which has been isolated on a number of occasions from the lower genital tracts of cows and bulls as well as aborted fetuses. Although generally regarded as a nonpathogenic saprophyte, there have been reports implicating the organism in disease conditions.

One study on reproductive tracts collected at slaughter reported a significantly higher isolation rate from the oviducts of repeat breeder cows than from those slaughtered for other reasons. Culture positive animals also had a higher incidence of salpingitis and bursal adhesions (21).

Others however, have reported that *A. laidlawii* may be a common contaminant on the serosal surface of oviducts following removal of the tracts at slaughter (12, 39). Care is therefore needed during sampling to ensure that oviduct flushings have not been inadvertantly contaminated.

In spite of some evidence to the contrary (32, 34) it is still generally accepted that *A. laidlawii* does not play an important role in bovine infertility at this time. However, there have been a sufficient number of isolations from aborted fetuses (6, 40) to warrant additional investigations into the possible role of the organism in bovine abortion.

Bovine Reproductive Disease Associated With Ureaplasmas

A) *Cows*

a) *Early Studies*

Although Mycoplasma infection has been known for many years, bovine ureaplasma infection has only recently been recognized.

The first isolation of ureaplasmas from the bovine urogenital tract occurred in Britain in 1967 and an 11% incidence in the vagina of apparently healthy cows was reported (42). The first report associating ureaplasma with bovine genital disease was made in 1974. Ureaplasmas were recovered from nine of eleven vulvar swabs taken from cows developing a purulent discharge shortly after breeding as well as from the frozen semen used to inseminate one of the cows (5).

Other studies at this time however, failed to show a correlation between ureaplasma infection and infertility in the cow. Ureaplasmas were recovered from 14% of cervicovaginal mucus samples taken from young beef heifers without evidence of reproductive disease. As well, the organisms could not be recovered from eighty uterine samples taken from infertile animals (28). A later study compared the incidence of mycoplasmas and ureaplasmas in the cervicovaginal mucus of fertile and infertile cows. The incidence in fertile cows was 5.6% for mycoplasmas (predominantly *Mycoplasma bovigenitalium)* and 15.3% for ureaplasma. A higher incidence of 11.4% for mycoplasmas and 27.2% for ureaplasmas was found in the "repeat breeders" but the differences were not statistically significant (35).

b) *Ureaplasma and Granular Vulvitis*

In 1975 a field study was carried out in Ontario in response to numerous complaints from veterinarians and farmers that a new disease was causing serious problems in many dairy herds. The disease was characterized by a white sticky discharge from the vulva of cows which had been bred four to five days previously. The condition spread quickly to involve a large percentage of cows in the herd and recurrent infections were common. The main complaint eventually heard from many herdsmen was that the majority of their cows had a "dirty" discharge at heat and herd fertility rates were poor. The field study involved clinical evaluation of the disease in almost 2800 cows in a large number of herds as well as a detailed culture study of 81 cows in 16 herds (10).

Clinical examinations revealed very early that the "new" disease was, in fact, a more severe form of granular vulvitis. This disease was first described in 1887 and despite a number of subsequent studies, there was little agreement in the literature as to the etiology, clinical severity or resulting effect on fertility (24). The general consensus among most workers was that the disease was not a significant cause of infertility (30, 37).

The clinical syndrome in the Ontario study was found to have both acute and chronic forms easily distinguished by the presence or absence of a purulent vulvar discharge.

The acute form was observed when the disease first appeared in a herd. The main presenting sign was the sudden onset of a sticky purulent vulvar discharge. The volume of the discharge varied from a small amount observed on the tail or vulvar hairs to a larger volume a few days later which appeared to pool in the vagina before emptying behind the recumbent cow in 60 to 100 ml amounts.

Examination of the vulvar epithelium during the acute stage revealed an inflamed sensitive hyperemic mucosa with small 1 to 2 mm raised granules usually clustered around the clitoris. Purulent material was usually also observed in the ventral commissure. In severe cases the granularity extended up the lateral walls of the vulva and occasionally involved the dorsal commissure. Coalescence of the granules produced raised ridges resulting in a corrugated and very roughened vulvar mucosa. The granularity did not appear to extend cranially to involve the vaginal epithelium.

The vulvar discharge persisted for three to ten days before the disease progressed to a chronic form. In many cases the acute form would reappear at subsequent heats, resulting in repeated signs of cloudy estral mucus.

The chronic stage was characterized by an absence of a purulent discharge and a gradual decline in the severity of both the hyperemia and granularity. Occasionally an excessive discharge of clear mucus was observed which made heat detection difficult for many owners. The granularity gradually disappeared over the next few weeks and the vulvar epithelium returned to normal within six weeks to three months. Re-infection however, was a common occurrence. The disease became endemic within many herds and numerous re-infections were observed over a four year period. Previously infected cows were generally less severely affected on re-infection, with the acute stage being short or missed entirely. The chronic form of the disease is currently the most common with acute cases being relatively sporadic.

A characteristic component of the disease, observed in approximately 10% of affected animals was the formation of discrete raised white epithelial inclusion cysts, 2 to 5 mm in diameter and usually arranged in rows or clustered on the dorsolateral wall of the vulva or in the dorsal commissure. The cysts did not appear to form in the clitoral area but were observed on two occasions in small numbers on the lateral vaginal wall and outer cervical ring. A creamy white exudate could be expressed from the cysts during the acute stage with the contents tending to become dry and inspissated later in the chronic form.

They cysts had been described on only one previous occasion in two herd outbreaks of "concomittent granular vulvitis, palate lesions and respiratory illness in Connecticut dairy cattle" (48). The etiology of the condition was not determined but the outbreaks were observed prior to the recognition of bovine ureaplasma infections. What significance or part the cysts have in the disease has not been determined but it seems likely that they are formed as a result of proliferation of the inflamed epithelium.

Cultures taken from 81 cows in 16 affected herds, revealed for the first time, a strong association between ureaplasma infection and a specific bovine genital disease, bovine granular vulvitis (38). Ureaplasmas were isolated from 100% of acute cases, 74% of chronic cases and 23.5% of apparently normal herd mates. *Mycoplasma bovigenitalium* and *Hemophilus somnus* were also isolated from more diseased cows than normal herdmates (Table 1).

Significant differences were not observed in the isolation rates of other bacteria (Streptococci and *E. coli*). *Corynebacterium pyogenes* was isolated from a low percentage of cows with a purulent discharge.

TABLE I

Bacteria and Mycoplasma Isolations From Vulvar Swabs of 81 Cows From 16 Herds Affected With Granular Vulvitis

Clinical Classification	Total Cultured	Ureaplasma % Positive	Mycoplasma bovigenitalium % Posiitve	Coryne. pyogenes % Positive	Hemophilus somnus % Positive
Normal	34	23.5	0	0	0
Chronic Vulvitis	27	74	7.7	7.7	0
Acute Vulvitis	20	100	20	16.6	25

TABLE III

Conception Rates Associated With Bovine Granular Vulvitis In Four Dairy Herds

Herd Status	Number of Records Examined	1st Service Conceptions %	2nd Service Conceptions %	Services per Conception
Before disease appeared	494	55.9	74.9	1.83
During acute form	230	28.8	51.4	2.71
During intensive treatment	51	51.7	73.7	1.81
During chronic form with sporadic treatment	343	42.2	69.4	2.16

The disease incidence in one practice area was closely monitored over a seven month period, with a definite seasonal variation being observed (Table II). During mid summer the incidence reached a low of 37% with the average for the summer months being slightly less than 50%. During the winter months with more confined housing the incidence reached a high of 75% with the average being 61%.

In cows examined because of a failure to conceive to one or more breedings, the average incidence was slightly higher; 69% during the summer and 75% during the winter.

TABLE II

Seasonal Incidence of Bovine Granular Vulvitis (Based on 2784 observations)

Season	In All Cows Examined	In Repeat Breeder Cows
June - August (Summer)	44.6%	69.1%
September - December (Winter stabling)	61.1%	75.0%
Average	54.5%	69.8%

The influence of the disease on fertility was evaluated in four herds by examining 1118 reproductive records for a four year period. With the exception of one farm where natural service was temporarily used, all herds utilized artificial insemination.

The acute form of the disease when widespread in herds had a severe effect on fertility. In one 35 cow herd, first service conception rates dropped by 48% (from 69% to 21%) with five (5) cows being subsequently culled due to persistent infertility. A second 300 cow herd experienced a 30% (64% to 34%) drop in first service conceptions over a two month period immediately following the appearance of the disease. On the average in all herds, first service conceptions during the acute form were reduced by 27% resulting in 2.7 services per conception (Table III).

In one thirty cow herd first service conceptions over the winter months during the acute stage were 25%. During the following summer months the owner introduced natural service with a young bull and first service conceptions improved to 65%. By fall, conception rates were again declining and natural service was discontinued. First service conceptions over the next eight months again plummetted to 17%.

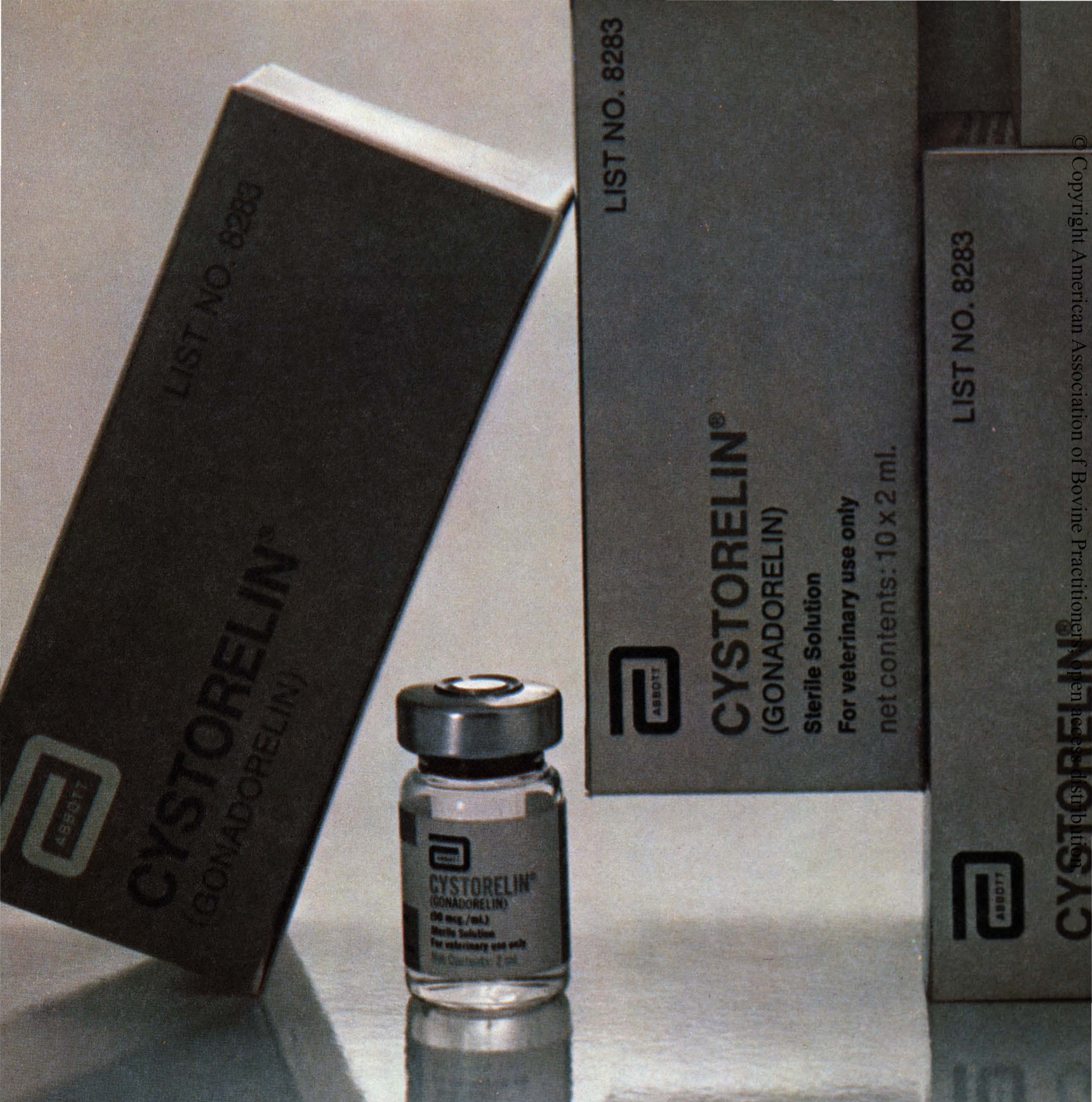
Treatment with conventional douches, suppositories and uterine infusions were used initially in an attempt to control the disease and improve conception rates. Products containing nitrofurazone, chloramphenicol, chlorhexidine, 0.2% Lugol's iodine or acriflavine solutions were judged to be of little value. When it was determined that ureaplasmas were associated with the disease, it was found that intrauterine infusions with approximately 1 gm tetracycline solution*, 24 hours postbreeding, resulted in a significant improvement in conception rates.

In herds where conceptions were greatly reduced, intensive treatment was instituted and all affected cows were treated 24 hours after artificial insemination. Conception rates with this treatment returned to the level observed prior to the appearance of the disease (Table III). Later with the chronic form of the disease, treatment was more sporadic and limited to only repeat breeders and cows with a purulent discharge. Conception rates with this form of therapy improved but remained approximately 10% lower than levels for the herds prior to the infection.

The findings from this field study were similar in many respects to those reported earlier. However, there were a number of findings which were different from those reported in the literature, and these merit further discussion.

Since the first description in 1887, few diseases have remained as controversial as bovine granular vulvitis. There has been little past agreement as to the etiology,

* *Reverin Suspension, Hoechst Pharmaceuticals, Montreal, Quebec.*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clinical severity or possible effects on fertility. It has also been stated that "no disease of the reproductive system of cattle has been so unproductive of results on research as granular venereal disease" (37).

One of the factors contributing to the controversy may be the failure to adequately characterize the syndrome. In our opinion, granular vulvitis should *not* be viewed as a single entity, but rather a disease with considerable clinical variation including both acute and chronic forms. The differentiation of the disease as to severity and duration of infection is very important, both for the ease of isolating ureaplasma and for the subsequent effect on fertility. It is well recognized that the mild chronic form, common in a large percentage of cows, may not be associated with a demonstrable effect on fertility. This should not be interpreted however, as meaning that granular vulvitis in general, is an unimportant reproductive disease. The acute form when widespread in a herd can have serious effects on fertility, comparable in many cases to that seen with Campylobacter infection.

Another variable which may be involved in the effect on fertility is the possible existence of strain differences between ureaplasma isolates. Both virulent and avirulent strains have been demonstrated by intramammary inoculation and some variation in the degree of pathogenicity also observed (22, 23).

Only one previous study has been reported on the influence of granular vulvitis on fertility following artificial insemination (45). Severely affected cows had a 10% reduction in 60 day non returns, and mildly affected 3% when compared to controls. Severe cases were reported to have many nodules plus inflammation. It is not clear whether a purulent discharge was present in all or any severe cases. Our findings of much lower conceptions with acute cases may be due to the fact that our "acute" classification was much more restricted. Many of our chronic cases also had numerous nodules and inflammation (hyperemia) and the resulting 13.5% reduction in conceptions compares closely to the previous findings.

Some clarification is also needed with respect to Infectious Pustular Vulvovaginitis (I.P.V.). In many field cases, acute granular vulvitis was initially erroneously diagnosed as I.P.V. since this was thought to be the main cause of vulvitis associated with a mucopurulent discharge. It is now recognized that the volume and duration of the mucopurulent discharge is greater in most cases of acute granular vulvitis than with I.P.V.

The diseases can be differentiated readily on clinical signs since granular vulvitis is a productive raised lesion while I.P.V. is associated with necrotization and erosion. A further cause of confusion is the fact that granular vulvitis may follow I.P.V. infection (27). In one herd where this occurred, followup cultures revealed that all cows developing granular vulvitis were also positive for ureaplasma infection (10).

Treatment of granular vulvitis has been no less controversial. The most common type of therapy used has been in the form of various douches usually containing either irritant drugs or antibiotics. Autogenous bacterins have also been reported to be of value. The tremendous variability in past treatments has prompted some to question the value of any form of therapy (37).

In affected herds in this study, utilizing artificial insemination, the treatment procedure was directed toward reducing possible mechanical transmission of infection from the vulva to the uterus. Uterine infusions with a tetracycline, twenty-four hours after service appeared to be effective in improving conception rates.

The use of a "double rod" technique by inseminators to minimize the possible contamination of the pipette as it passes through the vulva, has also been used with apparent benefit in many herds. Many severely affected farms also utilized "cleanup" bulls in an attempt to improve fertility rates. In most cases the introduction of natural service was followed by a dramatic improvement in conception rates but the favourable response in some cases was temporary. Since the improvement was similar to that observed with postbreeding antibiotic infusions, it likely resulted from a reduction in the mechanical transmission of the organisms from the vulva to the uterus, rather than improved heat detection.

c) *Experimental Transmission Studies*

Following the field study which demonstrated an association between ureaplasma infection and granular vulvitis, experimental studies were carried out to determine pathogenicity under controlled conditions.

In the initial study, granular vulvitis was consistently reproduced in virgin heifers following inoculation of the vulva without prior scarification (11). Clinical signs appeared one to five days postinoculation and once infected it was demonstrated that the heifers could become carriers for as long as seven months. There was no indication of colonization of the upper reproductive tract in sexually rested heifers following vulvar inoculation.

A second study determined the effects of cervical and uterine inoculation of virgin heifers with ureaplasma (12). Granular vulvitis was produced in a high percentage (14 of 16) of heifers following an incubation period of three to four days. As well, over 50% of inoculated animals had histopathologic evidence of endometritis and/or salpingitis during the first seven days postinoculation. Also important was the finding that although evidence of disease was produced, the organisms were cleared from the uterus after seven to nine days and remained only in the vulva. This finding suggests the need for caution in the interpretation of negative uterine or cervicovaginal mucus cultures from infertile cows. Failure to recover ureaplasmas from uterine swabs or cervicovaginal mucus taken at the time of return to estrum or later, may not be grounds for eliminating the organisms as a cause of the preceding infertile breeding. The localization of infection in the vulva which can persist for as long as 200 days, can be a possible source of re-contamination of the uterus at each breeding. If introduced, or repeatedly re-introduced into the uterus at each breeding, the persistence of the organisms for up to seven days may be sufficient time to create an abnormal uterine environment for the fertilized egg. The recovery of ureaplasma in pure culture from embryo transplant flushings containing fertilized but degenerate ova supports this concept (40). The improvement in fertility seen in cows with granular vulvitis following postbreeding infusions with tetracycline may have been due to a more rapid elimination of the organisms from the uterus.

Although the vulva is the preferred site for culture in cases of granular vulvitis care should be taken in interpreting positive cultures obtained from vulvar swabs of clinically normal animals. Up to 20% of normal cows may carry ureaplasma in the vulva which makes interpretation of single isolates difficult. Positive recoveries from the upper reproductive tract may be viewed as more significant since the organisms are rarely found in this location in normal animals (7, 39).

The existence of both pathogenic and non-pathogenic strains of ureaplasma has been demonstrated by intramammary inoculation (22) but until more pathogenicity tests are developed and utilized the controversy concerning the role of ureaplasma in bovine infertility will no doubt persist.

The role of ureaplasma in pregnancy termination also needs further assessment. Recoveries have been made from the stomach contents (6, 29) and lungs (40) of aborted fetuses, as well as vaginal mucus (6) and maternal caruncles (40) of the dams immediately following the abortion. Preliminary experimental inoculation studies have demonstrated that ureaplasmas are capable of causing abortion, stillbirths, the birth of weak infected calves at term and placentitis (31). A high incidence of early fetal loss in the first ninety days of pregnancy has been observed in herds with granular vulvitis and ureaplasma has been recovered from uterine swabs taken from the cows on return to estrum (40).

B) *Bulls*

A number of studies throughout the world have demonstrated the presence of ureaplasmas in the preputial cavity and semen of bulls at artificial insemination (A.I.) units. In the initial British studies the organisms were recovered from 100% of ten preputial cultures and 84% of 32 cultures taken from raw semen of bulls reported to have normal fertility (43). A lower incidence was reported in each of two Canadian studies. In a culture study of bulls in A.I. units in Ontario, 35% of 132 preputial cultures, 24% of 140 raw semen cultures and 14% of 42 processed semen samples were positive (33). A similar study in Alberta involving animals in artificial insemination units and a group of range bulls, reported that 29% of 267 preputial samples and 23.2% of 168 semen samples were positive (29). Studies in Czechoslovakia reported the recovery of ureaplasma from 46.5% of 202 semen samples from bulls in regular service at artificial insemination units. Mycoplasmas, predominantly *Mycoplasma bovigenitalium,* were also present in approximately 27% of the samples (26). Comparison of positive samples with those that were negative revealed a significant correlation between semen contamination and impaired spermatozoa motility (25).

Although the significance of ureaplasma and mycoplasma in bulls and the effect of subsequent contamination of semen on subsequent fertility has not yet been determined, studies are underway to document shedding characteristics and to develop methods of control. It is known that there is considerable variation between A.I. centres in the proportion of positive bulls and positive semen samples produced (26, 29, 40). Recent studies have also demonstrated that there is a significant and unpredictable variation in the numbers of organisms present in semen collected at different times from the same bull (49). Further variation occurs due to dilution, processing and freezing with the end result being that only a small percentage of processed straws from a given collection may contain these organisms. Quantitative assessments, moreover, have demonstrated that numbers of organisms should be determined since positive samples may contain only a few organisms or more than 1,000 colony forming units (26, 40).

The preputial cavity appears to be the main source of semen contamination in bulls collected with a sterile artificial vagina. However, it has been demonstrated in some bulls, that the urethra may also be heavily colonized (49) suggesting that any procedures instituted to reduce the number of organisms in the prepuce may not always be effective in reducing semen contamination.

Although seminal vesiculitis has been consistently produced following direct inoculation with ureaplasma, there is little indication thus far, that carrier bulls, in general, have a higher incidence of disease in the upper reproductive tract (49).

One difficulty in formulating control procedures for ureaplasma in semen is that there is no rapid method for differentiating pathogenic and non-pathogenic strains. For this reason, it is not known what percentage of semen isolates are pathogenic or what level can be considered safe in semen. It has been demonstrated by intramammary inoculation that semen isolates may differ in pathogenicity. Of six isolates tested, two were found to be pathogenic with one producing mastitis rapidly and the other after a period of several days (23).

Until better methods of determining pathogenicity are developed, all ureaplasma isolates from semen should be regarded as potentially significant and attempts made to eliminate the organisms. The addition of lincospectin to extenders has been used to control mycoplasmas in semen but was not found to be as effective against ureaplasma (46). The further addition of minocycline hydrochloride appeared to effectively control mycoplasmas and ureaplasmas without being detrimental to sperm quality (47). Unfortunately, the antibiotic can only be used safely with milk extenders (1). Further studies are currently underway to test other antibiotics for possible use in egg-yolk extenders.

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Panel Discussion

Question: In the dairy herds where you were following did you happen to be able to trace any of, or relate the infection rate all back to the bulls that were bred, by analysis of the bull numbers, Number 1 and number 2, do you use tetracycline oil based product which we can't get in the states, does anybody work with PP Iodine or do you know of any other type of control agent, whether it is antibiotic or antibacterial?

Answer: I understood the first question you are wondering if we could relate it to particular bulls initially, is that it? Right back at the start when we still had not determined what we were dealing with, I had a handful of bulls that I knew what was going to happen when they were used. I did not know why but you could relate it to a specific unit sires at one time or natural services. As for the second part of the question, we have, as I indicated, tried to avoid everything possible even before we realized what we were dealing with, and found them not very effective. As far as other tetracycline preparations that might be available to you people, I think our assessment of them was they were somewhat effective but probably not as effective as the one we were using, so I am not sure just how you people can handle that.

Used locally I think that if you use strong enough solutions to burn if off but as far as infusions, no they were not effective. We did not find them to be effective as post infusions. Topically the preparation that I mentioned is very mild to the mucosal surface and does a real nice job and clinically my impression is that the other products do not do nearly as well, but I am not sure what the answer to your dilemma is. It was the first product that I was using which is the oil based tetracycline the same as what I was post-infusing with. We just put a little bit on the cotton swab instead of infusing it. Initially we tried things like this also, but it was going through our herds in a very severe form, then, because it was their first exposure after we determined what was going on in the first few herds, and we found that if we infuse the cow or swabbed her or anything like that, we cleared it up in three or four days. And so she is two weeks or so away from her next heat cycle that he wishes to breed her and by then she is reinfected and we did not gain anything. We withheld milk and so on. So this is why I have stressed a post-infusion in my initial presentation. Now we are double riding this sort of thing and not doing very much post-infusion and I think this is probably the way you people can handle this that do not have some such product readily available. It is a more logical route, it just took us a while to work around it, because that is what we are doing now. We are not treating very often at all, we are trying to eliminate the contamination.

Question: What are you using as a swab and for transport medium in that you culture from the prepuce and what are your rod sizes and are you using the double rodding?

Answer: I will handle the second question and let Dr. Doig answer the first one. We are just using a conventional inseminating rod that has a larger diameter, larger inside bore and most of our straws are the half cc size, half ml size and there is an insemination rod that fits I think a universal gun that has a larger inside diameter than the regular infusion inseminating pipette. The technicians cut the tapered end off and insert that. Now I would hope that some time in the future somebody will come up with a commercial unit that is more suitable and in our embryo transfer field we do something simpler than that and I find easier. It is time-consuming for the people who make it up for me and that is we use the drinking straw and cover it with one layer of foil and attach a string to it and individually package them and autoclave them. The end is covered that way and you do not have to worry about carrying any contamination in on the very end of the rod the way when they are inseminating with the cut off rods. But it is not practical on a large scale as you can appreciate. It takes time to make these up. But hopefully some company will get on the road and make one for us.

The other one was about culturing bulls. Well as we showed, the transport media culturette, do not send in a dry swab. Preferably if you are going to bother taking this swab, take the time to have somebody drive it to the laboratory. We know from bitter experience that you are wasting your time by sending it in the mail.

Question: Was there a mastitis problem in any of these infected herds with this organism?

Answer: Ureplasma experimentally is one of the ways they test pathogenicity with regards to mastitis, but no. We have not associated mastitis with this syndrome.

Question: I believe you mentioned that the disease is seen in dogs. How is the lesion observed in the male dog and is that possibly one of the means of transmission in the dairy barn?

Answer: The dog in the barn is purely a mechanical transmitter from his tongue to another cow. The dog does not get infected. Ureaplasma in the dog as a cause of infertility does not produce a granular vulvitis but it will produce a penoposthitis. But the only way we can demonstrate infertility with ureaplasmic dogs is culture. There are no clinical signs other than purulent discharges but the farm dog should not be infected with the bovine ureaplasma. He merely mechanically transmits it as we could do with scoping if not clean.

Question: Dr. Doig, are you seeing this condition in virgin heifers, say 10-month heifers that have never been serviced?

Answer: Yes, once it gets rolling in the barn the disease will appear at about 4 months of age in the other end of the barn. It is seen as early as a month of age once it gets in the barn. In humans it is interesting that newborn infants will be cultured positive at birth then culture negative but culture positive at puberty. Where it has been in the interim I have no idea. So then I think it probably has spread by aerosol. Interestingly, in the 1920's it was stated in a very extensive monograph on granulovulvitis that this was transmitted in nonpasteurized milk. Leave that for what it is, but it certainly will appear in heifers the other end of the barn.

Question: I found that after a double riding program has been started in a barn, it may still take 5 to 6 breedings to get that cow in calf. Now if it is eliminated from the uterus, is it being reintroduced by the semen?

Answer: Well it may not be totally eliminated from the uterus, we have said if it is in the uterus, somebody put it there. That is probably true in the