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A Clinical Review of Bovine Ureaplasmosis

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INTRODUCTION
Ureaplasma has recently been associated with several bovine disease conditions. It has been isolated from field cases of calf pneumonia, keratoconjunctivitis, mastitis, seminal vesiculitis, granular vulvitis, endometritis, salpingitis and abortions. The role ureaplasma plays in each of these conditions is still under study, but its part in bovine reproductive disease is becoming better understood. While once considered a part of the non-pathogenic microflora of the lower reproductive tract, it has now been proven as a cause of granular vulvitis and is very closely associated with other reproductive disorders. Its importance in repeat breeders, long term infertility and decreased fertility rates are now being recognized. With improved means of diagnosis and better understanding of the organism, the total role ureaplasma plays in reproductive disease may soon be realized.

ORGANISM
Ureaplasma and Mycoplasma make up the two genera in the family Mycoplasmataceae. They are the smallest free-living organisms known to man ranging from 0.2-1.0 μm in diameter. While similar to bacteria in their ability to live and reproduce on artificial media, they differ in that they lack a rigid cell wall. This accounts for their poor Gram staining ability and pleomorphic appearance. Both genera require sterol for growth. However, the formation of tiny colonies of ureaplasmas (formerly called T-mycoplasmas because of this colony characteristic) on artificial media led to the initial differentiation between the mycoplasmas and ureaplasmas. Other differences such as optimal growth at pH 6, inhibition by thallium acetate (a common bacterial suppressant), and hydrolysis of urea have lead to the current classification: Ureaplasma. The medium presently used in most diagnostic labs for ureaplasma identification is called Hayflick's medium. It contains a combination of mycoplasma broth, yeast extract, horse serum, urea crystals, phenyl red and penicillin. The organism grows on Hayflick's agar or broth at 37°C with 4% CO₂. Positive broth cultures turn “hot pink” without evidence of turbidity, usually within 18-24 hours. The presence of turbidity indicates bacterial contamination which may cause a false positive reading. Positive agar plates can be observed at 24-48 hours with a dissecting microscope, or can be directly observed by staining with one drop of a 1:1 mixture of 10% urea and 2.8% MnCl₂. Within a few minutes the colonies will form a black precipitate due to their urease activity.

Currently, the species Ureaplasma urealyticum has been proposed for the human isolates, of which eight serotypes have been identified. At least eleven bovine serotypes have been identified and controversy exists as to whether these should be placed in the human species, classified as a subspecies, or placed in a totally different species. Gel electrophoresis comparisons of bovine and human isolates reveals significant differences between the two, possibly enough to warrant the naming of a new species. Both virulent and avirulent strains of bovine ureaplasma have been demonstrated by means of intramammary injection. While ureaplasma is not a common cause of mastitis in the cow, injection into the teat serves as a good means of evaluation of virulence. The existence of avirulent strains is no doubt confusing the theories and tests for pathogenesis,
clinical signs, and incidence of this disease.

Ureaplasma was first isolated from human non-gonococcal urethritis in 1954. Subsequently, researchers have found ureaplasma in the urogenital tracts of cows and bulls. The organism has been isolated from field cases of bovine granular vulvitis, endometritis, salpingitis, abortion, seminal vesiculitis, calf pneumonia, keratoconjunctivitis and mastitis. The precise role of ureaplasma in each of these disease states remains to be learned, but its importance in bovine reproductive disorders is becoming more and more apparent.

**FEMALE BOvine GENITAL INFECTION**

The first bovine ureaplasma isolation was made in 1967 from scrapings of the vaginas, urethras, and urinary bladder walls of cows at slaughter. The association with bovine genital disease was made in 1974 when ureaplasmas were isolated from 9 of 11 vulvar samples from cows with purulent vulvar discharge shortly after breeding. Further study of granular vulvitis in 1979 revealed two forms of the disease, acute and chronic, readily differentiated by the presence or absence of purulent vulvar discharge.

The acute form is usually seen 3–6 days post-breeding, spreading rapidly to other cows in the herd. Often first noted is a sticky, purulent, vulvar discharge on the tail, vulvar hairs, or in a 60–100 ml pool behind recumbent cows. During this stage, the vulvar epithelium is inflamed and hyperemic with varying degrees of granularity. Granules are usually 1–2 mm in diameter, raised and clustered around the clitoris. Occasionally the granularity extends up the lateral vulvar walls but rarely does it spread cranially to involve the vaginal epithelium. Histopathologic exam of the granules shows them to be diffuse and focal accumulations of lymphoid cells within and beneath the squamous epithelium. Vaginal discharge generally persists 3–10 days before the disease progresses to the chronic form. Reappearance of a cloudy discharge during subsequent heats is often noted.

The chronic form of the disease is characterized by absence of purulent discharges and decline in the severity of the hyperemia and granularity. The granularity gradually declines, returning to normal in weeks to months, but relapses are common. Often remaining is a swollen vulva with excessive mucous discharge making heat detection difficult. Previously infected cows are generally less severely infected and may or may not show a short acute phase.

One other clinical sign seen in about 10% of infected animals is the formation of discrete, raised, white cysts. These cysts are approximately 2–5 mm in diameter, often seen in clusters or rows along the dorsolateral wall of the vulva or in the dorsal commissure. Histologic examination shows epithelial inclusion cysts similar to those commonly found in dog skin. Expression of the nodules during the acute stage reveals a creamy, white exudate. Later, the contents become inspissated and are only indicative of a past granular vulvitis syndrome.

While nodules, cysts, and mucous discharge make up the primary clinical signs and lesions associated with granular vulvitis, the main problem encountered is the organism's effect on fertility. Three major effects have been associated with ureaplasma granular vulvitis: early embryonic death, abortion, and repeat breeders. Precise data is not available in this area due to lack of experimental study. However, many practitioners have noted that in herds showing signs of granular vulvitis, cows determined pregnant at 40–45 days by rectal palpation have returned to estrus between days 60 and 80. These cows usually exhibit a purulent vulvar discharge at this time. Uterine cultures taken from these cows at estrus have been positive for ureaplasma, sometimes in pure culture.

While ureaplasma has been incriminated as a cause of bovine abortion, further study is needed to verify the exact role the organism plays. In one study, four pregnant cows were given intra-amnionic inoculations of ureaplasma at four, five, seven, and eight months of gestation. Three cows, inoculated at four, seven, and eight months aborted 113, 25, and 29 days post-inoculation respectively. The cow inoculated at five months carried to near term and delivered a weak calf. All four cows had retained placentas. All four fetuses showed pneumonia and amnionitis, and ureaplasma was isolated from three of the four lungs. In a later field study of abortion cases associated with ureaplasma infection, the organism was isolated from the lungs of all cases of abortion during various times of gestation.

Ureaplasma was cited as the cause of repeat breeding and overall decreased fertility in On-
tario dairy herds. Fertility was measured in terms of first service conception rates. During the acute stage of the disease, first service conception rates dropped an average of 27% from 55.9% before the disease appeared to 28.8% during the acute form. In one herd a first service conception rate of 25% was increased to 65% by the use of a new young bull. Unfortunately, conception rates declined to 17% within six months possibly indicating ureaplasma infection of and spread by the bull.

**MALE BOVINE GENITAL INFECTION**

Ureaplasma was isolated from the preputial cavity and seminal fluids of bulls in 1969. Failure to isolate the organism from testicles, vas deferens, and urethral mucosa of bulls at this time led to the suggestion that organisms from the prepuce contaminated the seminal fluid. More recently ureaplasma has been isolated from raw semen, commercially processed and frozen semen, preputial swabs and washes, urethral mucosa and urinary bladder mucosa. The organism has been found in seminal vesicles, ampullae, and other upper reproductive tract organs only experimentally, and has not yet been associated with any disease process occurring there. But in a recent study 11 yearling holstein bulls were inoculated with a ureaplasma broth culture in one testicle and necropsied at various intervals. Seminal vesiculitis was seen from three days post-inoculation until the experiment ended eight weeks later. Histologically an acute inflammation was seen at three days, chronic active lesions were seen at one to four weeks, and chronic interstitial fibrosis seen at four to eight weeks. Ureaplasma was isolated from the seminal vesicles of these bulls only up to four weeks post-inoculation. Since the organism could not be isolated from advanced cases of the disease, a true cause-effect relationship between ureaplasma and field cases of chronic seminal vesiculitis cannot be made at this time. Therefore, at present, no disease states or clinical signs or lesions have been positively associated with ureaplasma infection of the bull.

**INCIDENCE**

The exact incidence of *Ureaplasma* spp. in bovine herds has not been determined. A 1975 Canadian report stated that ureaplasma appeared to be a part of the normal flora of the cervico-vaginal region. The organism was isolated from 88/633 samples taken from clinically normal one and two-year-old bred heifers in Alberta and Saskatchewan. Another study of 81 cows from 16 Ontario dairy herds isolated ureaplasma in 8/34 clinically normal cows, 20/27 cows with moderate vulvar hyperemia and granules, and 20/20 cows with acute vulvar hyperemia, granules, and purulent discharge. The disease was studied for seasonal variation by observing 2784 cows in 16 herds over a 7-month period. A variable rate of infection was found with a midsummer low of 37% infection and a midwinter high of 75%.

Researchers in England found 23/88 bulls sampled in one artificial insemination center and four bulls on separate farms to be ureaplasma-positive from preputial swabs. A Canadian study isolated ureaplasma from 46/132 preputial swabs, 34/140 raw semen, and 6/42 processed semen samples. Clearly, further study is needed to determine the clinical incidence of the disease in the United States and other countries.

**TRANSMISSION AND PATHOGENESIS**

The question of transmission is one that is essential for control of the disease, and while no one answer has been definitely proved, several theories have been brought forward. An increased incidence of the disease during the winter months with more confined housing is suggestive of a direct or close contact transmission. In two cases the farm dog was suspected of spreading the disease by licking vulvas. Certainly the bull must be considered as a prime source of transmission of granular vulvitis. While transmission via contaminated semen has not yet been proven, the finding of increased incidence of granular vulvitis immediately post-breeding would seem to suggest transmission during natural breeding. Since the organism has been found in processed semen, transmission during artificial insemination should also be kept in mind.

The role of the site of deposition of the organism was the topic of two separate studies on disease pathogenesis. In one study granular vulvitis was consistently reproduced in virgin heifers following inoculation of the vulva without prior scarifications. Clinical signs occurred one to five days post-inoculation and it was found that carriers could occur as long as
seven months post-inoculation. There was no evidence of upper reproductive tract infection in these heifers. Effects of cervical and uterine inoculation were determined in a second study.\textsuperscript{18} Granular vulvitis was produced in 14/16 heifers following a three to four-day incubation. More importantly, greater than 50\% of inoculated animals showed histopathologic evidence of endometritis and/or salpingitis up to seven days post-inoculation. It was also found that while the organism was cleared from the uterus seven to nine days post-inoculation, it could still be found on the vulva greater than 200 days later. This brings up an important point in the pathogenesis of the disease. If introduced, or repeatedly re-introduced into the uterus at each breeding, the persistence of the organism in the uterus for up to seven days may be sufficient time to create an abnormal uterine environment for the fertilized egg.\textsuperscript{11} The recovery of ureaplasma in pure culture from embryo transplant flushings containing fertilized ova supports this concept. This could easily be correlated with the repeat breeder syndrome most commonly associated with granular vulvitis. An abnormal environment would prevent ova implantation and a normal cycle would ensue.

**DIAGNOSIS AND TREATMENT**

Perhaps of greatest importance to both the producer and practitioner is diagnosis, and prevention or treatment of the disease. One problem currently encountered is that few labs are set up to successfully isolate the organisms. Presently, both agar and broth for ureaplasma isolation require several pH adjustments and sterilizations and have shelf lives of only one month. Also, proper collection and transport of samples is of utmost importance. Specimens should be collected as aseptically as possible. Culturette swabs or any swab with some provision for moisture, such as Amies or Stuarts transport media are suitable for transport, but for best results a tube of HayBick's media with urea should be used. Hopefully, this will be readily available at diagnostic labs in the future. Rapid delivery to the lab is essential in correct diagnosis and all samples should be sent on ice. The organism will not withstand more than 24 hours transport time and should never be sent by mail.

Another problem is proper interpretation of culture results. Preputial swabs are commonly taken for isolation of ureaplasma in bulls. However, care must be taken interpreting negative cultures since it has been demonstrated in several bulls that the urethra may be heavily colonized and may harbor organisms after the prepuce has been cleared.\textsuperscript{11} Similar problems are encountered when evaluating results from cows. While vulvar swabs are the most common method of diagnosis, it must be kept in mind that up to 20\% of clinically normal cows may carry ureaplasma in the vulva. Positive uterine cultures are highly significant because the organism only remains in the uterus seven days after inoculation. Unfortunately, cultures are rarely positive for the same reason.

Efforts at disease control are normally directed towards two goals: prevention of infection of the cow and treatment of the infected cow. Prevention can consist of clearing infected bulls to eliminate shedding of the organism, or eliminating the organism from semen before inseminating with it. Cleanup of infected bulls would involve extensive treatment using preputial infusion and/or parenteral injection. While little research has been done in this area, in vitro tests have showed the organism to be especially sensitive to tetracycline, declomicin, tylosin, rosaramicin and minocycline hydrochloride.\textsuperscript{19,20} Unfortunately, little is known of how this will apply to parenteral or preputial administration.

Known shedders of the ureaplasma organisms can be handled by incorporation of certain antibiotics in the semen extenders. A combination of minocycline hydrochloride and linospectin were found to be effective against ureaplasma and mycoplasmas.\textsuperscript{21} Unfortunately, this combination is only compatible with milk extenders. Additional studies are under way to develop methods of controlling the organism in other extenders.

Control of the disease in herds with infected cows can be handled by one or more methods. The use of a "double rod" technique by inseminators to minimize possible contamination of the pipette as it passes through the vulva has been used with apparent benefit in many herds.\textsuperscript{11} Many severely affected farms also utilize "cleanup" bulls in an attempt to improve fertility rates.\textsuperscript{11} Effects with this approach are often temporary and may be due to a reduction in mechanical transmission. Perhaps the most widely practiced approach is the use of uterine douches, suppositories, or infusions containing...
either irritant drugs or antibiotics. Products containing nitrofurazone, chloramphenicol, chlorhexidine, 0.2% Lugol's iodine, or aeriilafine solutions were judged to be of little value. When it was determined that ureaplasmas were associated with the disease it was found that intra-uterine infusion with 1 gram tetracycline suspension, 4 24 hours post-breeding resulted in a significant improvement in conception rates. 5 Perhaps this process functions to more rapidly eliminate the temporary colonization of ureaplasma in the uterus. In one study in herds where conceptions were greatly reduced, all affected cows were treated 24 hours after artificial insemination. 6 Conception rates with this treatment returned to the level observed prior to the appearance of the disease. 8 Later, with the chronic form of the disease, treatment was more sporadic and limited to only repeat breeders and cows with purulent discharge. Conception rates with this form of therapy remained 10-12% lower than the level observed prior to the appearance of the disease. 9

CONCLUSION

The role ureaplasma plays in granular vulvitis and other reproductive disorders is becoming better understood. Its incidence, transmission and pathogenesis are currently under study and more knowledge in these areas will lead to improved means of diagnosis and treatment. The sooner this knowledge can be passed on to practitioners and herdsmen, the sooner the disease can be totally recognized and hopefully brought under control.

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