Dairy Split Session II

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Bovine Reproductive Failure Associated with Ureaplasma Diversum

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Introduction

Ureaplasmas are part of the group of microorganisms designated the Mycoplasmatales, more often simply referred to as the mycoplasmas. Thus far, eleven ureaplasma serotypes have been serologically identified and appear distinct from the eight serotypes of *Ureaplasma urealyticum* isolated from humans. For this reason, a new name *Ureaplasma diversum* has been proposed for the bovine isolates.

Ureaplasmas share many of the characteristics of other mycoplasmas which are the smallest free living organisms. They are characterized by the absence of a rigid cell wall and are therefore resistant to many antibiotics, most notably Penicillin. Like other bacteria they can live and produce on synthetic media although the growth requirements are very specific.

Both virulent and avirulent strains have been demonstrated by intramammary inoculation, but a reliable method of identifying pathogenic serotypes by serological or other means has not yet been developed.

Ureaplasmas were considered for many years to be part of the nonpathogenic microflora of the lower reproductive tract. More recent studies have demonstrated an association between Ureaplasma and disease, and it is now apparent that Ureaplasmas may play a more important role in bovine reproductive failure than had previously been recognized.

Clinical Forms

A. *Granular Vulvitis (infertility) Syndrome*

Bovine granular vulvitis, characterized by the formation of discrete raised red to brownish coloured granules in the vulvar mucosa, has been a recognized clinical syndrome for many years.

Over the years, there has been controversy over the role of disease in infertility, due in part to a failure to correlate the severity of the lesion with the effect on fertility. The mild form characterized by only a few granules may have little or no effect on fertility while the acute form with associated hyperemia and a purulent discharge can have a severe effect.

When the disease first appears in a herd, the predominant clinical feature is a profuse sticky mucopurulent vulvar discharge. In the most acute form, the discharge may pool in the vagina and empty behind the recumbent cow in 60 to 100 ml amounts. In many cases rectal palpation may precipitate an emptying of the purulent material in the absence of palpable abnormalities. Less severe discharges may be noted on the tail or vulvar hair or occasionally may only appear as purulent flecks in otherwise clear estral mucus.

The vulvar epithelium during the acute purulent stage is inflamed, sensitive and hyperemic. Small 1-2 mm raised granules are evident, usually most prominent around the clitoris. Purulent material may often be observed in the ventral commissure. In severe cases the granularity may extend dorsally along the lateral walls of the vulva and occasionally involve the dorsal commissure. Coalescence of the granules produces raised ridges resulting in a corrugated and pebbled

vulvar mucosa. The granularity does not appear to extend cranially to involve the vaginal epithelium.

The vulvar discharge persists for three to ten days before the disease progresses to a chronic form. In many cases the acute form will reappear at subsequent heats.

The chronic form is characterized by an absence of purulent discharge and a gradual decline in the severity of both the hyperemia and granularity. Occasionally an excessive discharge of clear mucus may be observed which makes heat detection difficult for many owners. The granularity gradually disappears over the next few weeks and the vulvar epithelium returns to normal within six weeks to three months. Reinfection however is common. The disease may become endemic within many herds and numerous infections can be observed over a prolonged period. The clinical signs are generally less severe during reinfection, with the acute stage being short or apparently absent. A characteristic finding observed in approximately 10% of affected animals is the presence 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.

When the acute purulent stage of the disease predominates in a herd, there can be a severe effect on fertility. First service conception rates often drop to below 20% and may remain low for a four to six month period. When the disease progresses to the chronic form, herd conception rates usually gradually increase but may remain 10-15% lower than normal for the herd.

Ureaplasmas appear to principally colonize the vulva and vagina and are only rarely found in the upper reproductive tract of normal animals. This appears to be true for both pathogenic and non pathogenic strains.

The localized vulvar infection is believed to be a possible source of recontamination of the uterus at each breeding through mechanical transmission on the insemination pipette. If the organisms are mechanically introduced or repeatedly reintroduced into the uterus at breeding, their persistence for at least seven days may be sufficient time to create an abnormal uterine environment for the fertilized egg.

Once the uterine exposure has occurred, one of three clinical syndromes can result; (i) loss of the fertilized egg prior to day 14 and a return to estrum at the normally expected time, (ii) apparent multiplication of the organisms in the developing fetal fluids with an early embryonic death occurring between 40 and 90 days postbreeding, (iii) abortion at any stage of pregnancy or the delivery of weak infected calves.

B. *Early Embryonic Death and Abortion*

The initial studies carried out on herds affected with granular vulvitis gave no indication of an increase in pregnancy loss even in cows conceiving during the acute stage of the disease. More recently however, there have been indications that in some herds the infection may be associated with a high incidence of early embryonic death.

In herds where the syndrome was observed, cows confirmed pregnant by palpation per rectum at 40 to 45 days returned to estrus between 60 and 80 days, usually with evidence of a purulent vulvar discharge. Uterine culture taken from cows returning to estrus and transported immediately to the laboratory have been positive for ureaplasma, in some cases in pure culture. Endometrial biopsies taken from affected cows have also demonstrated an inflammatory response in the endometrium.

Abortions, incriminated as being due to Ureaplasma infection have occurred sporadically in affected herds and may not be associated with the granular vulvitis syndrome. The abortions appear to occur at any stage of pregnancy and are associated both clinically and experimentally with a chronic placentitis and fetal pneumonitis. The latter finding although not specific, is sufficiently characteristic of Ureaplasma infection to alert the pathologist to culture for the organism. The lung, stomach contents, cotyledons and maternal caruncles appear to be culture sites of choice.

The observations both clinically and experimentally that calves infected in utero can be carried to term and appear weak after delivery, suggests that further studies are needed on the role of the infection in neonatal calf disease.

C. *Infection in the Bull*

Ureaplasmas have been found to be common contaminants of the preputial cavity. Recovery rates of 29-100% have been reported from preputial swabs taken from bulls at AI centres. Raw semen samples from carrier bulls were also commonly contaminated (23-84%) and the organism was found to survive routine processing and freezing, being present in at least 14% of the samples. Ureaplasma contamination of semen appears to result mainly from the penile and preputial mucous membranes. However, in one study six of ten bulls examined were found to have urethral colonization, at least half of which were carrying the organism in the urinary bladder.

Although venereal transmission no doubt can occur, it is also apparent that even virgin bulls can be Ureaplasma carriers. In most cases, preputial and semen contamination has no significant detrimental effect on fertility, suggesting the organisms are nonpathogenic commensals. However, in clinically normal carrier bulls it has been demonstrated that there is a significant and unpredictable variation in the number of organisms present in semen collected at different times from the same bull. Additional variation can occur due to dilution, processing, and freezing of semen resulting in only a small percentage of processed straws from a given

collection containing the organisms. Not only may a limited number of straws from any one ejaculate be positive but the sample should be quantitated since positive samples may contain only a few organisms or more than 1000 colony-forming units. With this appreciation of the shedding characteristics it is also apparent that even if a bull was shedding a potential pathogen, the number of infective straws may be sufficiently low that a 60 day nonreturn rate may appear normal.

In most cases ureaplasma does not appear to cause clinical disease in carrier bulls. Experimentally both acute and chronic inflammatory lesions have been observed at necropsy in the distal and proximal urethra of carrier bulls in association with positive ureaplasma culture. Clinical signs however were not apparent.

Clinical signs suggestive of urethritis have been observed in bulls being used for "clean-up" breeding in ureaplasma infected herds with the onset being associated with a decrease in both libido and fertility.

Experimentally, ureaplasma has been found to be pathogenic for the seminal vesicle following direct inoculation and positive cultures have also been made from field cases of seminal vesiculitis. These findings suggest that ureaplasma may play a role in the seminal vesiculitis syndrome but field studies to confirm this have not been done. The experimental finding that ureaplasma persists in an inflamed gland for only four weeks, suggests that cultures must be done early in the course of the disease if the organism is to be recovered. For this reason, establishing a cause effect relationship between Ureaplasma infection and field cases of chronic seminal vesiculitis will be difficult.

Treatment and Control

A. *Cows*

Since Ureaplasma infection appears to be primarily localized in the vulva, treatment and control procedures should be directed at minimizing the potential for mechanical transmission to the uterus at the time of breeding. Eliminating the infection from a herd is extremely difficult due to the chronic nature of the disease.

Minimizing uterine transmission can be attempted in a number of ways. In all cases where the disease is diagnosed, all insemination or treatment pipettes should be protected as they pass through the vulva through a "double rod" technique. This technique using plastic drinking straws or a commercial double pipette system is usually effective in increasing the herd fertility rate.

Antibiotic therapy (tetracycline or spectinomycin) directed locally at the vulva or in the form of uterine infusions have also been used with apparent success. Local treatment of the vulva using douches or creams is designed to control the infection and minimize the numbers of organisms present at the time of breeding. Uterine infusions are designed to treat an existing endometritis or when used 24 hours postbreeding, to minimize potential uterine transmission that may have occurred. Rigorous antibiotic therapy is usually only needed during the acute form when herd fertility rates are extremely poor. During the chronic form, the double-rod technique will usually achieve fertility rates close to the previous farm average. Unfortunately in many instances, appropriate therapy is not instituted until the disease has been present in the herd for some time. In this instance a number of cows may not respond due to chronic and occasionally irreversible lesions already being present in the endometrium or oviducts. Sham embryo transplant flushings with warm saline have been found to be of value in some individual cases where chronic productive endometritis is present.

The nutritional status of the herd should also be evaluated since an imbalance or deficiency could be expected to compound the problem. A correlation between Vitamin E and selenium deficiency and the incidence of the disease was made in New York dairy herds. An improvement in fertility with less clinical signs appeared to follow supplementation of deficient herds.

B. *Bulls*

To date an effective treatment for eliminating the carrier state in bulls has not been developed. This plus the inability to easily differentiate nonpathogenic strains has made control in the bull difficult.

Until methods of determining pathogenic strains are developed, a "safe level" of ureaplasma in semen cannot be determined. However, due to the demonstrated pathogenic potential of bovine ureaplasmas, all semen isolates should be viewed as potentially significant especially from bulls with a suspicious history or if present in levels of at least 100 CFU. Due to the variability that can occur between straws, a number of samples of processed semen should be evaluated to determine the bull's shedding characteristics.

In AI centres the approach has been to control any ureaplasma contamination that might occur in processed semen through the addition of antibiotics. Initially the antibiotic lincospectin was added to extenders to control Ureaplasmas. More recently the further addition of minocycline hydrochloride appeared to effectively control both mycoplasmas and Ureaplasmas without being detrimental to sperm quality. Unfortunately at the present time this antibiotic only appears to be compatible with milk extenders. Additional studies are under way to develop methods of controlling the organism in other extenders and it is hoped that in the near future it will be possible to control Ureaplasma in all processed semen marketed in North America.

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