Trichomoniasis: Diagnosis, Pathogenesis, Treatment and Control

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Trichomoniasis is a veneral disease of cattle caused by *Tritrichomonas fetus* (*T. Fetus*). Its clinical signs are repeat breeding at both regular and irregular intervals and, occasionally, abortion. These are easy to observe in dairy cattle. However, in beef cattle on western ranges, the first indication of a problem is usually finding over 20-40% of the cows open at the time of fall pregnancy check. By this time the significant economic damage has already been done. Economic losses are from a reduction in calving rates and lower weaning weights of calves due to delayed conception. A recent report estimated that losses due to trichomoniasis in the state of Oklahoma alone approached 5 to 7 million dollars annually.

Incidence

Data is limited on the incidence of trichomoniasis. A 7.3% infection rate has been reported in slaughtered bulls in Florida. Another study in Oklahoma reported a 7.8% infection rate in bulls presented for sale at auction. These figures agree closely with the incidence of 7.5% in over 800 bulls in Arizona, Utah, Colorado, Idaho and Wyoming. These infection rates reflect only selected populations of bulls within these areas.

Primary infection rates of over 40% have been reported in cow herds in Australia. No reports are available on herd incidence in beef herds in the United States. At Colorado State University the number of isolations of trichomoniasis has increased. Whether this simply reflects an increased awareness or an actual increase in the incidence of trichomoniasis is not known.

Infection rates have not been determined for dairy cattle, but trichomoniasis is mostly limited to dairies that use bulls in natural service. Artificial insemination (AI) decreases the likelihood of occurrence and spread of trichomoniasis in dairy cattle.

Pathogenesis

Bulls are the primary vectors of trichomoniasis. The organism is harbored in the epithelial crypts of the penis and prepuce as surface contaminants; consequently, it does not evoke an immune response in bulls. Because these crypts do not develop until after the bull is about four years of age, younger bulls usually do not become permanent carriers.

In cows, *T. fetus* may be associated with vaginitis, cervicitis, endometritis, or pyometra, and a transient infertility of from two to five months. Occasionally abortion occurs. The cows then conceive and may carry a calf to term even if bred to infected bulls; rarely will a cow carry the infection completely through a gestation. Cows are susceptible to reinfection after a variable period of immunity. Reinfection rates of 0%, 56.3%, 72.2%, 75%, and 100% were reported when the interval to re-examination was 1, 9.2, 10.3, 13.2, and 19.7 months respectively. Thus it is doubtful if immunity lasts beyond 15 months in any circumstance.

In beef cows, the signalment of trichomoniasis is more striking than in dairy herds in most instances. In beef cows, 30% or more may be open in the fall, but the disease is insidious in dairy herds. There are several reasons for these differences. First, most cows develop temporary immunity 2 to 5 months after they become infected. Since pen-mated dairy cows are with bulls as soon as they calve, many are bred by 30-40 days post partum and a significant number become immune by 120 days or less. These cows with a short infective period may not have an excessively extended calving interval. Others with longer infective periods would have extended calving intervals and decreased productivity.

In addition, young bulls are often used for natural service in dairy herds. These are less likely to become carriers than older bulls. A third related factor is transmission frequency. Very active breeding bulls with moderate numbers of trichomonads resident in their prepuce are less likely to transmit trichomonads in sufficient numbers to infect susceptible cows. Young bulls are likely both to harbor smaller numbers of the organism if they are permanently infected, and to be more active sexually than older bulls.

Diagnosis

When *Trichomonas fetus* is suspected in a herd with an infertility problem, culture of preputial samples from the bull is more likely to isolate the organism than culture of cervical mucus from the cow. In most cases the cow will rid herself of the organism in 4-5 heat cycles, while susceptible

bulls remain infected indefinitely.

Collection of Samples

In dairy herds, diagnosis of trichomoniasis can be accomplished practically by several methods. These include culture of cervical mucus from recently bred cows, culture of preputial smegma from bulls, direct microscopic observation of pyometra fluids, culture of pyometra fluids, and direct examination of abomasal contents of aborted fetuses.

Trichomonads colonize mucosal crypts of the penis and preputial membrane; consequently, sampling methods must be effective in removing them from the crypts. Numerous methods have been described to obtain smegma samples from the bull. Some of these are:

1. swabbing the penis with gauze pledgets on a rod.
2. swabbing and washing the exteriorized penis with gauze pledgets.
3. douching the sheath with sterile saline.
4. aspiration of smegma with a dry pipette.

In our experience the most satisfactory method of sample collection is the dry pipette technique if several bulls are to be tested. Trichomonads were isolated experimentally in Australia from 139 of 143 (97%) preputial samples from infected bulls using this method.

Both preputial and cervical samples can be collected using an ordinary insemination pipette attached to a 10 cc syringe with a rubber or plastic adaptor. To prevent contamination of the pipette as it is introduced into the prepuce, an ordinary plastic drinking straw may be inserted into the preputial opening and used as a sheath for the pipette. This apparatus can be gas sterilized if desired, but this is not necessary if fresh pipettes are used out of newly-opened containers.

The hair around the preputial opening should be trimmed away prior to introducing the pipette. The straw should be introduced into the prepuce first, then the pipette, attached to a 10-12 cc dry syringe, is introduced through the straw to the full extent of the preputial sac. A negative pressure is created in the syringe with one hand while the other guides the tip of the pipette. The pipette is then moved back and forth vigorously scraping the area of the dorsum of the glans penis as constant negative pressure is maintained on the syringe.

The quality of the collected sample has a direct influence on results. The preputial sample in the pipette should be cloudy and blood tinged. Clear samples usually do not contain epithelial cells or blood, indicating the scraping was not deep enough to remove the trichomonads from the epithelial crypts.

Once collected, the sample should be transferred into a vial containing 2 cc of USP Ringer's lactate or USP saline. Bang's tubes should not be used for this purpose. The Ringer's lactate or saline can be drawn back and forth into the pipette to flush the smegma out. If the animals are also to be tested for vibriosis, Clark's transport medium is used. One cc of the material can be used to inoculate Clark's transport media for transportation to the laboratory. The other cc is used to inoculate the Diamond's medium.

Transport of Sample to the Laboratory

USP Ringer's lactate or USP saline should always be used as the transport medium for trichomonad organisms. In our experience Diamond's medium **is not** good for transport of trichomonads from the field. Samples can either be refrigerated at about 4°C or kept at room temperature for transport. We have been able to isolate *T. fetus* from refrigerated samples in USP saline or Ringer's lactate for up to 72 hours and from samples maintained at room temperature for up to 96 hours. It is always best however, to get the sample to the laboratory as soon as possible (the same day the samples are collected). Survival of *C. fetus* is not as good over time as survival of *T. fetus.*

Culturing T. Fetus

Diamond's medium is used to culture *T. fetus.* The sample in Ringer's lactate or saline should be mixed thoroughly and then **carefully** layered on the surface of the Diamond's medium. This can be done either by pipetting the material onto the medium or pouring it gently down the side of the tube.

The sample is then incubated at 37°C for 24 hours. At this time the culture should be checked for bacterial growth. If there is substantial bacterial growth, indicated by a cloudy appearance in the medium, the culture should be read at this time. If there is no bacterial growth or if there is growth only in the upper portion of the medium leaving the bottom part clear, the culture should be incubated for an additional 24 hours and be read at 48 hours. The trichomonads grow at the very bottom of the medium. A few drops are removed from the bottom of the tube, placed on a slide and covered with a cover slip. The sample is examined at 100x total magnification for trichomonads which have irregular, jerky movements associated with continual rolling. Confirmation is made by examination at 450x total magnification, at which time the characteristic features of *T. fetus* can be identified. These features include three anterior flagellae and a single trailing flagellum attached to an undulating membrane which runs the length of the organism.

Treatment

Treatment efforts need to be directed primarily toward the infected bull rather than the cow because of the self limiting nature of the disease in cows. Local treatment of the penis and prepuce has given way to systemic treatment by compounds containing the imidazole ring. Some of these compounds are licensed in the United States for the treatment of histomoniasis in turkeys. Dimetridozole (Emtryl®, Salisbury Laboratories, Charles City, Iowa) has been effective both orally and intravenously. The oral dose is 50 mg/kg daily for five days given in boluses or by drenching. It is ineffective as a feed additive because it is unpalatable and the bull may not eat enough. As a

consequence, subtherapeutic doses may induce drug resistance. Intravenously, dimetridozole is administered as a single injection of 50 mg/kg. Intravenous injection is not without risk because the drug must be dissolved in 20% sulfuric acid. Treated bulls may become dyspneic, ataxic, or collapse.

The drug most recently advocated for treatment is ipronidazole hydrochloride (Ipropran®, Hoffman-LaRoche Inc). It is soluble in water so it can be given by intramuscular injection. This agent is easily oxidized so it should be mixed in glassware, not metal containers. It should also be mixed a short time before use and not be allowed to stand, mixed, more than a day before use. It is mixed as a 50% solution and recommended dosages have been 30 g/bull and 15 g/cow or heifer. However, this dosage is not adequate because some bulls become positive again after 1-2 months of treatment. With Ipropran®, it is necessary to pretreat bulls with broad-spectrum antibiotics for 3-4 days to eliminate preputial bacteria that inactivate ipropran. Ipropran has not been critically evaluated in the United States. Preliminary studies are now being conducted at CSU to test its effectiveness. So far, bulls were temporarily negative following an initial single treatment with 30 mg ipropan but became positive again after about 30 days. Three were re-treated at 30 gm/day for 3 days and 3 at 15 gm/day for 3 days. All remained negative.

None of the ipronidazole ring compounds have been approved for use in cattle.

Prevention and Control

The maintenance of closed herds with the introduction of only virgin bulls and cows would be an ideal way to prevent trichomoniasis. However, this is not always possible. Practical prevention and control can be usually achieved by using only 3-year-old or younger bulls for breeding. This method of control is effective because young bulls are not as likely as old ones to become permanent carriers. In dairy herds, this practice with bulls is often followed routinely because of the danger of keeping older bulls. This along with the practice of AI has effectively controlled trichomoniasis in most of the dairy industry. In many beef operations, however, some older bulls are used. In these herds, all bulls over 4 years of age should be routinely tested for trichomoniasis as a part of the breeding soundness examination. The status of the herd would thus be monitored to detect either infection or reinfection.

When trichomoniasis is diagnosed in dairy herds, it can be controlled most effectively by AI. However, in some herds, management levels may be inadequate to have a successful AI program. In these, natural breeding programs must be tailored to eliminate the infection.

First, bulls should be tested by preputial culture and infected ones eliminated. In addition, any bull older than 2½ or 3 years of age should be sold and as other bulls reach this age they should be sold as well.

Cows are segregated into "exposed" and "nonexposed" groups. Cows that calve are seldom carriers until they are bred to infected bulls, so these can be placed in the "nonexposed" groups as soon as they calve, and before such exposure occurs. Virgin bulls are used to breed these cows. Under no conditions should bulls from the "exposed" groups be placed with "nonexposed" cows. As the "nonexposed" cows become pregnant, they can be placed with "exposed" cows until they calve to expedite nutritional programs.

A year or two of this kind of management should result in the elimination of trichomoniasis from a herd.

Bulls in all groups should be monitored regularly by preputial mucus culture. If they become infected, they should be sold immediately and replaced with virgin yearling bulls.

Culling of open beef cows at the time of pregnancy test also effectively reduces a primary source of infection. Thereafter, cattle that abort prior to calving should also be culled. For commercial cattlemen, these recommendations are generally accepted because maintenance of a nonproductive cow is costly. Unfortunately, the culled cattle are often sold through auctions, and serve as a source of infection for other livestock producers. Because of this problem it has been advocated that trichomoniasis become a reportable disease.

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