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ANIMAL HEALTH UPDATES

Animal Health Group

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Quarterly Newsletter

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Bovine Trichomoniasis (Trichomonosis)

Introduction

Bovine venereal *Trichomoniasis* is caused by *Tritrichomonas foetus*, a flagellate protozoan parasite. The normal hosts of *T. foetus* are cattle (*Bos taurus*, *B. indicus*).

Etiology & Epidemiology

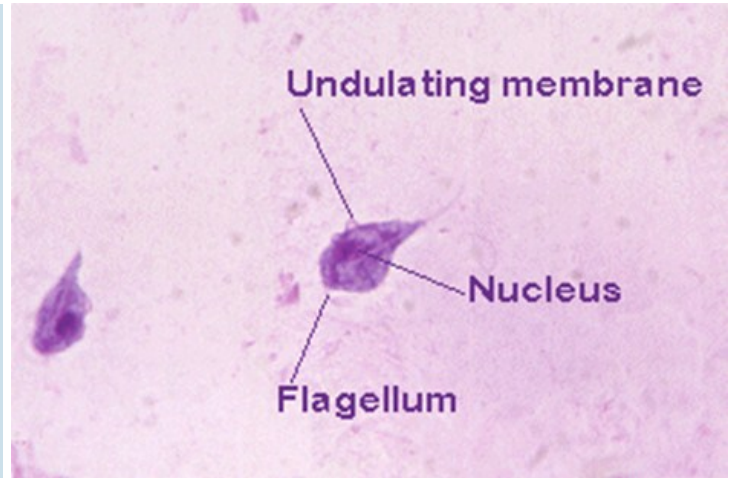
Tritrichomonas foetus is pyriform, 8–18 μm long and 4–9 μm wide, having three anterior and one posterior flagellae, with an undulating membrane. It is often misdiagnosed as *Giardia* spp., but the movement of the organism is quite different. *T. foetus* movement is jerky and random while *Giardia* movement resembles a “falling leaf” and seems more deliberate.

Distribution

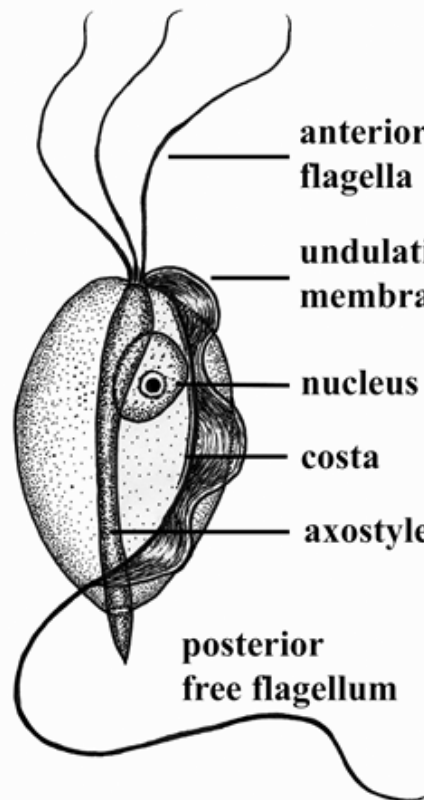
Bovine Trichomoniasis was first reported in France in 1888. It has a worldwide distribution especially in countries where natural service is commonly practised. However with the use of AI, the disease is seen rarely in many countries. The reports from India are scanty.

Life cycle

T. foetus consists of only one trophozoitic form and has a simple life-style. The trophozoites can adopt a spherical shape, known as a pseudocyst. The formation of pseudocysts occurs during times of stress



Giemsa stained *T. foetus* X1000 (Source: http://www.link.vet.ed.ac.uk/parasitology/InfectionAndImmunity/P_05DiagnosisOfProtozoa/)



Morphogenic structure of *Trichomonas foetus* (Left)

T. foetus is indistinguishable from *T. suis* (found in nose and gut of pigs) according to morphology. *T. foetus* causes chronic large bowel diarrhoea in cats. Other trichomonads occurs infrequently in humans in the gums, trachea, bronchi or in the intestinal tract.

either inside the body of the host or in culture media and also during adhesion to and subsequent phagocytosis of sperm cells. Pseudocysts can generate multinucleated organisms that, under favourable environmental conditions, release single organisms. *T. foetus* does not form cysts and cannot survive outside the host.

Economic impact

The economic impact of Trichomoniasis are due to five main factors: (1) Reduced number of calves due to early embryonic loss or abortions;

(2) Reduced weaning weight due to delayed conception;

(3) Culling and replacement of infected cattle;

(4) Loss of bulls due to culling of infected bulls, and

(5) Genetic losses from culling infected high genetic merit cattle.

In the US, it is estimated that each infected cow in a dairy herd leads to an estimated economic loss of \$665. Beef herds with 20–40% *T. foetus* positive bulls reported a reduction of 14–50% in annual calf crops, 4–10% in monetary return per calf born, and 5–35% in the return per cow.

Transmission

Transmission of infection occurs by coitus, Artificial Insemination (AI), or by gynaecological examination of cows.

Transmission in bulls

Bulls carry the protozoa in small numbers in the preputial cavity, in the fornix and, around the glans penis and preputial membranes. It also localizes in the secretions (smegma) of the epithelial lining of the penis and distal portion of the

urethra. *T. foetus* does not occur in the epididymes, ampullas, seminal vesicles, pelvic urethra or testes. There are no lesions of diagnostic significance in bulls and the parasite does not affect either semen quality or sexual behaviour. *But* semen may become contaminated with organisms from the skin of the penis. The infective dose for three to seven year old bulls is 10^6 organisms, but some can be infected with as little as 10^2 organisms. In bulls under 3–4 years old, infection may be transient. For bulls older than 3–4 years, spontaneous recovery rarely occurs, resulting in a permanent source of infection in herds. It may perhaps be as a result of the development of epithelial crypts in the preputial cavity of older bulls. The chronically infected bulls show no gross lesions. The parasite transmission rate from male to female at breeding may be as great as 42%. *T. foetus* is rarely transmitted by artificial insemination of cattle if appropriate procedures for bull testing and hygiene are practiced.

Transmission in cows

The infective dose for cows is usually 10^3 organisms. Infection in previously non-infected cows is most likely to occur when the time interval between services is less than 20 minutes. It was shown that a single service by an infected bull resulted in 95% infection of previously uninfected susceptible cows. Interestingly, transmission from infected cows/heifers to bulls appears less efficient. Passive transmission (transmission of the organism from infected to non-infected cows via a non-infected bull) can occur but the transmission rate is low.

T. foetus is found in all regions of the reproductive tract of cows,

where trophozoites attach to the surfaces of the epithelial cells lining. Examples of colonization in heifers and cows include the vagina, uterus, and oviduct.

Pathogenesis and clinical signs

The pathogenesis of pregnancy loss is not yet well understood. A likely cause of abortion is the direct cytotoxic insult of maternal endometrium and/or fibroblasts and the foetal chorionic trophoctoderm. Another potential virulence factor is the extracellular cysteine proteinases that are elaborated by *T. foetus*.

In the infected cow, the initial lesion is vaginitis, which can be followed in pregnant animals by invasion of the cervix and uterus. On a herd basis, cows may, following infection, exhibit irregular oestrous cycles, uterine discharge, pyometra, or abortion. When abortion occurs, it is usually within the first third to one-half of gestation. Repeat breeding or infertility of individual cows can last up to 5 months. The majority of infected cows will clear the infection if given 120 to 150 days of sexual rest, but their immunity to the disease is weak; therefore, they can be re-infected the next season. Very rarely, an infected cow may carry a normal calf to term and not clear her infection, remaining infected, and a carrier to the next breeding season.

Grossly, the degree of autolysis in foetus and placenta can vary from mild to marked. Foetuses may have no discernible lesions; however, enlarged livers and non-inflated, enlarged, firm lungs may be present on some. To a lesser extent, *T. foetus* is located in amniotic, allantoic, or abomasal fluids from the infrequently aborted foetuses. Sometimes, foetus and membranes may

not be completely expelled, corpus luteum and the cervical seal of pregnancy may be retained. This leads to maceration of the foetus, and the chronic catarrhal and purulent endometritis. It usually ends in permanent sterility.

No clinical signs are present in infected bulls. During the first two weeks of infection, a scant purulent preputial discharge may be noted.

Diagnosis

Diagnosis of Trichomoniasis takes into account clinical history of the herd, which includes signs of early abortion, repeated returns to service and irregular oestrous cycles, and is confirmed by detection of the protozoan by culture and/or polymerase chain reaction (PCR).

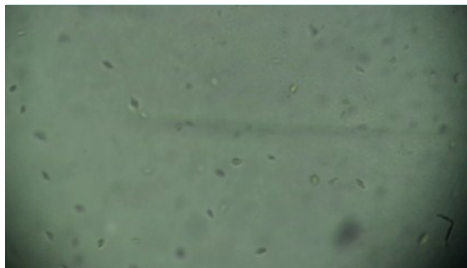
Agent identification

Identification by direct examination or culture is the prescribed test by OIE for international trade.

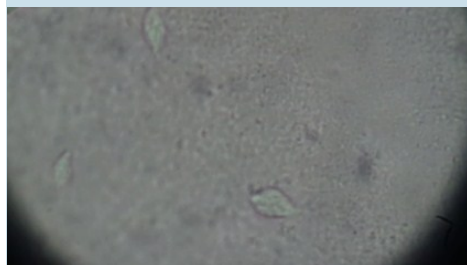
In infected herds, the most reliable material for diagnosis is either preputial or vaginal washings or scrapings. The organisms are numerous in the aborted foetus and uterus several days after abortion. In recently infected cows, they are plentiful in the vaginal mucus 12–20 days after infection. In the infected bull, two weeks of sexual rest is recommended before a bull is tested. This allows the organism time to build up to a detectable level.

Commercial culture test kit (InPouch™ TF)

Where a combination of convenience and sensitivity is required, the culture test kit may be used. The kit consists of a clear flexible plastic pouch with two chambers. The upper chamber contains special medium into which the sample is introduced. Field samples for direct inoculation into the



T. foetus examined in 10X in InPouch™ kit



T. foetus examined in 40X in InPouch™ kit

culture pouch would normally be collected by the preputial scraping technique. Samples collected by preputial washing require centrifugation before introduction of the sediment into the upper chamber. Following mixing, the medium is forced into the lower chamber, and the pouch is then sealed and incubated at 37°C. Microscopic examination for trichomonads can be done directly through the plastic pouch.

As the parasites multiply, white sediment along the sides and bottom of the chamber will become visible. If *T. foetus* organisms are present, they will be identifiable by their distinct features which can be seen using a 10X or 40X objective lens.

Diagnosis by InPouch™ TF can be done at NDDDB R & D, Hyderabad and at Translational Research Platform for Veterinary Biologicals (TRPVB), Chennai.

Polymerase chain reaction (PCR) assays

PCR assays are capable of detecting very low numbers of parasites from laboratory cultures of the organism in the presence or absence

of preputial material. However, in the presence of preputial material, a higher number of parasites are required to yield a positive PCR result; this is most likely due to inhibition by components of the preputial smegma. Diagnostic specificity of the PCR test depends in part, on the specificity of the primers.

Immunological tests

Several immunological tests have been developed for the diagnosis of bovine Trichomoniasis. However, they are limited in use and are not recommended for the detection of *T. foetus* in individual animals.

An immunohistochemical technique using a monoclonal antibody has also been evaluated to specifically label *T. foetus* in formalin-fixed, paraffin-embedded sections of placenta and foetal lung from bovine abortions.

Sample collection

It is important to avoid faecal contamination while collection of samples, as this may introduce intestinal protozoa that may be confused with *T. foetus*. Contamination of samples should be minimised by removal of extraneous material and soiled hair from around the preputial orifice or vulva; however, cleansing of the area, particularly with disinfectants, is to be avoided, as this may reduce diagnostic sensitivity.

Sampling from bulls

Samples can be collected from bulls by scraping the preputial and penile mucosa with an artificial insemination pipette or brush, by preputial lavage, or by washing the artificial vagina after semen collection.

(For video on sample collection from bulls by **InPouch™ TF** test, please visit NDDDB Dairy Knowledge Portal :<http://www.dairyknowledge.in/file/video> or NDDDB youtube: <https://youtu.be/GL64N2D2v2A>)

Sampling from heifers & cows

Samples from cows are collected by washing the vagina, or by scraping the cervix with an artificial insemination pipette or brush. The best time to collect samples from the vagina of heifers and cows is 12-19 days post-coitus. In cases of pyometra, *T. foetus* is nearly always present. Other collection times are just after abortion or just prior to oestrus.

Where samples must be submitted to a laboratory and cannot be delivered within 24 hours, a transport medium containing antibiotics should be used (e.g. a thioglycollate broth media with antibiotics) or the field culture plastic pouch. During transportation, the organisms should be protected from exposure to daylight and extremes of temperature, which should remain above 5°C and below 38°C.

Treatment

Currently there is no approved treatment for cattle infected with Trichomoniasis. The treatment of a bull, though possible, is tedious and it is better to cull the animal. Acriflavin is recommended for local application if only the penis is infected. It may be necessary to inject acriflavin in the urethra if parasites are present there. Berenil (100-150 ml of 1 % solution) infusion into the prepuce for five consecutive days has also been practiced in bulls. Metronidazole @ 75 mg/kg thrice at 12 hr interval is effective in treating infected cows.

Prevention and control

Adoption of AI practices and eliminating the infected animals(especially the bull) from the herd are the most effective means of control. The parasite may survive even in frozen semen. The salient points to be noted to protect herds from Trichomoniasis are:

1. Purchase only virgin bulls.
2. Quarantine and test the bull for Trichomoniasis while purchasing a bull that has prior breeding experience.
3. Pregnancy check cows in a timely manner after the breeding season to identify a potential problem early.

Significant animal diseases reported to OIE (Apr–June'15)

No	Disease Outbreak	Countries reporting
1	Bluetongue	Australia
2	Highly pathogenic avian influenza	Canada & Hong kong(new strain), Kazakhstan, Mexico
3	Foot & Mouth Disease	Taipei, Namibia
4	Rabies	Honduras
5	Haemorrhagic Septicaemia	Kazakhstan
6	Sheep & Goat pox	Kazakhstan
7	Contagious Equine metritis	South Korea
8	Lumpy Skin disease	Saudi Arabia
9	African Swine Fever	Ukraine

Source: www.oie.int

4. Purchase only virgin heifers and/or cows from a reputable source.
5. Keep fences in good repair to prevent accidental contact with potentially infected cattle.
6. Keep good records of a herd's reproductive efficiency. The records can help identify a possible problem.

Vaccination

It is documented that immunisation with a vaccine containing killed cells of *T. foetus* prevents genital infection in most bulls up to the age of five years. However, it does not effectively prevent or cure infection in bulls older than five and a half years. Vaccination reduces the rate of abortion by around 30% only.

Presently no vaccination is available in India against Trichomoniasis.

Sources

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