

Neosporosis and Trichomoniasis: Major Protozoan Diseases Causing Abortion in Cattle

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Abstract: This paper summarizes the major protozoan diseases that cause abortion in cattle resulting in serious economic impact. Among the causes, protozoan diseases: neosporosis and trichomoniasis were reviewed with special focus. Besides, the paper enumerates other possible etiological agents of bovine abortion; describes the ways of transmission, infection and the probable pathogenesis through which these agents bring abortion; the appropriate diagnostic approaches; the measures that should be taken in preventing and controlling of the major protozoan causes of abortion in dairy cattle and its global and national status.

Key words: Abortion • Bovine • Economic Impact • Neosporosis • Trichomoniasis

INTRODUCTION

Abortion is the most important condition that limits cows' ability to produce a calf and considerably erodes the profit. Abortion in dairy cattle is commonly defined as a loss of a fetus between the age of 42 days and approximately 260 days of gestation. The principal agents causing protozoal abortion in dairy cattle are *Neospora caninum* and *Trichomonas foetus* [1].

Neosporosis is a disease caused by recently recognized protozoan parasite, *Neospora*. Since its first recognition in 1984 in dogs in Norway [2] and the description of a new genus and species, *N. caninum* [3], neosporosis has emerged as a serious disease of cattle and dog worldwide. Until 1988, infection with the parasite was misdiagnosed as *Toxoplasma gondi* because of morphological similarities between these two parasites [3]. Although *Neospora* have morphologic similarities to *T. gondi*, they are antigenically different from *Toxoplasma* in the tissue cyst stage when neospora often have a thicker cyst-wall.

Bovine trichomonosis is one of the most prevalent sexually transmitted diseases in cattle. Causative agent of cattle trichomonosis is *T. foetus*. This parasite is an extracellular, a non-invasive flagellate protozoan that colonizes only in low oxygen tension environment of

reproductive tract mucosa in cattle. The infection varies in cows from a mild vaginitis or cervicitis to endometritis, transient or permanent infertility and abortion causing significant economic losses [4]. Infertility is the primary manifestation of *T. foetus* infection which was clinically evidenced as a high percentage of non-pregnant cows [5].

Therefore, the objective of this paper is to review the biology, epidemiology, pathogenic effect, clinical sign, diagnosis, treatment, control and prevention; and global and national status of neosporosis and trichomoniasis.

Major Protozoan Diseases Causing Abortion in Cattle:

Protozoan diseases are a significant cause of abortion and infertility in domestic ruminants. Toxoplasmosis is a protozoan disease that causes rarely abortion in cattle but is a major abortifacient in sheep [6]. Felids are the definitive hosts and there are wide ranges of intermediate hosts in this disease [7]. Sarcocystosis caused by sarcocystis species cause a common, frequently asymptomatic infection in cattle [8]. Carnivorous definitive hosts spread the infection through their feces and domestic ruminants are intermediate hosts. A similar, recently recognized protozoa, *Neospora* species, has emerged as an important cause of reproductive disease, especially as an abortifacient in dairy cattle [9]. The venereally transmitted *T. foetus* is an

important cause of pregnancy loss in naturally bred cattle throughout the world [10]. In the absence of effective methods for vaccination or treatment, control of these parasites is based on management procedures to reduce infection and transmission.

Neosporosis

Aetiology: *Neospora caninum* is a protozoan parasite, which has been reported to cause abortions in cattle worldwide [11]. It is a primary pathogen and one of the most efficiently transplacentally transmitted organism in cattle [12].

Life Cycle: The life cycle is typified by 3 known infectious stages. These are: tissue cysts, tachyzoites and oocysts. Tissue cysts and tachyzoites are the stages found in the intermediate host and they occur intracellularly [13]. Tissue cysts are often round or oval in shape and found primarily in the central nervous system. Extra neural tissues, especially muscles, may contain tissue cysts [14].

Dogs are both the intermediate and definitive hosts for *N. caninum* [15]. Most dogs are infected by ingesting *Neospora* together with infected tissues of animals usually cattle, sheep, goat, horse and also dogs, whose tissue contains tissue cyst. The environmentally resistant

stage of the parasite, the oocyst, is excreted in the feces of dogs and coyotes in an unsporulated stage [16]. All three infectious stages of *N. caninum* (tachyzoites, bradyzoites and oocysts) are involved in the transmission of the parasite. Carnivores probably become infected by ingesting tissues containing bradyzoites and herbivores probably become infected by the ingestion of food or drinking water contaminated by *N. caninum* sporulated oocysts. Transplacental infection can occur when tachyzoites are transmitted from an infected dam to her fetus during pregnancy [1]. In general, the life cycle of *N. caninum* is summarized as follows (Figure 1):

Epidemiology: The parasite is wide spread in the US, but the majority of case reports have come from California, where *N. caninum* is considered to be the number one cause of abortion in dairy cattle. In some herds in the US, up to 90% cattle are infected [12]. *Neospora caninum* infections have also been reported from most parts of the world including Australia, New Zealand, Europe, Korea, Japan, Thailand and the Americas.

Although *Neospora* infections occur in both dairy and beef cattle, most reports attributing significant numbers of abortions to this infection have been associated with dairy cattle, particularly those in dry lot dairies [17]. It is possible that the environment of the dry

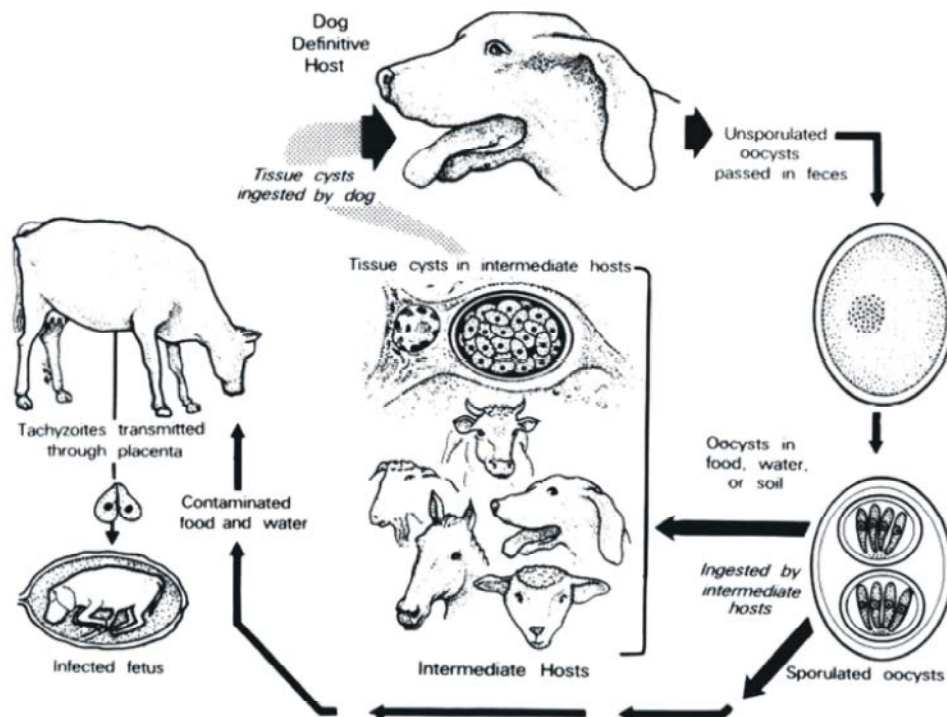


Fig. 1: Life cycle of *Neospora caninum* Source: [61]

lot dairy is more conducive to the spread and transmission of this disease. Cattle in dry lot dairies are densely populated and fed a variety of harvested feeds and commodities, which are frequently stored on or around the dairy prior to being mixed and fed. These feeding practices offer many opportunities for fecal contamination of individual ration components, which would then be mixed and fed, efficiently exposing much of the herd [18].

Neospora caninum-associated abortion in bovine herds may have an epidemic or an endemic pattern. There are reports that in the years after an epidemic abortion outbreak, the affected herd may experience endemic abortions [19]. Abortion outbreaks have been defined as epidemic if the abortion outbreak is temporary and if 15% of the cows at risk abort within 4 weeks, 12.5% of the cows abort within 8 weeks and 10% of the cows abort within 6 weeks [20]. In contrast, an abortion problem is regarded as endemic if it persists in the herd for several months or years. It is likely that these two patterns of *N. caninum*-associated abortion are related to two routes by which infections can cause abortion [21].

Transmission: Cattle could get infected by oocysts either transplacentally from an infected dam to her fetus during pregnancy or postnatally by ingestion of food or drinking water contaminated by sporulated oocysts [21]. Most epidemiology studies conducted on Neosporosis in cattle indicate that vertical transmission is the major mode of transmission within a herd [22].

Neospora caninum is one of the most efficiently transplacentally transmitted parasites among all known microbes in cattle. Björkman *et al.* [23] traced the familial history of *N. caninum*-seropositive dairy cows in a herd in Sweden and found that all infected animals were the progeny of two cows that were bought when the herd was established 16 years earlier. A strong evidence for transplacental transmission of *N. caninum* has been obtained by comparison of seroprevalence in dams and their progeny. In cattle and other ruminants, there is no transfer of antibodies from the dam to the fetus, not even through a placenta that has been damaged by an infectious process [11]. Therefore, detection of specific antibodies in precolostral serum indicates in utero synthesis of antibodies by the fetus. However, a finding of no antibody in the fetus is not conclusive of the absence of infection, because the fetus might have been infected late in gestation, leaving insufficient time for antibody synthesis. Rarely, it is possible for a seronegative dam to give birth to a seropositive calf; this

may be because the cow has been infected for some time and the level of antibodies has declined to an undetectable level [24].

The ingestion of sporulated *N. caninum* oocysts from the environment is the only demonstrated natural mode of infection in cattle after birth [16]. To date, cow-to-cow transmission of *N. caninum* has not been observed. At present there is no evidence that live *N. caninum* is present in excretions or secretions of adult asymptomatic cows. Neonatal calves may become infected after ingestion of milk contaminated with tachyzoites [25] and *N. caninum*-DNA in milk, including colostrum, has been demonstrated [26]. However, there is no conclusive evidence that lactogenic transmission of *N. caninum* occurs in nature [27].

Pathogenic Effect: Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows. There is a rise in antibody titers 4 to 5 months before parturition. These observations strongly suggest reactivation of latent infection. Little is known of the mechanism of reactivation. It is likely that there is parasitemia during pregnancy leading to fetal infection. However, *N. caninum* has never been identified in histologic sections of adult cows and there is a single report of isolation of viable *N. caninum* from the brain of an adult cow [28].

Little is known at the present time concerning the pathogenic effect of infection in cows following infection with a natural route (oral) and using the naturally transmitted stage of the parasite (oocyst) although oocysts are infective to cattle by the oral route. Results of experiments with dairy or beef cows inoculated parenterally with *N. caninum* tachyzoites indicate that the fetus can become infected and sometimes diseased by 4 weeks after inoculation of the parasite. Even at the early stage, there were lesions in placenta and the central nervous system and encephalitis was the predominant lesion. The extent of damage to placenta directly due to multiplication of *N. caninum* and by immune response is not known [29]. Gestational age may determine the outcome of infection. Fetuses infected early in pregnancy are likely to die [30].

Clinical Signs: In cattle the main clinical sign is abortion between 3 and 9 months of gestational age, with a mean age of 5- 6 months [31]. Also, *Neospora* may cause foetal death; retention or resorption early in gestation and, in some instances; only skeleton or mummified foetuses are aborted or retained until full gestation. Mummification

appears to be an important clinical finding in outbreaks of *N. caninum* associated abortions in cattle [32] but calves may also be born alive with clinical signs or be born clinically normal but chronically infected. Repeated abortion in cows due to neosporosis is infrequent [33]. Additionally, Thurmond and Hietala [12] reported a decreased *N. caninum* induced abortion risk in subsequent pregnancies, especially in heifers. They attributed this fact to the selective culling of cows that aborted.

Diagnosis: In the first years after discovery of *N. caninum*, the diagnosis was made by means of clinical signs and histopathology, describing lesions found in CNS, muscles and other organs of the fetuses. This kind of diagnosis had the problem that *N. caninum* was misdiagnosed as *T. gondii* due to the close structural similarities [31]. Because this similarity, it was necessary to develop specific tests to differentiate between *N. caninum* and *T. gondii*. Thus, immunohistochemistry, serology and molecular biology have been important in supplying specific tests to diagnose exactly, rapidly and sensitively those animals with neosporosis [34].

Treatment: Several studies have been carried out to find the best treatment for *Neospora* infection or its effects on the species affected. However, there is currently no commercial chemotherapy available. A well known problem is that drug treatment for neosporosis in cattle leads to milk withdrawal when used in lactating dairy cows. However, this problem is not very important in other species, in which the duration of chemotherapeutic treatment has less impact. Another problem in all species is the resistance of tissue cysts and bradyzoites to the drug; there is no 100% guarantee of effectiveness in clearance of these stages of the parasite in the animal [35].

Early treatment with drugs used to treat toxoplasmosis show some success in treating neosporosis in cattle. The drugs include: clindamycin (antirobe) (11-22 mg/kg); potentiated sulfonamides (example: Tribissen co-Trimoxazole) (60mg/kg/day) and pyrimethamine (Daraprim) (1mg/kg/day). In addition, supportive treatment is essential using Aspirin or related drugs and low doses of corticosteroids [25].

Prevention and Control: Control programs at the national, regional and farm levels are being developed in different countries to control neosporosis [36]. Control programs should incorporate a cost-benefit calculation comparing

the expenses of testing and control measures with the benefit of reduced economic losses due to *N. caninum* infection or abortion [37]. Since, at present, neosporosis is not considered a zoonotic disease, no special measures are recommended at this stage from a public health point of view.

In *N. caninum* -free herds, prevention of the introduction of the infection through standard biosecurity measures is the primary goal, whereas in *N. caninum*-infected herds, control programs are based on decreasing the vertical transmission in a herd by reduction of the number of seropositive cattle and/or decreasing the risk of horizontal transmission of *N. caninum* principally by controlling the definitive host population as a source of oocyst contamination [38]. Different control measures have been suggested, ranging from no action taken to the improvement of biosecurity on the farm, the introduction of new alternatives in the reproductive management of the herd, vaccination and the so-called “test and cull” strategies [39].

Global and National Status of Neosporosis: *Neospora caninum* is recognized world-wide as an important infectious cause of abortion in, primarily, cattle and of clinical disease in dogs [1]. Worldwide seroprevalences of *N. caninum* in dairy cattle are summarized in Table 1.

A cross-sectional study of *N. caninum* infection was conducted in major milksheds of Ethiopia. Cattle (n=2334) from 273 farms were bled and the sera screened for antibodies against *N. caninum* using a commercial ELISA kit. Herd and individual animal level data were collected from farm records and a semi-structured questionnaire format. The overall animal level seroprevalence was 13.3%, while the prevalence at farm level was 39.6%. In urban and peri-urban smallholder dairy farms, the seroprevalence was 14.9%, while 12.9% and 9.8% reactors were found among commercial dairy farms and breeding cattle, respectively. At farm level, 35.7% of urban and peri-urban farms, 47.5% of the commercial farms and five of the breeding farms were found to have at least one infected animal. Purchased cows and cows with history of maternal reproductive disorders were associated with seropositivity at the individual animal level. Crossbred cattle (Holstein-Friesian crossed with indigenous zebu) were associated with lower risk than pure breeds. A trend of prevalence increment was observed for large herd sizes. Other factors that were associated with seropositivity were: presence of farm dogs for more than 5 years, access to farm by wild carnivores and compromised farm hygienic status. Abortion, retention of foetal membrane

Table 1: Serologic prevalence of *N. caninum* antibodies in dairy cattle

Country	No. of animals (Relevant details)	No. of herds	% Positive	Test ^a	Reference(s)
Argentina	189 (abortion)	19	64.5	IFAT	[62]
Australia	266	1	10.2	ELISA	[63]
Brazil	100	3	46.0	ELISA	[64]
Canada	2, 037	23	21.9	ELISA	[65]
Costa Rica	2, 743	94	43.3	ELISA	[66]
France	1, 373	13	10.4	ELISA	[67]
Japan	145 (abortion)	-	20	IFAT	[68]
New Zealand	600 (abortion)	1	50	ELISA	[69]
United Kingdom	95 (abortion)	1	60	ELISA	[70]
United States	1, 000	16	14.7	ELISA	[71]
Vietnam	200	>30	5.5	ELISA	[72]

and metritis were the most frequently reported clinical reproductive disorders among seropositive cattle. Together, these findings indicate that *N. caninum* infection is highly prevalent, widely distributed and clinically important in dairy and breeding cattle of Ethiopia. *N. caninum* should be considered an important infectious cause of reproductive disorders in Ethiopian cattle and the risk factors for exposure identified here should be used as basis for implementing control measures that could limit the transmission of this infection [40].

Trichomoniasis

Aetiology: Trichomoniasis is a contagious venereal disease of cattle caused by the protozoan parasite *T. foetus* and is characterized by pronounced infertility following natural breeding, including early embryonic death (EED), abortion and pyometra and is responsible for major economic losses in beef cattle enterprise [41].

Life Cycle: The life cycle of trichomonads is simple. They reproduced by longitudinally fission [42]. Bulls, once infected remain so permanently, the organisms inhabit the preputial cavity and transmission to the cow occurs during coitus. From vagina trichomonads reach the uterus via the cervix to produce a low-grade endometritis intermittently organisms are flushed in to the vagina after two to three days before oestrus. Infection is usually followed by early abortion, the organisms being found in the amniotic and allantoic fluid. Subsequently cows appear to “self cure” and in most cases appear to develop a sterile immunity [43].

The organism lives in the crypts on the mucosal surface of the penis and prepuce of the bulls. Cows acquire the protozoa up on insemination by infected bulls. The organism infects the lining of the uterus, vagina, oviducts and cervix, making it possible for cows to transmit the diseases to other bulls if inseminated by more than one animal [44].

Epidemiology: Bovine trichomoniasis has a worldwide distribution and it is a venereal disease transmitted at coitus. *T. foetus* is known to occur in cattle, but where it is also present in other animals and whether it may be transmitted from them to cattle by a non-venereal route remain to be determined. *T. foetus* may or may not survive in frozen bovine semen, depending on the conditions. It survives in some media but not in others. Rapid freezing and high salt concentration are deleterious and the stage in the population growth curve is important, the protozoa being much more sensitive to injury when frozen during the initial and logarithmic phases than at the peak of the curve and for some time thereafter. Temperature fluctuation during storage is deleterious. Glycerol appears to be toxic at refrigerator temperatures but not at either subfreezing or incubator 37°C temperatures [42].

Normally one might expect the overall prevalence of trichomoniasis to be high since it is venerably transmitted by bulls, which do not show clinical signs. In fact the advent of supervised schemes of artificial insemination has largely eradicated the disease and today it is limited to areas where there are many small farms each with their own bulls or to countries where veterinary supervision is limited [43].

Transmission: Since trichomoniasis is a venereal disease it is transmitted from infected bulls to heifers or cows during mating. Under natural breeding conditions bulls often develop persistent asymptomatic infections and become parasite vectors thus act as carriers of the infection for life [45]. Benchimol *et al.* [46] demonstrated that *T. foetus* interact with sperm cells provoking damage and death of these reproductive cells in *in vitro* conditions.

Bulls are long-term carriers of the infection. They carry the infection in the folds of the penis or in the fonix area of the prepuce. Younger bulls are less likely to become permanent carriers than are older bulls, but may

still transmit the organism to susceptible females. Yearling bulls have been found by several investigators and practitioners to be culture positive on occasion [47].

Cows are also potential sources of new infections and maintaining the organism in a herd. Usually they only harbour the organism for a few heat cycles after infection or pregnancy loss. Sexual rest of at least four months has been prescribed for infected cows. Female cattle do not usually become carriers of *T. foetus* but usually clear the infections after aborting, maintenance of these parasite is thus by the clinically normal carrier male [48].

The sexual contact in this case includes AI. There is no satisfactory way to treat semen from infected bulls to make it safe for use in AI. [49]. Although not likely, transmission is possible by AI using frozen semen. The organism does not normally inhabit the urethra but could be found in the semen if infectious preputial fluid drained in to the artificial vagina at the time of semen collection. The diseases are limited to areas where there are many small farms each with their own bulls or to countries where veterinary supervision is limited [43].

Pathogenic Effect: Bulls infected with *T. foetus* are entirely without symptoms. Semen quality and sexual behavior are not affected. In bulls, the organism is only found on the penis and membranes inside the sheath. It localizes in the smegma, or secretions, of the penis, sheath and end of the urethra [50]. *T. foetus* does not normally inhabit semen, but semen may become contaminated with organisms from the skin of the penis. Crypts, or microscopic folds within the skin surface of the penis and sheath, are sites for localization of the organism. Because these crypts become deeper as the bull ages, there is a definite association between age and infection. Mature bulls are more adapted to become infected and stay infected. Strong evidence exists that once a bull is infected with trichomoniasis, he is infected for life [51].

As bulls infected with trichomoniasis are asymptomatic, the visible effects of the disease present themselves in the cows; [51] although, as is the case with bulls, cows do not appear ill while infected and overt signs of uterine or vaginal infection are not seen. Infection of the cow takes place at breeding. *T. foetus* organisms adhere to vaginal lining cells first with their posterior flagella, then with their body. The organism has adapted itself in several ways that enhance this attachment. Adherence to vaginal cells seems to be optimal at pHs of 6.0- 7.5, which is the normal pH range of the bovine vagina. Also, *T. foetus* adheres better to highly

keratinized (tougher) cells, which are present in greatest numbers during standing heat [52]. After the protozoa attach to the lining cells of the vagina, they form colonies which spread to the uterus and oviducts (Fallopian tubes) [50]. The uterus reacts to this colonization with an inflammatory response. The time interval from initial infection to the maximum inflammatory response means that the cow or heifer likely will conceive to the breeding in which the infection was transmitted. Inflammation due to trichomoniasis usually takes 50-60 days. In some cases, infection may take an extended period of time to overtake the fetus and abortion may not occur until 7-8 months of gestation. This is relatively uncommon [50].

Clinical Findings: In bulls there are no clinical signs once the infection is established [43]. On initial infection the prepuce may be inflamed and swollen and there may be a mucopurulent discharge. Orchiditis rarely occurs usually all clinical signs of infection ceases in about two weeks after infection but the bull remains a non-clinical carrier of the trichomonads, generally for life [48].

In cows the symptom of trichomoniasis is early abortion, which is a characteristic feature although this is often undetected because of the small size of the foetus and the case may present as one of an irregular oestrus cycle. Other clinical signs are those of purulent endometritis or a closed pyometra and in these cases, the cow may become permanently sterile [43].

Diagnosis: Various methods have been developed to accurately diagnose *T. foetus* in cattle. The most common techniques include serological assay for antibodies, light microscopic evaluation, culture of organism and molecular-based analyses of preputial washings from the sheath of infected bulls and cervico-vaginal secretions from female cattle [53]. Cobo *et al.* [45] detected *T. foetus* antigens immunohistochemically and reported that changes in lectin binding pattern may have been the consequence of either an inflammatory reaction or the effects of *T. foetus* enzymes such as neuraminidase and cysteine proteinase. Corbeil *et al.* [45] concluded that serologically tested breeding bulls could carry non *T. foetus* trichomonads in the prepuce causing false positive diagnoses for trichomoniasis in breeding bulls as well as in non-breeding bulls.

Treatment: There is currently no approved, effective treatment for trichomoniasis in cows or bulls. Since the disease is self-limiting in the female only symptomatic treatment and sexual rest for three months are normally

necessary [43]. Cows that have aborted early during the gestation period should be treated with the antibiotics intrauterine. In positive female the treatment to the genital tract is on the same lines as that advocated for pyometra. The use of broad spectrum antibiotics is indicated, however, the results are uncertain; the affected animals may act as carriers for in definitive period. If not culled this animals may be segregated, treated and bred only by AI [54].

The affected bulls may be treated with broad spectrum antibiotics infused through the prepuce. Only bulls of exceptional value should be treated and reused only after they are proved negative to strict diagnostic test. Treatment of bulls is expensive tedious and time consuming therefore, it is best to sell them for slaughter [54].

Prevention and Control: It has been generally recommended to slaughter infected bulls because treatment is difficult and the results are poor. Infected cows usually clean themselves; isolation and sexual rest for not less than three months followed by tests for infection before entrance into the herd are usually sufficient safety precautions. In practical use of AI rather than natural breeding will aid control of trichomonad abortion [48].

Effective control can be established by the following methods: breed virgin bulls to virgin heifers; elimination of infected bulls from the herd; breeding in large herds must be done by AI using semen from clean bulls; clean bulls must not be used for natural service or any cow or heifer ever bred to an infected bull; good breeding performance in cows is not a definite evidence of freedom trichomonad infection; maintenance of accurate service registers for bulls and cows; examine all mature females that have had any chance of infection or are suspected for any reason; suspend all other services for 6 to 8 weeks; all infected bulls and cows may be segregated and culled and examine all cows before service [47].

Global and National Status of Trichomoniasis: Bovine trichomoniasis is widespread around the world, especially in Asia, Australia, South America and South Africa, where natural service by bulls is used as a major means of breeding. For example, four of 80 aborted fetuses (5%) in 12 dairy herds in Beijing, China, between 2008 and 2010 were positive [55]. Five of 140 cows (3.5%) were positive in the province of Formosa, Argentina [56]. The incidence of infected bulls in South Africa varied from 0.9% in the southern Orange Free State to 10.4% in the north-western Cape Province, with an average of 7.1% [57]. In contrast, the disease has been dramatically decreased or even

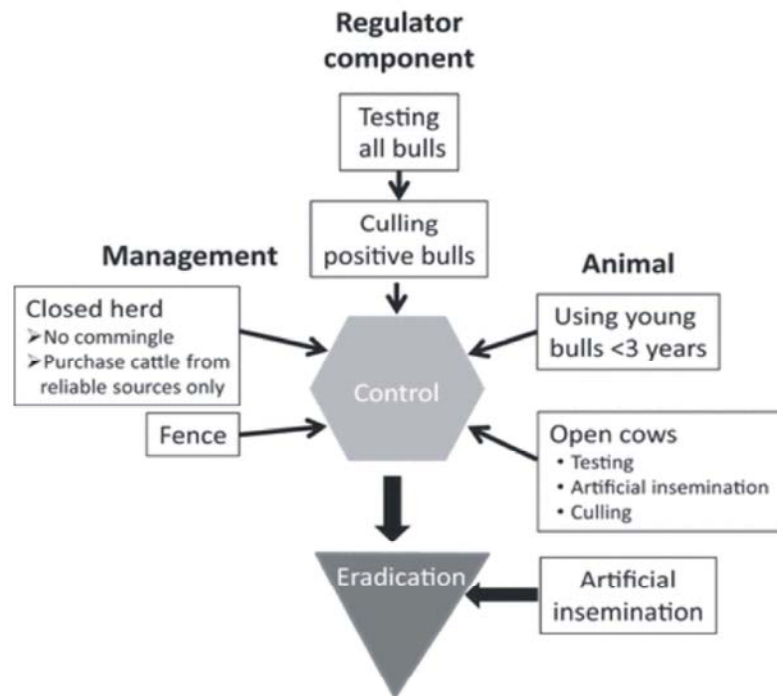


Fig. 2: A comprehensive approach to control and eradicate bovine trichomoniasis [60]

eradicated from some regions, such as many European countries, where artificial insemination is widely practiced. Only two cases have been reported in the UK over two decades [58]. A hotspot of *T. foetus* infection in Europe is northern Spain, where natural breeding is still a common practice. In Principado de Asturias and Leon province, 32% (33/103) and 2.9% (2/70) of bulls were positive, respectively [59]. Artificial insemination itself does not stop disease spread. The protozoan survives freezing in the liquid nitrogen required for conservation of semen if it is able to contaminate the semen. In the USA, especially in the Midwest and west, the disease is endemic. As a result, Mississippi and all states west of the Mississippi River, except Minnesota and Iowa, have already enforced state rules/regulations to curtail the disease according to the approach in Figure 2 [60].

CONCLUSION

Abortion is of great concern to the farmer because the fetus is lost and could have subsequent negative effects on the future productivity of affected animals. There are a number of possible causes of abortion among which protozoan causes are very important in that they can be transmitted to other animals in the herd. The major protozoan causes of abortion have worldwide distribution except trichomoniasis which is limited to regions and countries still using natural service. The major protozoan causes of abortion are neosporosis and trichomoniasis. There are different methods to differentiate protozoan causes of abortion including clinical signs of the case and serological testing. Since protozoan diseases results in abortion and infertility, there exists heavy loss in dairy industry; therefore control measures should be implemented.

Therefore, having the above conclusion in mind, the following points are forwarded as recommendation:

- Preliminary investigation must be done on the presence of the parasite especially in areas where there is a high incidence of abortion without clearly determined causes and appropriate control measure should be undertaken.
- The diagnostic laboratory facilities and skills should be improved.
- Examination of semen before AI of breeding cows should be done.
- The use of communal bulls for service should be avoided unless and otherwise the bulls are tested regularly for the major infectious causes of abortion.

REFERENCES

1. Dubey, J.P., G. Schares and L.M. Ortega-Mora, 2007. Epidemiology and control of neosporosis and *Neospora caninum*. *Clinical Microbiology Reviews* 20: 323-367.
2. Bjerkås, I., S.F. Mohn and J. Presthus, 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Z. Parasitenkd*, 70: 271-274.
3. Dubey, J.P., J.L. Carpenter and C.A. Speer, 1988. Newly recognized fatal protozoan disease of dogs. *Am. Vet. Med. Assoc.*, 192: 1269-1285.
4. Benchimol, M., R.S. Fontes and A.J.B. Dias, 2007. *Trichomonas foetus* damages bovine oocytes *in vitro*. *Vet. Res.*, 38: 399-408.
5. Mardones, F.O., A.M. Perez, A. Martinez and T.E. Carpenter, 2008. Risk factors associated with *Trichomonas foetus* infection in beef herds in the Province of Buenos Aires, Argentina. *Vet. Parasitol.*, 153: 231-237.
6. Christensen, B.W., M. Drost and M.H.T. Troedsson, 2009. Female reproductive disorders. In: Smith, B.P. (Eds): *Large Animal Internal Medicine*. Mosby, Elsevier, St. Louis, pp: 1419-1469.
7. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2008. Diseases associated with protozoa. 10th Edn. In: *Veterinary Medicine: A Textbook of Diseases of cattle, horses, sheep, pigs and goats*. Saunders Elsevier; pp: 1483-1540.
8. Taylor, M.A., R.L. Coop and R.L. Wall, 2007. *Veterinary Parasitology*. Third Edn.
9. Gumber, S., D.R. Aradhana Sharma and J. Singh, 2002. Neosporosis- an emerging cause of bovine abortion- a review. *Veterinary Practitioner*, 3(2): 94-100.
10. Bon Durant, R.H., 2005. Venereal diseases of cattle: Natural history, diagnosis and the role of vaccines in their control. *Vet. Clin. N. AM. Food A.*, 21: 383-408.
11. Dubey, J.P., 2003. Review of *Neospora caninum* and neosporosis in animals. *Korean J. Parasitol.*, 41: 1-16.
12. Thurmond, M.C. and S.K. Hietala, 1997a. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion on subsequent abortion in dairy cattle. *Am. J. Vet. Res.*, 58: 1381-1385.
13. Dubey, J.P., D.E. Hill, D.S. Lindsay, M.C. Jenkins, A. Uggla and C.A. Speer, 2002. *Neospora caninum* and *Hammondia heydorni* are separate species/organisms. *Trends Parasitol.*, 18: 66-69.

14. Dubey, J.P., 2004. Toxoplasmosis a waterborne zoonosis. *Vet. Parasitol.*, 126: 57-72.
15. Gondim, L.F.P., L. Gao and M.M. McAllister, 2002. Improved production of *Neospora caninum* oocysts, cyclical oral transmission between dogs and cattle and *in vitro* isolation from oocysts. *J. Parasitol.*, 88: 1159-1163.
16. Gondim, L.F.P., M.M. McAllister, R.C. Anderson-Sprecher, C. Björkman, T.F. Lock, L.D. Firkins, L. Gao and W.R. Fischer, 2004. Transplacental transmission and abortion in cows administered *Neospora caninum* oocysts. *J. Parasitol.*, 90: 1394-1400.
17. Roger, D.G., M.S. McCullough, W.S. Shain and M. L. Anderson, 1993. Endemic protozoal abortions in a dairy cow herd. *Agri-practice.*, 14: 16.
18. Nietfeld, J.C., J.P. Dubey, M.L. Anderson, M.C. Libal, M.J. Yaeger and R.D. Neiger, 1992. *Neospora*-like protozoan infection as a cause of abortion in dairy cattle. *J. Vet. Diagn. Invest.*, 4: 223-226.
19. Pfeiffer, D.U., N.B. Williamson, M.P. Reichel, J.J. Wichtel and W.R. Teague, 2002. A longitudinal study of *Neospora caninum* infection on a dairy farm in New Zealand. *Prev. Vet. Med.*, 54: 11-24.
20. Schares, G., A. Bärwald, C. Staubach, P. Söndgen, M. Rauser, R. Schröder, M. Peters, R. Wurm, T. Selhorst and F.J. Conraths, 2002. p38-avidity-ELISA: examination of herds experiencing epidemic or endemic *Neospora caninum*-associated bovine abortion. *Vet. Parasitol.*, 106: 293-305.
21. Trees, A.J. and D.J.L. Williams, 2005. Endogenous and exogenous transplacental infection in *Neospora caninum* and *Toxoplasma gondii*. *Trends Parasitol.*, 21: 558-561.
22. Barling, K.S., M. Sherman, M.J. Peterson, J.A. Thompson, J.W. McNeill, T.M. Craig and L.G. Adams, 2000. Spatial associations among density of cattle, abundance of wild canids and seroprevalence to *Neospora caninum* in a population of beef calves. *J. Am. Vet. Med. Assoc.*, 217: 1361-1365.
23. Björkman, C., O. Johansson, S. Stenlund, O.J.M. Holmdahl and A. Uggla, 1996. *Neospora* species infection in a herd of dairy cattle. *J. Am. Vet. Med. Assoc.*, 208: 1441-1444.
24. Frössling, J., A. Uggla and C. Björkman, 2005. Prevalence and transmission of *Neospora caninum* within infected Swedish dairy herds. *Veterinary Parasitology*, 128(3-4): 209-218.
25. Davison, H.C., C.S. Guy, J.W. McGarry, F. Guy, D.J.L. Williams, D.F. Kelly and A.J. Trees, 2001. Experimental studies on the transmission of *Neospora caninum* between cattle. *Res. Vet. Sci.*, 70: 163-168.
26. Moskwa, B., K. Pastusiak J. Bien and W. Cabaj, 2007. The first detection of *Neospora caninum* DNA in the colostrum of infected cows. *Parasitol. Res.*, 100: 633-636.
27. Dijkstra, T., H.W. Barkema, M. Eysker and W. Wouda, 2001. Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. *Int. J. Parasitol.*, 31: 209-215.
28. Sawada, M., H. Kondo, Y. Tomioka, Park, C. Morita T. Shimada A. and T. Umemura, 2000. Isolation of *Neospora caninum* from the brain of a naturally infected adult dairy cow. *Vet. Parasitol.*, 90: 247-252.
29. Buxton, D., M. McAllister and J.P. Dubey, 2002. The comparative pathogenesis of neosporosis. *Trends Parasitol.*, 18: 546-552.
30. Parish, S.M., L. Maag-Miller, T.E. Basser, J.P. Weidner, T. McElwain, D.P. Knowles and C.W. Leathers, 1987. Mylitis associated with protozoal infection in newborn calves. *J. Am. Vet. Med. Assoc.*, 191: 1599.
31. Dubey, J.P. and D.S. Lindsay, 1993. Neosporosis. *Parasitol. Today*, 9: 452-458.
32. Campero, C.M., D.P. Moore, H. Lagomarsino, A.C. Odeón, M. Castro and H. Visca, 2003. Serological status and abortion rate in progeny obtained by natural service or embryo transfer from *Neospora caninum*-seropositive cows. *J. Vet. Med. B*, 50: 458-460.
33. Anderson, M.L., C.W. Palmer, M.C. Thurmond, J.P. Picanso, P.C. Blanchard, R.E. Breitmeyer, W. Layton, M. McAllister, B. Daft, H. Kinde, D. Read, J.P. Dubey, P.A. Conrad and B.C. Barr, 1995. Evaluation of abortions in cattle attributable to neosporosis in selected dairy herds in California. *J. Am. Vet. Med. Assoc.*, 9: 1206-1210.
34. Baszler, T.V., S. Adams, J. Vander-Schalie, B.A. Mathison and M. Kostovic, 2001. Validation of a commercially available monoclonal antibody-based competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to *Neospora caninum* in cattle. *J. Clin. Microbiol.*, 39: 3851-3857.

35. Barr, C., I. Bjerkas, D. Buxton, P.A. Conrad, J.P. Dubey, J.T. Ellis, M.C. Jenkins, S.A. Johnston, D.S. Lindsay, L.D. Sibley, A.J. Trees and W. Wouda, 1997. Neosporosis, Report of International Neospora Workshop. *Comp. Con. Educ.*, 19: 120-126.
36. Ortega-Mora, L.M., A. Fernández-García and M. Gómez-Bautista, 2006. Diagnosis of bovine neosporosis: recent advances and perspectives. *Acta Parasitol.*, 51: 1-14.
37. Reichel, M.P. and J.T. Ellis, 2006. If control of *Neospora caninum* infection is technically feasible does it make economic sense? *Vet. Parasitol.*, 142: 23-34.
38. Hall, C.A., M.P. Reichel and J.T. Ellis, 2005. Neospora abortions in dairy cattle: diagnosis, mode of transmission and control. *Vet. Parasitol.*, 128: 231-241.
39. Conraths, F.J., G. Schares, L.M. Ortega-Mora and B. Gottstein, 2007. Control measures: neosporosis, pp: 279-287. In L.M. Ortega-Mora, B. Gottstein, F.J. Conraths and D. Buxton (ed.), *Protozoal abortion in farm ruminants. Guidelines for diagnosis and control.* CAB International, Oxfordshire, United Kingdom.
40. Asmare, K., F. Regassa, L.J. Robertson and E. Skjerve, 2013. Reproductive disorders in relation to *Neospora caninum*, *Brucella* spp. and bovine viral diarrhoea virus serostatus in breeding and dairy farms of central and southern Ethiopia. *Epidemiol. Infec.*, 193(1-3): 85-94.
41. Hafez, E.S.E., 1980. *Reproduction in farm animals*, 4th ed. Britain. Tindall. London, pp: 511-512.
42. Levine, N.D., 1985. *Flagellates the trichomonads, veterinary protozoology*. 1st ed. Iowa State University Press. Ames. Iowa, pp: 59-64.
43. Urquhart, G.M., J. Armour, J.L. Dunean, A.M. Dunn and F.W. Jennings, 1996. *Veterinary Parasitology*. 2nd ed. Black Well Sciences. UK, pp: 220-222.
44. Thomas, M.W. and W.M. Harmon, 1994. Bovine trichomoniasis; General information, Diagnosis and Control. *Agri. Practice.*, 11: 13-17.
45. Corbeil, L.B., C.M. Campero, K.V. Hoosear and R.H. BonDurant, 2008. Detection of trichomonad species in the reproductive tracts of breeding and virgin bulls. *Vet. Parasitol.*, 154: 226- 232.
46. Benchimol, M., I.A. Rosa, R. Da Silva Fontes and A.J.B. Dias, 2008. *Trichomonas* adhere and phagocytose sperm cells: Adhesion seems to be a prominent stage during interaction. *Parasitol. Res.*, 101: 597-604.
47. Bon Durant, R.H., 1985. Diagnosis, treatment and control of bovine trichomoniasis. *Compend Cont Ed Pract Vet.*, 7: S179-S188.
48. Ristic, M. and M.I. Lan, 1981. *Disease of cattle in the tropics.* Martinus Nijhft Publishers. Netherlands, 6: 578-582.
49. Bearden, H.I. and J.W. Fuquay, 2000. *Applied animal reproduction* 5th ed., Hall Inc. London, pp: 352.
50. Kimsey, P.B., 1986. Bovine trichomoniasis. In: Morrow DA (Ed.): *Current Therapy in Theriogenology*, 2nd ed. Philadelphia, WB Saunders, pp: 275-9.
51. Kimberling, C.V., J. Cheney and W. Cunningham, 2005. Bovine trichomoniasis: "the silent rustler." Proceedings, The Range Beef Cow Symposium XIX December 6, 7 and 8, 2005, Rapid City, South Dakota.
52. Corbeil, L.B., J.L. Hodgson, D.W. Jones, R.W. Corbeil, P.R. Widders and L.R. Stephens, 1989. Adherence of *Tritrichomonas foetus* to bovine vaginal epithelial cells. *Infection and Immunity*, 57: 2158-2165.
53. Grahn, R.A., R.H. BonDurant, K.A. Van Hoosear, R.L. Walker and L.A. Lyons, 2005. An improved molecular assay for *Tritrichomonas foetus*. *Vet Parasit.*, 127: 39-47.
54. Sane, C.R., N. Luktukes, A.S. Kaikini, V.B. Hukeri, B.R. Deshpande, D.P. Vethankar and S.R. Kodapali, 1982. *Reproduction in farm animals.* Varghese Publishing House, pp: 330-334.
55. Yang, N., X. Cui, W. Qian, S. Yu and Q. Liu, 2012. Survey of nine abortifacient infectious agents in aborted bovine fetuses from dairy farms in Beijing, China, by PCR. *Acta. Vet. Hung.*, 60: 83-92.
56. Mancebo, O.A., A.M. Russo, L.L. Carabajal and C.M. Monzon, 1995. Persistence of *Tritrichomonas foetus* in naturally infected cows and heifers in Argentina. *Vet. Parasitol.*, 59: 7-11.
57. Erasmus, J.A., J.A.L. De Wet, H.E. Van Der Merwe and G.C.J. Pienaar, 1989. Bovine trichomoniasis in the north Western Cape Province, western Transvaal and the Orange Free State. *J. S Afr. Vet. Assoc.*, 60: 51-52.
58. Taylor, M.A., R.N. Marshall and M. Stack, 1994. Morphological differentiation of *Tritrichomonas foetus* from other protozoa of the bovine reproductive tract. *Br. Vet. J.*, 150: 73-80.

59. Mendoza-Ibarra, J.A., S. Pedraza-Díaz, F.J. García-Peña, S. Rojo-Montejo, Ruiz-Santa- J.A. Quiteria, E. San Miguel-Ibáñez, V. Navarro-Lozano, L.M. Ortega-Mora, K.Osoro and E. Collantes-Fernandez, 2012. High prevalence of *Tritrichomonas foetus* infection in Asturiana de la Montaña beef cattle kept in extensive conditions in Northern Spain. *Vet. J.*, 193: 146-151.
60. Yao, C., K.D. Bardsley, E.A. Litzman, M.L. Hall and M. R. Davidson, 2011. *Trichomonas foetus* infection in beef bull populations in Wyoming. *J. Bacteriol. Parasitol.*, 2: 117.
61. Dubey, J.P., 1999. Neosporosis in cattle, biology and economic impact. *J. Am. Vet. Med. Assoc.*, 214: 1160-1163.
62. Venturini, M.C., L. Venturini, D. Bacigalupe, M. Machuca, I. Echaide, W. Basso, J. M. Unzaga, C. Di Lorenzo, A. Guglielmone, M.C. Jenkins and J. P. Dubey, 1999. *Neospora caninum* infections in bovine foetuses and dairy cows with abortions in Argentina. *Int. J. Parasitol.*, 29: 1705-1708.
63. Haerdi, C., M. Haessig, H. Sager, G. Greif, D. Staubli and B. Gottstein, 2006. Humoral immune reaction of newborn calves congenitally infected with *Neospora caninum* and experimentally treated with toltrazuril. *Parasitol. Res.*, 99: 534-540.
64. De Melo, C.B., R.C. Leite, Z.I.P. Lobato and R.C. Leite, 2004. Infection by *Neospora caninum* associated with bovine herpes virus 1 and bovine viral diarrhoea virus in cattle from Minas Gerais State, Brazil. *Vet. Parasitol.*, 119: 97-105.
65. Bergeron, N., G. Fecteau, J. Paré, R. Martineau and A. Villeneuve, 2000. Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec. *Can. Vet. J.*, 41: 464-467.
66. Romero, J. J., S. Van Breda, B. Vargas, G. Dolz and K. Frankena, 2005. Effect of neosporosis on productive and reproductive performance of dairy cattle in Costa Rica. *Theriogenology*, 64: 1928-1939.
67. Pitel, P.H., S. Pronost, M.F. Legendre, G. Chatagnon, D. Tainturier and G. Fortier, 2000. Infection des bovins par *Neospora caninum*: deux années d'observations dans l'Ouest de la France. *Le Point Vet.*, 31: 53-58.
68. Koiwai, M., T. Hamaoka, M. Haritani, S. Shimizu, T. Tsutsui, M. Eto and I. Yamane, 2005. Seroprevalence of *Neospora caninum* in dairy and beef cattle with reproductive disorders in Japan. *Vet. Parasitol.*, 130: 15-18.
69. Pfeiffer, D.U., N.B. Williamson and M.P. Reichel, 2000. Long-term serological monitoring as a tool for epidemiological investigation of *Neospora caninum* infection in a New Zealand dairy Herd, pp: 616-618. *Proceedings of the 9th Symposium of the International Society for Veterinary Epidemiology and Economics*, Breckenridge, CO.
70. Dannatt, L., 1997. *Neospora caninum* antibody levels in an endemically-infected dairy herd. *Cattle Practice*, 5: 335-337.
71. Lehenbauer, T.W., S.J. Rodgers, R.G. Helman and J.T. Saliki, 1998. Epidemiology of *Neospora caninum* infection in Oklahoma beef and dairy cattle. *Proc. 31st Ann. Conv. Am. Assoc. Bovine Pract.*, 31: 225.
72. Huong, L.T.T., B.L. Ljungström, A. Uggla and C. Björkman, 1998. Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes in southern Vietnam. *Vet. Parasitol.*, 75: 53-57.