Economic Impact, Diagnostic Investigations And Management Of Protozoal Abortions In Farm Animals

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Economic impact of abortions depends on direct costs and value of foetuses lost. Indirect costs include those associated with establishing the diagnosis, re-breeding cows that aborted, possible loss of milk yield, and replacement costs if cows aborted are culled. A low rate of abortions is usually observed on farms and 3 to 5 abortions per 100 pregnancies per year is often considered "normal." However, the loss of any pregnancy can represent a significant loss of income to the producer and appropriate action should therefore be taken to prevent abortions and to investigate the cause of abortions that may occur. Protozoan abortions are of utmost important in the changing scenario of farm practices and also in the wake of O.I.E. guidelines, to screen bulls before using for breeding purposes. Protozoan parasites cause abortions in farm animals include neosporosis, trichomonosis, toxoplasmosis and sarcocystosis. *Neospora caninum*, a recently recognized protozoan has become cause of bovine abortion in most of the countries. Although the organism shares a close relationship to *Toxoplasma gondii*, yet both of these agents induce biologically distinct diseases. *Neospora* associated abortions have also been reported in horses and goats. There is no clinical case report of disease in humans. In other words the diseases is not of zoonotic importance at present, dogs are the only proven definitive host for *N. caninum* and horizontal transmission to cattle occur via oocysts shed in the faeces of the dogs. There is no horizontal cow to cow transmission. Trichomoniosis is a venereal disease of cattle, characterized primarily by early pregnancy loss and, occasionally, by abortion and pyometra. *Toxoplasma gondii* induced abortions are very common in sheep**.** This parasite is species-specific. All felids, mostly the common domestic cat, are its final hosts*. Sarcocystis spp.* commonly infects cattle but only rarely and with massive infection does it cause abortion. Carnivores shed this protozoan in their faeces as a very resistant stage which survives in the environment and is ingested with forage. The paper reviews the economic impact diagnostic dilemmas and causes of protozoal abortions in farm animals.

Key Words: Abortions, Protozoan, Neosporosis, Trichomonosis, Toxoplasmosis and Sarcocystosis.

Abortion in dairy cattle is commonly defined as a loss of the foetus between the age of 42 days and approximately 260 days. Pregnancies lost before 42 days are usually referred to as early embryonic deaths, whereas a calf that is born dead between 260 days and full term is defined a stillbirth. A low rate of abortions is usually observed on farms and 3 to 5 abortions per 100 pregnancies per year is often considered "normal." However, the loss of any pregnancy can represent a significant loss of (potential) income to the producer and appropriate action should therefore be taken to prevent abortions and to investigate the cause of abortions that may occur. It is ideal to have every pregnant cow go to term and have a healthy calf. However, some losses due to abortion are expected and the maximum loss of 3% is acceptable. The economic impact of abortions depends on direct costs and value of fetuses lost. Indirect costs include those associated with establishing the diagnosis, re-breeding cows that aborted, possible loss of milk yield, and replacement costs if cows that aborted are culled.

Common Causes of Abortion

While infectious agents are perhaps the most frequently thought of cause of abortions in domestic animals, there are other factors which may cause a proportion of pregnancies to terminate with an abortion. Genetic abnormalities in the fetus which may also cause obvious physical changes in the fetus that may result in abortion and are not very frequently diagnosed. Heat stress can affect reproductive performance in a dairy herd, although it will generally cause conception problems rather than abortions. Toxic agents may also cause abortions or early embryonic deaths. Cattle are susceptible to fertilizer nitrites and nitrates or the nitrates found in plants under certain conditions (e.g. drought-stress). Some mycotoxins, Ergot alkaloids can cause abortions in cattle. Although the cause of many abortions is never determined, abortions caused by diseased agents represent the most commonly diagnosed cause of abortions in many laboratories. Among various diseases responsible for causing abortions, parasitic causes especially protozoan are very important.

Protozoal causes of Abortion:

There are following important protozoan parasites responsible for causing abortions in domesticated animals. Neosporosis, Trichomoniasis, Toxoplasmosis, Sarcocystosis and Miscellaneous Protozoan Parasites.

Neosporosis:

This is a recently recognized disease caused by a protozoan, *Neospora caninum.* Neosporosis once considered emerging disease, is now well established in most parts of the world. The literature on neosporosis in cattle and dogs has been reviewed by various authors (Dubey, 1992, 1999a, 1999b, 2003; Dubey and Lindsay, 1996; Wouda, 1998; Toolan, 2003; Romero and Frankena, 2004; Schetters, 2004; Sharma *et al*., 2004). In 1984, a new syndrome was recognized in three litters of dogs suffering from encephalitis and myositis in Norway (Bjerkas *et al*., 1984). But until 1988, it was misdiagnosed as *Toxoplasma gondii*. Later on, the causative agent was isolated in cultured cells from canine cases showing symptoms of neuromuscular disease and named as *Neospora caninum* (Dubey et al., 1988a, 1988b). However, neosporosis earned its significance due to its association with the storms of bovine abortions in United States (Thilsted and Dubey, 1989).

It is most common in dairy cattle. There are still many questions to answer about this disease. Infected dams only seem to abort when they are severely stressed during pregnancy. But the calves born from these dams are almost always infected and carry that organism for life and infect their offspring. The infection is not spread cow to cow within the herd. Uninfected cows can become infected by exposure to feed contaminated with dog faeces.

The majority of abortions seem to occur at 4-6 months of gestation after a period of stress to the dam resulting in lowered resistance. Serum from the dam can be used to determine if she has ever been exposed to *Neospora.* The aborted fetus, especially the foetal brain, is important in trying to confirm the diagnosis.

The etiological agent, *N. caninum* is a recently recognized obligate intracellular coccidian protozoa belonging to family sarcocystidae and Phylum Apicomplexa. *N. caninum* and *T. gondii* share several common antigens but the four *N. caninum* antigens (17, 29, 30 and 37 kDa) are not recognized in lysates prepared from *T. gondii* (Bjerkas *et al.,* 1994). Although the organism shares a close relationship with *T. gondii* structurally, immunologically and genetically (McAllister *et al.,* 1996), yet both these agents induce biologically distinct diseases; *T. gondii* mainly causes disease in sheep and humans whereas neosporosis is a major disease of cattle (Dubey, 2003).

Parasite Epidemiology

Bovine neosporosis has been reported as a leading cause of bovine abortions in most countries of the world (Dubey and Lindsay, 1996). The disease has been reported from most of the Asian countries but no case of neosporosis had been reported in Indian subcontinent (Dubey and Lindsay, 1996) although there are few suspected reports of neosporosis in India (Agrawal *et al*., 1997). Antibodies to *N. caninum* were found in 9.6% of cows, 5.1% of heifers, and 5.0% of calves from Punjab, India, suggesting postnatal transmission of *N. caninum* on the farm (Meenakshi *et al* 2007). Antibodies to *N. caninum* were determined in dogs from Punjab State, India, using commercial monoclonal antibody-based competitive ELISA and found in 16.8% of the animals (Sharma *et al* 2008). Neospora associated abortion has also been reported in horses (Dubey and Porterfield, 1990) and goats (Barr *et al.,* 1992; Dubey *et al.,* 1992).

Immunity

Clinical outcome of the transplacental foetal infection with *N. caninum* is likely to be determined by maternal and foetal immune response which involves humoral and cell mediated immune factors (Lunden *et al.,* 1998). CD_4^+ T Cells , interferon gamma and macrophages significantly inhibit multiplication of *N. caninum* tachyzoites *in vitro* indicating that cell mediated immune response plays an important role in reducing multiplication of *N. caninum* and thus combating the parasitemia (Lunden *et al.,* 2002).

Factors for transmission of disease

There are many factors that may affect the seroprevalence and may explain the occurrence of simultaneous abortions in dairy herds. The dogs can act as both definitive host as well as intermediate host for *Neospora caninum* (Basso *et al.,* 2001). Based upon this fact it is plausible that cattle are infected by exposure to canine oocysts shed in faeces. Various epidemiological studies have indicated that the presence of dogs on farm was the most important risk factor for the occurrence of *N. caninum* abortions in the cattle (Pare *et al.,* 1998; Bartels *et al.,*1999). A significantly positive association has been found between frequency of *N. caninum* antibodies in cattle and presence of dog on farm (Schares *et al.,* 2004). Presence of poultry (Bartels *et al.,*1999), pigeon (Mcguire *et al.,* 1999) and rabbit (Ould *et al.,*1999) on the farm premises had been found to be positively associated with *N. caninum* seropositivity, as these animals could serve as mechanical vector of oocysts particularly when they were grubbing about freely. In addition poultry and pigeon might play a role as intermediate hosts for parasite by which a dog may become infected after eating dead birds. Abortion is most likely to occur during the first pregnancy following infection with future pregnancies less likely to result in abortion. The proportion of seropositivity in cows increased gradually with age/ gestation number (Sadrebazzaz *et al.,*2004).

Clinical Symptoms

Neospora infection may either result in congenital infection in calf or foetal loss without any clinical illness in adults. Foetal loss may be in form of foetal mummification (Larson *et al.,* 1994), abortion, stillbirth or a stillborn calf with clinical signs (Anderson *et al.,* 1995). Outcome of the foetal infection will depend upon the stage of pregnancy in which infection occurs. Infection in early gestation caused abortion whereas in the late gestation produced congenitally infected but clinically normal calves (Toolan, 2003). The immunocompetence in the foetus develops at about 17 weeks of gestation. Williams *et al.* (2000) in experimental study found that abortion occurred in heifers exposed to *N. caninum* infection in 10^{th} week of gestation whereas heifers exposed to infection at 30^{th} week of gestation gave birth to live, full term but congenitally infected calves. The reason could be that tachyzoites multiply rapidly in non-immune foetus causing infection of foetus and placenta resulting in abortion or mummified foetuses whereas in immunocompetent foetus, tachyzoites multiply at slower rate and may become encysted in central nervous system and hence resulting in birth of live, full term but congenitally infected calves.

Although dogs of any age may be affected, clinical signs are mostly seen in young and congenitally infected pups. These dogs develop hind limb paresis that progresses to ascending paralysis. Hind limbs are more severly affected than front limbs (Cuddon *et al.,* 1992).Depending upon site parasitized other neurological signs can be difficulty in swallowing, paralysis of jaw, muscle flaccidity and atrophy (Dubey and Lindsay, 1996).

Diagnostic techniques

Definitive diagnosis of *N. caninum* abortion is difficult because in most of the cases, infection does not result in abortion. Even the demonstration of *N. caninum* infection histologically or immunohistochemically do not give conclusive evidence of organism as a cause of abortion (Thurmond *et al.,* 1999). However, when tests for other common aborting conditions like brucellosis, leptospirosis, salmonellosis, vibriosis and IBR are negative, neosporosis may be suspected. Conventional microscopy or histopathological examination of tissue cannot differentiate it from other apicomplexans particularly *T. gondii.* Recent use of immunological and molecular diagnostics has made it possible to detect neosporosis with high precision.

Histopathological diagnosis: A presumptive diagnosis of the neosporosis can be based on the presence of characteristic multifocal non-suppurative inflammatory lesions in brain and heart of aborted foetus (Wouda, 2000). For optimal diagnostic results, brain, heart and liver should be included in the histological studies. Characteristic focus of mononuclear cell infiltration surrounding a central area of necrosis is associated with intracellularly dividing tachyzoites and not usually associated with tissue cysts.

Demonstration of the parasite: As the other apicomplexans like *T. gondii* and *Sarcocystis sp*. may also cause similar lesion, the histological diagnosis should be confirmed by demonstration of parasite in tissues using either immunohistochemistry (IHC) or molecular techniques to detect parasite DNA.

Immunohistochemical examination (IHC): IHC can be performed on formalin fixed paraffin embedded tissue sections. The advantage of method is that the presence of parasite can be related to the lesions. However, this method is laborious and relatively insensitive. The rate of success depends on the number of sections made and the amount of time spent on the microscopic examination (Wouda, 2000). This method can be used to differentiate the parasite for other apicomplexans spp .

Molecular techniques: PCR detection of *N. caninum* infection in fetal tissue has been found to be more sensitive than IHC (Innes *et al.,* 2000; Okema *et al.,* 2004). Several PCR methods have been designed to detect *N. caninum* DNA such as standard PCR (Kaufmann *et al.,*1996), Real time PCR (Collantes *et al.,* 2002) and Multiplex PCR (Schatzberg *et al.,* 2003).

Serological diagnosis: The presence of antibodies in a host is only indicative of recent infection but a seroepidemiological approach by using various serological tests like ELISA, IFAT, DAT and immunoblotting allows one to assess an abortion problem at herd level (Jenkins *et al.,* 2002) along with information on epidemiology and life cycle (Atkinson *et al.,* 2000). Body fluids and sera can be examined for serological studies (Bjorkman and Uggla, 1999). Recently Meenakshi *et al* 2007 and Sharma *et al* 2008 found seroprevalence of *N.caninum* antibodies for the first time in India. There are several commercial ELISA kits available for serological diagnosis.

Indirect fluorescent antibody test (IFAT): IFAT was the first serological test used for the demonstration of the antibodies to *N. caninum* using culture-derived tachyzoites as antigen (Dubey *et al.,* 1988b). This test has been used as a reference test in evaluation of other tests as it showed very little cross reactivity with other coccidian parasites (Frossling *et al.,* 2003).

Direct agglutination test (DAT): DAT is another serological test not requiring any species-specific secondary antibody so can be used for a wide number of host species (Pachkam *et al.,* 1998; Romand *et al.,* 1998). Because of its simplicity and versatility the DAT had the potential of replacing IFAT as first hand test in many situations (Bjorkman and Uggla, 1999). An immunocomb test, which correlates very well with the results of the ELISA and IFAT tests, has been recently assessed in the Kilkenny laboratory. The result of this assay indicates a score from 0 to 6. A score of 3 or greater is significant in a recently aborted animal (Toolan, 2003).

Prevention and Control measures:

No treatment can prevent abortion or vertical transmission of infection in cattle. However, treatment of experimentally infected calves with Ponazuril (toltazuril-sulfone) resulted in considerable decrease in antibody concentration as well as reduced clinical signs in these calves (Kritzer *et al.,* 2002). Sulfonamides, pyremethamine and clindamycin either alone or in combination have partial success in treating canine neosporosis (Knowler and Wheeler). However, no treatment can prevent transplacental transmission in bitches (Dubey and Lindsay, 1996). Two principles that can be employed to prevent *N. caninum* infection in cattle is by limiting vertical and horizontal transmission (postnatal infection). Only mean to prevent vertical transmission is raising heifers from seronegative cows. Hall *et al.* (2005) recorded decrease in seropositivity of *N. caninum* antibodies from 10.2% to negligible adopting method of selective culling of seropositive animals. In herds with low prevalence, culling of all the seropositive cows and their offsprings is recommended but in herds with high prevalence more stress should be given towards culling of congenitally infected calves through precolostral serological testing. Horizontal transmission can be prevented by restricting the free access of dogs and wild animals like foxes & coyotes to feeding areas and feed stores to reduce the chance of faecal contamination of feed and water sources. Also try to avoid the access of dogs to calving areas and dispose off all the possible source of infections like dead foetuses and placental membranes carefully. Avoid feeding of colostrum from infected dam to calves. Serological and faecal testing of dogs in the surrounding areas should be done.

Dog faeces may be examined by sucrose flotation for oocysts and, if positive, sent to a specialist laboratory for identification of species. But dogs shed oocyst sporadically and also not all the dogs that shed oocyst have antibodies in blood. Many experimentally infected dogs had shed very small numbers of oocysts, making diagnosis by examination of the faeces very difficult (McAllister *et al.,* 1998). Because the disease is not contagious, the relationship between higher stocking density and seroprevalence is probably due to more cows coming into contact with the same contaminated feed or water compared to herd exposure when animals are spread over a larger area.

Vaccination: There is no proven vaccine to prevent *N. caninum* abortion in cattle . However, vaccination of mice before pregnancy with killed *N. caninum* tachyzoites has been shown to block transplacental transmission of infection when mice were challenged with *N. caninum* infection in pregnancy (Innes *et al.,* 2001). Bovilis-Neoguard (Intervet), a killed vaccine is available commercially in European countries (Andrianarivo *et al.,* 1999). A field trial conducted using this vaccine showed a reasonable decrease in abortion rate in cattle (Romero *et al.,* 2004).

Trichomoniosis: Trichomoniasis is caused by the protozoan *Tritrichomonas fetus*. This organism is spread at breeding (venereal) only. The majority of cows clear themselves of the infection after several estrus cycles. Bulls tend to remain infected and carry the organism from one breeding season to the next. Trich usually results in early embryonic death, which appears as repeat breeders and infertility.

Tritrichomonas foetus is confined to all regions of the reproductive tract where trophozoites multiply by binary fission to form two daughter trophozoites. In cows, the trophozoites attach to the surfaces of epithelial cells lining the reproductive tract. Examples of colonization in heifers and cows include the vagina, uterus, and oviduct. *T. foetus* can be found in secretions from these sites, including the mild mucopurulent discharge associated with vaginitis and endometritis. Bulls carry the protozoa only on the penis and preputial membranes, localizing in the secretions (smegma) of the epithelial lining of the penis, prepuce, and distal portion of the urethra. There are no lesions of diagnostic significance in bulls and the parasite does not affect either semen quality or sexual behavior. A scant purulent preputial discharge may be noted within the first two weeks of infection, but generally, the infected bull serves as an asymptomatic carrier of the parasite.

Older bulls tend to become permanent carriers of *T. foetus*, perhaps as a result of the development of epithelial crypts in the preputial cavity of older bulls. The parasite transmission rate from male to female at breeding may be as great as 42%. The prevalence of the diseases has been reduced due to application of arthlical insinuation pradies used all over the world. *T. foetus* is rarely transmitted by artificial insemination of cattle if appropriate procedures for bull testing and hygiene are practiced.

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 trophectoderm. Another potential virulence factor is the battery of extracellular cysteine proteinases that are elaborated by *T. foetus*. At physiologic pH, these enzymes are very active against a wide variety of proteins including immunoglobulin, fibronectin, and lactoferrin. Although *T. foetus* can bind immunoglobulin molecules in a nonspecific manner, whether this binding offers the parasite any protection from specific immune attack or whether it precedes proteinase degradation of immunoglobulin is not known.

Overt clinical signs are rare as the apparent infertility due to embryonic death is the most common result. Pyometra and abortion often are the first signs of trichomoniasis noticed in a herd, but they occur in relatively few animals. When abortion occurs, it is usually within the first third to one-half of gestation.

Grossly, the degree of autolysis in fetuses and placentas can vary from mild to marked. Placentas are edematous, but otherwise unremarkable. Fetuses may have no discernible lesions; however, enlarged livers and non-inflated, enlarged, firm lungs may be present on some fetuses. Emphysematous bullae involving the splenic and hepatic capsules and the parietal peritoneum have been reported. Because infection is inapparent in bulls and mild vaginitis is found only occasionally in cows, a definitive diagnosis requires the identification of parasites in infected animals. *T. foetus* is best located in preputial or vaginal secretions and, to a lesser extent, amniotic, allantoic, or abomasal fluids from the infrequently aborted fetuses. The flagellated parasites can be identified by direct microscopic examination of these fluids. More commonly, samples are inoculated into one of the several media, most notably Diamond's or Claussen's media, and allowed to grow in vitro until sufficient numbers of parasites are present to allow detection by light microscopy. In bulls, the organisms are in the prepuce, frequently in small numbers. Microscopic examination of preputial smegma for trichomonads is the most common method to confirm a herd diagnosis. The characteristic aimless, jerky motion of the flagellate is diagnostic. Culturing increases sensitivity markedly over direct exam. Even with culturing, there is a 10-20% probability than an infected bull will be missed by a single culture; hence, multiple cultures are recommended. In the field, the In-Pouch media system (Biomed Diagnostics, Santa Clara, CA, U.S.A) are convenient to use and have a long shelf-life.

Prevention and control: Artificial insemination has reduced the incidence of trichomoniasis over the past three decades and has proven to be the best control measure available. Other control measures are possible if artificial insemination is not feasible. The herd should be divided into exposed and unexposed groups. The exposed group should be treated for recognizable uterine disease and allowed three months of sexual rest. One may recommend eliminating all bulls greater than three years of age and using only younger bulls for mating. This is based on the relative lack of susceptibility of young bulls to trichomonad infection.

Vaccination: Immunoprophylaxis for bovine trichomoniasis has been a priority due to the prevalence of the disease and its economic impact. A killed T. foetus vaccine is available which can be used in both cows and bulls. Two infections are required, 2-4 weeks apart, prior to breeding season. Extensive field testing reveals that when used properly, protection can be obtained in over 90% of the animals vaccinated. Annual vaccinations are required. Natural immunity in the cow will develop after 1-3 heat cycles, but is of short duration (6-12 months). The available vaccine in USA (Trich Guard-Fort Dodge) is partially efficacious in the cow, but has no known efficacy in the bull; hence, disease control involves both identification and culling of infected bulls along with vaccination (prebreeding) of cows to decrease the incidence of infertility until the disease is eradicated from the herd. It is also important to remember that younger bulls are less susceptible to persistent infection than are older bulls.

A new bovine vaccine composition comprising an immunogenically active component having inactivated bovine Trichomonas cells or antigens derived therefrom, in combination with an effective amount of an immunogenically suitable adjuvant; and a veterinary pharmaceutically acceptable carrier or dilent is available in USA. The vaccine composition is useful to prevent *Tritrichomonas* (Trichomonas), e.g., *T. foetus*, infection in bovine, and may also be combined with other vaccine compositions or therapy.

Diagnosis: Diagnostic samples can include the foetus, placental fluids and cervical mucus from the dam. Preputial scrapings from the bulls can also be used to grow and identify *Trichomonas.*

An immunohistochemical technique using a monoclonal antibody was evaluated as a diagnostic tool to specifically label *Tritrichomonas foetus* in formalin-fixed, paraffin-embedded sections of placenta and fetal lung from bovine abortions. Trichomonads were demonstrated in tissues from each of 12 abortions due to *T. foetus* and none of 15 abortions due to other or unidentified causes. The antibody faintly labeled 1 of 3 other species of trichomonads (*Trichomonas gallinae*) but did not label other protozoa, bacteria, or fungi tested (Jack *et al* 1995).

A trichomoniasis polymerase chain reaction (PCR) assay was successfully established at the Central Veterinary Laboratory (CVL) Zimbabwe (Makaya *et al* 2002). Four preputial and 27 vaginal washings of bulls and cows obtained from communal cattle in Zimbabwe's Buhera and Wedza districts were tested using a PCR amplification assay as well as the traditional method of microscopy. Out of the 31 cattle tested, two bulls and three cows were positive on trichomoniasis PCR assay. The trichomoniasis microscopy method detected only one cow which was also positive with the PCR assay.

Reaching a diagnosis is only the beginning of the problem. No legal treatment exists for bovine trichomoniasis. Given the lifelong nature of most bull infections and lack of legal treatments, a veterinarian must recommend slaughter of infected bulls.

Toxoplasmosis:

Toxoplasma gondii is a widely prevalent protozoan of warm-blooded animals including humans, livestock and marine mammals (Dubey and Beattie, 1988). Toxoplasma induced abortions are very common in sheep.

Toxoplasma gondii is a common unicellular parasite of cats. This parasite is species-specific. All felids, mostly the common domestic cat, are its final hosts. Cats usually become infected by ingesting oocysts the oocysts continue developing in the cat's intestines to form schizonts and gamonts in the epithelial cells. Both of these stages greatly multiply the number of parasites in the cat's intestines. More oocysts are formed, which may infect any mammalian species, including sheep.

When nonfeline hosts ingest oocysts, they become intermediate hosts in which the parasite develops a tissue phase. The parasite penetrates the host animal through the blood vascular system. In the early acute stage (tachyzoite), the parasite multiplies quickly within host cells. Most animals respond by producing antibodies that limit the spread of tachyzoites and limit the pathogenic or damaging effect of these organisms. Usually, cysts form, which contain bradyzoites that are slower growing and may lie latent in the intermediate host. Humans or predators that consume meat containing these intermediate stages may become infected. This method of transmission may cause abortions, stillbirths, or CNS damage to human fetuses.

The intestinal stage of *Toxoplasma* normally does not cause disease. It is the tissue stage of the tachyzoites and bradyzoites in the sheep intermediate host that causes abortions, stillbirths, or the birth of weak lambs. Shepherds may experience all of the above conditions within their flocks, depending on the stage of pregnancy when a ewe becomes infected. Unfortunately, these same production losses may be caused by chlamydia, vibriosis, campylobacter, or leptospirosis. It is important for the producer to determine the cause of the lamb losses and take the correct actions to minimize future losses.

Diagnosis: Toxoplasmosis in sheep may be diagnosed by examining aborted fetuses, placental membranes, culturing *Toxoplasma*, or serological samples. Blood samples should be taken as soon as the abortion occurs and again one to two weeks later to test for changes in blood antibody titres. Many times, young ewes in their first pregnancy will experience abortions associated with *Toxoplasma*. If these ewes are kept and rebred, they often lamb normally, because they have developed a level of immunity as a result of their original infection.

In a random sample of 103 sheep farms in Ontario, 99% of the farms had some sheep serologically positive for *Toxoplasma gondii*, based on an enzymelinked immunosorbent assay (ELISA). The percent of sheep affected within farms ranged from 3.8% to 97.8%, with an average flock prevalence of 57.6 % (David *et al* 1991).

Two PCR based strategies were designed in which specific primers were used to amplify *T. gondii* mobile genetic elements (MGE's) revealing information on element size and positional variation(Terry *et al* 2001). The first PCR strategy involved the use of a standard two primer PCR while the second strategy used a single specific primer in a step-up PCR protocol. This approach was applied to *T. gondii* reference strains which were either acute virulent or avirulent to mice. The use of a standard two primer PCR reaction revealed the presence of a virulence related marker in which all avirulent strains possessed an additional 688 bp band. The single primer PCR strategy demonstrated that all virulent strains had identical banding patterns suggesting invariance within this group of strains. However, all avirulent strains had different banding patterns indicating the presence of a number of individual lineages within this group. The applicability and sensitivity of MGE-PCR in epidemiological studies was demonstrated by direct amplification of *T. gondii* from sheep tissue samples. All sheep isolates, tested in this way, gave identical banding patterns suggesting the presence of an endemic *Toxoplasma* strain on this farm.

Aurélien *et al*(2006) observed that ovines slaughtered in France may be highly infected by *T. gondii* with a potential risk of parasite transmission to humans by consumption of undercooked meat. Multilocus microsatellite analysis shows the predominance of type II in sheep as previously described in humans.

Even though there is no approved treatment for ewes exposed to or infected by *Toxoplasma*, the coccidiostat drug decoquinate may be fed to pregnant ewes as an aid to prevention of abortions due to toxoplasmosis. Injectable or oral sulfonamide antibiotics may be used to reduce/control abortions.

A vaccine is available in England, but it has not yet been approved for use in the United States. Farmers may reduce the incidence of infection in sheep by taking measures to reduce the chance of their animals' feed and water being contaminated by cat feces containing the *Toxoplasma* oocysts.

Sarcocystis: This protozoan organism commonly infects cattle but only rarely and with massive infection does it cause abortion. Infected dogs, coyotes, foxes and cats shed this protozoan in their feces as a very resistant stage which survives in the environment and is ingested with forage. *Sarcocystis*, which exists as numerous species, undergoes a coccidian-like life cycle with each having a distinctive definitive (usually carnivore) host which excretes sporocysts into the environment. Clinical sarcocystiosis is much less commonly diagnosed than toxoplasmosis and neither is it normally associated with fetal infection or abortion in either sheep or goats. However, infection is extremely common throughout the world and follows ingestion of food or water contaminated with sporocysts. Clinical signs, when seen, include fever, anaemia, inappetance and weight loss or reduced weight gain. Central nervous signs (hind limb weakness, ataxia, paresis), acute myopathy and death may occur. Diagnosis is difficult as infection is so common and clinical signs absent, mild or non-specific. Serology may be useful in some situations and histopathology/immunohistochemistry is valuable for confirming the cause of death. Control relies on preventing contamination of pasture and water with faeces of dogs, foxes and cats or by controlling access of young susceptible stock to contaminated land. Relatively little is known of the immunity induced by infection with *Sarcocystis* spp. but research indicates that protective immunity does develop and that cell-mediated mechanisms are probably important. It is likely that sarcocystiosis is under diagnosed as a problem and that better diagnostic methods are needed to show the true extent of the losses caused. Severely affected cows usually abort during the last trimester. **Diagnosis**: The best tissue sample is a caruncle (placentome) from the uterus submitted for histopathology or fluorescent antibody diagnosis. Sarcocystosis was diagnosed in an aborted bovine fetus. Immature and mature schizonts of *Sarcocystis* were disseminated in the vascular endothelium of all organs, but

especially the brain(Hong *et al* 1982). *Sarcocystis* organisms were also demonstrated by immunofluorescent techniques in sections of frozen tissues.

There are no effective vaccines. A *Sarcocystis* Neurona Vaccine (Killed protozoal vaccine) for vaccination of healthy horses as an aid in the prevention of neurologic disease (Equine Protozoal Myeloencephalitis) caused by subsequent exposure to the protozoan *Sarcocystis neurona* is available in USA .

Miscellaneous causes: Various protozoan parasites which are not directly responsible for causing abortion, occasionally results in abortion due to stress of disease. These includes *Theileria spp., Babesia Spp., Anaplasmosis spp., Trypanosoma spp.* and *Cryptosporidium spp.* In severe cases of the ileriosis lactation is reduced or ceases and abortion can occur (Dolan 1990). The parasites *Babesia (Theileria) equi* and *Babesia caballi* cause acute, subacute or chronic diseases in Equidae or neonatal babesiosis or abortion in mares (Heerden 1996; de Waal *et al*. 2004). By exclusion of other possible aetiological agents, strong circumstantial evidence is presented of *Trypanosoma evansi* infection being the cause of late gestation abortion and stillbirth in buffaloes (Lohr et al 1986). Correa (1978) reported bovine abortions associated with *Anaplasma marginale.*

Protozoan parasites are a significant cause of abortion and infertility in domestic ruminants. In the absence of effective methods for vaccination or treatment, control of these parasites is mainly based on management procedures to reduce infection and transmission.

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