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VOLUME III ISSUE II (For Private circulation only) July - Sept'14

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Disease - Neosporosis and its control in farms

Since its first recognition in Norway in dogs in 1984, Neosporosis has materialized as a serious disease of cattle and dogs worldwide and was defined as a new genus and species *Neospora caninum*. Abortions and neonatal mortality are a major problem in livestock sector and Neosporosis is a major cause of abortion in cattle.

Etiology

It is a protozoan parasite having wide host range infecting all the major domestic livestock species, companion animals, a few wild animals and even marine mammals. It is mainly associated with endemic and epidemic abortion in cattle. It is a sporadic cause of encephalomyelitis and myocarditis. Clinical Neosporosis has been reported in sheep, goats, deer, rhinoceros, and horses. Antibodies to *N. caninum* have been found in the sera of water buffaloes, red and gray foxes, coyotes, camels and felids.

Epidemiology

Occurrence

Early in 1990s, *N. caninum* was associated with abortion in pastured cattle in Australia and New Zealand. At present it probably has a worldwide occurrence.

Sero-positivity in herds may vary widely. Herd sero-prevalence from 7 to 70 % has been recorded. Within herds, aborting cows shows higher rate of sero-positivity than non-aborting cows. Abortion may be epizootic or sporadic in nature. In epizootic abortion, it is usually between 5-10%, but up to 33 % of cows may abort within a short period. In one study in Pakistan, *N. caninum* was present in 47% of aborting animals whereas in non-aborting cattle it was recorded as 39%.

In India, sero-prevalence of 12.61 and 9.97 % in dairy cattle and water buffaloes respectively were documented in Karnataka and Andhra Pradesh (2012). In another study in Ludhiana, 9.6% of cows, 5.1% of heifers, and 5.0% of calves were seropositive, which suggested postnatal transmission of *N. caninum* on that farm. A study on free-ranging mithuns in Nagaland revealed an overall prevalence of antibodies to *N. caninum* at 10%.

Distribution in different countries

N. caninum infections have been reported from most parts of the world causing abortion and neonatal mortality. Quantitative studies in the USA, New Zealand, the Netherlands and Germany indicate that 12 to 42% of aborted foetuses from dairy cattle are infected with *N. caninum*. Serological prevalence in cattle varies widely depending on geographical location and sensitivity of test used. In some herds, up to 87% of cows were seropositive. In one review which included 10 different countries, *N. caninum* associated abortions in cattle suggests that the median specific risk of abortion due to *N. caninum* infection in dairy cattle was 14.3%.

Life Cycle of N. caninum

In general, it is very similar in structure and life cycle to *Toxoplasma gondii*. Neosporosis is primarily a disease of cattle, and dogs and related canids are definitive hosts of *N. caninum*. Toxoplasmosis, on the other hand is primarily a disease of humans, sheep and goat, and, felids are the only definitive hosts.

The life cycle of *N caninum* having three known infectious stages; tachyzoites, tissue cysts, and oocysts. In intermediate hosts, tachyzoites and tissue cysts are found and they occur intracellularly. Tachyzoites are approximately 6 by 2 µm. Tissue cysts are found primarily in the central nervous system and are often round or oval in shape, up to 107 µm long. The tissue cyst wall is up to 4 µm thick, and the enclosed bradyzoites are 7 to 8 by 2 µm. Extra neural tissues, mainly muscles, may contain tissue cysts.

The oocysts are excreted in the feces of dogs and coyotes in an unsporulated stage. Oocysts sporulate outside the host in as few as 24 hours. They are the key in the epidemiology of neosporosis. They are environmentally resistant like the oocysts of other coccidians.

Transmission

Postnatal (horizontally & laterally) transmission may result by ingestion of tissue cysts or tachyzoites infected tissues or, by ingestion of sporulated oocysts contaminated food or drinking water. It may also transmitted transplacentally (vertically, congenitally) during pregnancy from an infected dam to her foetus. Transmission of the parasite from dam to offspring appears to be highly efficient and is quoted as ranging as high as 75–100%. Postnatal transmission rates are often quoted as being comparatively low. Exogenous transplacental transmission occurs after a primary, oocyst-derived, infection of a pregnant dam, while endogenous transplacental transmission occurs in a persistently infected dam after reactivation (recrudescence) of the infection during pregnancy.

Congenital infection is the major method of perpetuation in some herds. Calves born from congenitally infected cows are themselves congenitally infected and the infection is believed to be persistent and lifelong. Seropositive cows may have congenitally infected calves. Congenital infection can result in abortion, or the birth of a normal, infected calf. An infected cow can therefore give birth to a normal, infected calf at one pregnancy and abort in the subsequent pregnancy.

Other modes of transmission suggested are via milk and semen. Venereal transmission is possible, but unlikely since under experimental conditions large numbers of tachyzoites were necessary for infection. Dams naturally bred with experimentally infected bulls failed to seroconvert.

Tissue cysts and bradyzoites can survive up to 2 weeks at refrigeration temperature (4°C) but are killed by freezing. Oocysts were orally infective to cattle, goat and sheep, and, rodents such as mice, gerbils and guinea pigs.

Risk Factors

Age of cattle

The risk of being seropositive may increase with age or gestation number, suggesting that horizontal transmission of *N. caninum* is of particular importance in some herds.

Definitive hosts

In most epidemiological studies of dairy herds, the presence of farm dogs, either currently or within the past 10 years, or the number of farm dogs was a risk factor for seropositivity in cattle. Specific feeding habits of farm dogs, like feeding on aborted fetuses and placenta may increase the chance of seropositivity in cattle.

Intermediate hosts other than cattle

Not only cattle but also other intermediate hosts of *N. caninum* may present a source of infection for dogs and other canids. The presence of *N. caninum* DNA in naturally infected mice and rats suggests that these animals may be important sources of infection for dogs and other canids.

Feeding colostrum or milk

Experimental studies have demonstrated that neonatal calves may become infected by the ingestion of milk containing tachyzoites. However, cross-suckling of calves born to seronegative mothers on seropositive cows has not led to an infection.

Seropositivity of individual cattle

Seropositive cows are more likely to abort than are seronegative cows. Heifers may have an early abortion compared to older cows. The disease in dairy herds frequently occurs as an epizootic, with multiple abortions occurring in a 1-2 months period. Severely autolytic fetuses are aborted in the 5^{th} -7^{th} month of pregnancy in most reports.

Differences in the farm management (e.g. feeding, pasture management, cattle density and housing) may have an influence on infection risk.

Epidemic and Endemic N. caninum-associated Abortion

N. 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. 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. 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 *N. caninum* infections can cause abortion. Epidemic abortions are thought to be due to a primary infection of naïve dams with *N. caninum*, probably due to ingestion of feed or water contaminated with oocysts. Because pregnant dams may be exposed to contamination with oocysts almost at one time (point source exposure), exogenous transplacental foetal infection and the resulting abortions occur within a short period of time.

Recrudescence of a latent infection in the dam during gestation (resulting in endogenous transplacental foetal infection) may cause abortion. Latent infection in dams may have been acquired vertically or postnatally. If epidemic abortion is caused by an exposure to oocyst-contaminated feed or water, the observed variability regarding abortion risk may be explained by factors such as the infection dose, the pathogenicity of the parasite strain, and by the susceptibility of the dams (e.g., immune status, state of gestation). In many cattle herds with endemic abortion due to neosporosis, there is often a positive association between the serological status of mothers and their progeny; i.e., there is evidence that the major route of transmission in these herds is vertical. Several studies demonstrate that chronically infected seropositive cows can have more than a two fold-increased risk of abortion compared to seronegative dams.

Pathogenesis

The organism has a predilection for foetal chorionic epithelium and foetal placental blood vessels producing a foetal vasculitis and inflammation and degeneration of the chorioallantois, and widespread necrosis in the placentome. Tachyzoites penetrate host cells and are located in a parasitophorous vacuole. They can be found in macrophages, monocytes, vascular endothelial cells, fibroblasts, hepatocytes, renal tubular cells, and in the brain of infected animals. Cell death is by the active multiplication of tachyzooites.

Clinical findings

N. caninum causes abortion in cattle. Cows of any age may abort from 3 month gestation to term. Most neosporosis-induced abortions occur at 5-6 month gestation. Foetuses may die in-utero, be resorbed, mummified, autolysed, stillborn, born alive with clinical signs, or born clinically normal but chronically infected. Neosporosis-induced abortions occur year-round. Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows.

However, up to 95% of congenitally-infected calves born from seropositive dams remain clinically normal. Clinical signs have only been reported in cattle younger than 2 month of age. Hind limbs or forelimbs or both may be flexed or hyperextended. Neurologic examination may reveal ataxia, decreased patellar reflexes, and loss of conscious proprioception. Calves may have exophthalmia or asymmetrical appearance in the eyes. *N. caninum* occasionally causes birth defects including hydrocephalus and narrowing of the spinal cord.

Infected cows may have decreased milk production in the first lactation than uninfected cows, are prone to abort, and have a higher risk of being culled from the herd at an early age. There are indications that the N. caninum seroprevalence differs according to the cattle breed. Rate of abortion and immune responses after N. caninum infection might also be affected by the breed of cattle.

Diagnosis

Examination of the serum from an aborting cow is only indicative of exposure to *N. caninum* and histologic examination of the foetus is necessary for a definitive diagnosis of neosporosis.

The brain, heart, liver, placenta, and body fluids or blood serum are the best specimens for diagnosis and, diagnostic rates are higher if multiple tissues are examined. Although lesions of neosporosis are found in several organs, foetal brain is the most consistently affected organ. Because most aborted foetuses are likely to be autolysed, even semi-liquid brain tissue should be fixed in 10% buffered neutral formalin for histologic examination of haematoxylin and eosin (HE) stained sections. Immunohistochemistry is necessary because there are generally only a few *N. caninum* present in autolysed tissues and these are often not visible in H and E stained sections. The most characteristic lesion of neosporosis is focal encephalitis characterized by necrosis and non-suppurative inflammation. The efficiency of the diagnosis by PCR is dependent on the laboratory, stage of the autolysis of the foetus, and sampling procedures. Although immunohistochemical demonstration of *N. caninum* in lesions is the best evidence for etiology of abortion at the present time, its sensitivity is not beyond doubt. *N. caninum* DNA can be detected by PCR in formalin fixed paraffin-embedded bovine aborted brain tissue.

Other serologic tests that can be used to detect *N. caninum* antibodies are various Enzyme Linked Immunosorbent Assay (ELISA)s, tindirect fluorescent antibody test (IFAT), and *Neospora* agglutination test (NAT). Immunoblots are useful in detecting *N. caninum*-specific antibodies. Avidin-ELISAs designed to distinguish recent and chronic infections in cattle appear promising to distinguish endemic and epidemic abortion. Finding *N. caninum* antibody in serum from the foetus can establish *N. caninum* infection, but a negative result is not confirmative because antibody synthesis in the foetus is dependent on the stage of gestation, level of exposure, and the time between infection and abortion. Immunoblotting using *N. caninum* specific antigen improves diagnosis.

Although blood serum or any body fluid from the foetus may be used for serologic diagnosis, peritoneal fluid is better than other body fluids. In calves, pre suckling serum can be submitted for diagnosis of congenital infection.

Necropsy findings

Gross findings are of autolysis. The brain may be autolysed, but should still be submitted for examination along with heart, liver and placenta if available. Histological lesions are of multifocal encephalitis, myocarditis and periportal hepatitis. Liver lesions may be more prominent in epizootic abortions. Immunohistochemistry using anti-*N. caninum* serum is used to identify tachyzooites in tissues and the brain is the organ with the highest detection rate. Immunohistochemistry is specific but insensitive in diagnosing foetal neosporosis and maternal serology should be used in conjunction.

Serology using IFAT can confirm in individual cows. Because of the high prevalence of infection and the occurrence of congenital infection, care must be taken in extrapolating the results of a single positive diagnosis to problems of abortion. The high rate of natural congenital infection means that evidence of an aborted foetus is not proof of causation of abortion and foetal examination should be coupled with serological examination of aborting and non-aborting animals in the herd for statistical differences.

Differential diagnosis

T. gondii and *Sarcocystis cruzi* are 2 other protozoans that should be considered in the differential diagnosis of protozoal abortion in cattle. Immunohistochemical and detection of parasite DNA by PCR can distinguish them from *N. caninum*. *Sarcocystis cruzi* forms schizonts in vascular endothelium and is rarely (< 0.1%) found in aborted foetal brains, whereas *N. caninum* is usually located in extravascular tissues. Additionally, there are no immature schizonts in *N. caninum* infection in contrast with *S. cruzi* infections. Infection by *T. gondii* in bovine fetuses is rare.

Other causes of abortion for which differential diagnosis should be made are brucellosis, trichomoniasis, genital campylobacteriosis, Leptospirosis, IBR, mycotic abortion, listeriosis, epizootic viral abortion (borrelia-like spirochete), nutritional reasons etc.

Control

On farms with abortion problems, both maternal serology and abortion examinations should be carried out. In dairy herds, bulk milk testing could be used as an inexpensive tool for monitoring seroprevalence in lactating cows. This technique could adequately detect a 15% or higher intra herd seroprevalence in lactating cows.

N. caninum is efficiently transmitted vertically in cattle, perhaps for several generations. Congenitally infected cows are at high risk for abortion and abortion rates in infected herds can be substantially reduced by culling these animals.

The general control measures are:

(i) Quarantine and testing of replacement and purchased cattle.

(ii) Prevention of transmission from dogs and other potential definitive hosts.

(iii) Prevention of waterborne transmission: Since the source of water (pond versus well or public water supply) has been shown to be a probable risk factor for *N. caninum* in cattle and waterborne transmission has been demonstrated for the closely related parasite *T. gondii,* measures to prevent water contamination by faeces from the definitive hosts should be implemented.

(iv) Rodent control: Regular rodent control by appropriate measures should be implemented to reduce the potential risk of infection that may exist in a reservoir for *N. caninum* in rodents.

(v) Prevention of putative factors for disease recrudescence in congenitally infected cattle: Giving feed of mouldy fodder, which may contain mycotoxins, should be avoided.

(vi) Transfer of embryos from infected dams into uninfected recipients can prevent endogenous transplacental transmission of *N. caninum*. Embryo transfer should be done only to seronegative recipient cows.

Treatment

Treatment of cattle appears to be uneconomical due to the fact that it can only be used as a preventive measure and hence it must be long-term and likely produce unacceptable milk or meat residues or withdrawal periods. Currently, there is no chemotherapy for bovine neosporosis that has been shown to be safe and effective.

Vaccination

There is no commercial vaccine presently available for neosporosis. Mouse models are being used to test efficacy of killed and recombinant N. caninum vaccines.

Zoonotic potential of *N. caninum*

As two rhesus monkeys have been successfully infected with *N. caninum*, there is concern about the zoonotic potential of *N. caninum*. However, at present there is no firm evidence that it can successfully infect humans. Neither *N. caninum* DNA nor the parasite has been demonstrated in human tissues. Humans are not regarded as intermediate host of N. caninum. As yet, no accidental *N. caninum* infections in persons handling viable organisms have been reported.

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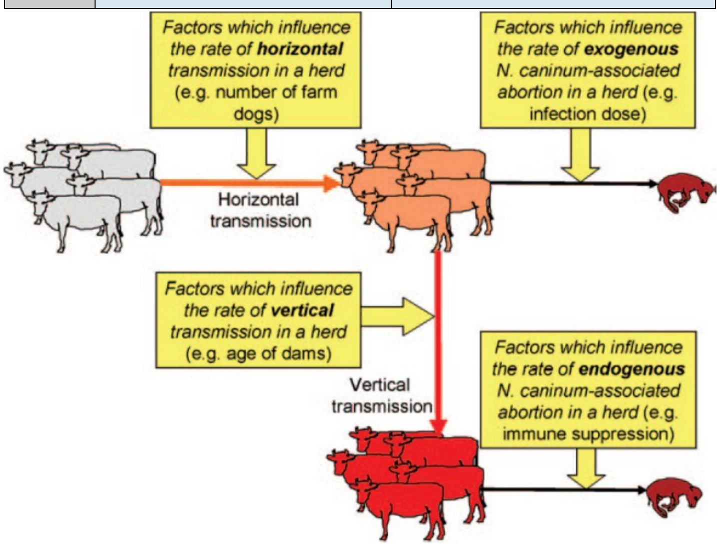
OIE - Significant animal diseases reported to OIE during Jul – Sep'14

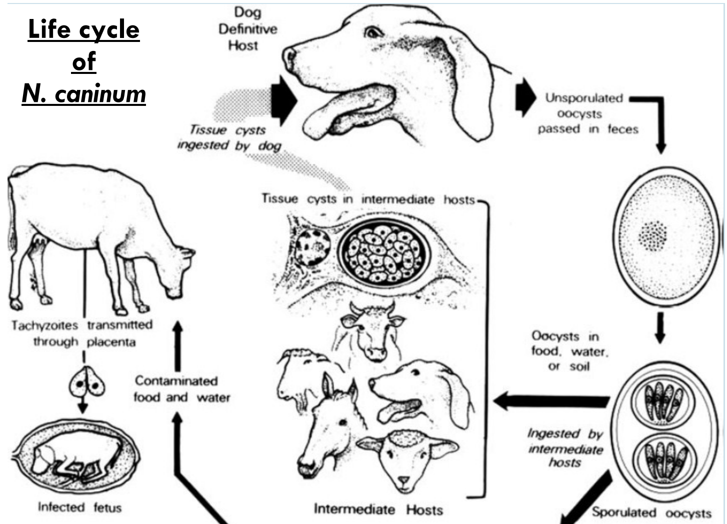
Sl.No	Disease Outbreak	Countries reporting
1	Lumpy skin disease	Azerbaijan
2	Bluetongue	Botswana
3	FMD	China (A) , South Korea (O)
4	Porcine Epidemic Diarrhea	Taipei
5	Bovine Anaplasmosis	French Polynesia
6	Anthrax	Kazakhstan, Poland
7	Classical swine fever	Mongolia
8	Infection with Perkinsus olseni	New Zealand
9	Newcastle disease	Sweden Source: www.oie.int



Overview of potential risk or protective factors influencing the horizontal or vertical transmission of *N. caninum* and the occurrence of exogenous or endogenous *N. caninum*-associated abortion. In the above diagram, naïve cattle are gray, postnatally infected cattle are orange, and vertically infected cattle are red.

Source : http://cmr.asm.org/content/20/2/323.full.pdf+html

Life cycle of *N. caninum*



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Epigenetic inheritance- A new angle to inheritance

Genetic information is encoded by stretches of DNA inside the chromosome of each cell. But another layer of information is encoded in epigenetic marks, which include chemicals such as methyl (CH_3) that attach to the DNA and to the histone groups that the DNA encircles. When these epigenetic marks bind to DNA in or hear genes, they often alter the amount of RNA or protein made from the gene.

Pollutants, stress, diet and other environmental factors can cause persistent changes in the mix of epigenetic marks in chromosomes and, in that way, can alter how cells and tissue behave. Surprisingly, some acquired changes can be passed on to descendants.

Epigenetic inheritance might play a role in health problems such as obesity and diabetes, as well as in the evolution of species.

Source: Scientific American , August 2014.

Pathogens northward bound

Rising arctic temperatures are helping pathogens spread and thrive in areas where they have not been before. Changing climate will hurt many species including man, raising the need for improved biosecurity. Some of the examples are as under:

Sweden: Mosquitoes have spread tularaemia to people a across the country.

Russia: Tick borne encephalitis cases in humans have risen 50 fold from the decade 1980-89 to 2000-09.

Canada:

(i) Distemper virus of seals has been discovered in otters.

(ii) Avian Cholera has killed hundreds of arctic birds.

(iii) Ticks have been seen in areas where they were never seen before.

(iv) lungworm infection in musk oxen has spread several hundreds of kilometers northwards.

For the same reason, the arctic is now being called the "Pandora's box of infectious disease and climate change" by some global health experts.

Source: Scientific American , August 2014.

Disclaimer : The views expressed in the articles of this issue are not that of NDDB but have been obtained from the source (s) mentioned at the end of each article.

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