A 6-month-gestation aborted fetus in a Holstein cow

Keywords: bovine neosporosis, *Neospora caninum*, abortion, immunohistochemistry, PCR

History
An 8-year-old 550 kg, Holstein cow had an abortion in the second trimester of gestation (approx 4 to 6 months of gestation). It was the first abortion of this animal, and in the previous 6 months, the farm owner reported 3 abortions in a herd of 122 animals. Routine herd prophylaxis includes annual vaccination against infectious bovine rhinotracheitis, bovine viral diarrhea virus, *Campylobacter fetus* subsp *fetus*, *Campylobacter fetus* subsp *veneralis*, *Leptospira interrogans* serovar Pomona, and *Histophilus somni* (Bioabortogen).

Clinical and Gross Findings
Gross postmortem examination was performed in an aborted fetus with moderate autolysis (Figure 1). The crown to rump length was 56 cm (estimated gestational age, 6 months). External malformations or dystocia-associated lesions were not observed. There was a diffuse red discoloration of the subcutaneous tissue, with scant adipose stores. Approximately 30 mL of serosanguineous fluid was found within the pleural and peritoneal space. The internal organs were grossly unremarkable. Multiple tissue samples from the gastrointestinal tract, heart, lung, liver, spleen, kidney, thymus, and brain were collected, fixed in neutral-buffered 10% formalin, and routinely processed for histologic examination.

Histopathologic and Microbiological Findings
Histologically, the cerebral cortex had multifocal discrete foci of necrosis (approx 200 to 300 μm in diameter), surrounded by a rim of glial cells, scant lymphocytes, and plasma cells (Figure 2). The heart had multifocal aggregates of lymphocytes, plasma cells, and macrophages expanding the interstitial space. Occasional extracellular and intracytoplasmic zoites were evidenced within the necrotic foci of the neuronal parenchyma and myocytes, respectively, on immunohistochemistry staining (*Neospora caninum* mouse monoclonal gp65 IgG1 isotype, catalog No. 5B6-25, 1:500 dilution; VMRD Inc). The skeletal muscle was multifocally infiltrated by perivascular and interstitial aggregates of lymphocytes, plasma cells, and macrophages, separating the muscle fibers and surrounding the blood vessels. An *N caninum*–specific PCR performed on fresh tissue samples from brain was positive.

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Morphologic Diagnosis and Case Summary

Morphologic diagnosis: (1) severe multifocal necrotizing encephalitis, lymphoplasmacytic, with intralesional extracellular protozoal zoites; (2) severe multifocal interstitial lymphoplasmacytic myocarditis with intracytoplasmic protozoal zoites; and (3) severe multifocal lymphoplasmacytic and histiocytic interstitial and perivascular myositis.

Case summary: bovine protozoal abortion caused by *N caninum* infection.

Comments

Bovine neosporosis is caused by *N caninum*, an intracellular, parasitic protozoan, from the phylum Apicomplexa. Bovine neosporosis has a worldwide distribution and is associated with abortion in dairy and beef cattle. *N caninum* has a heteroxenous life cycle, with dogs and wilds canids (Australian dingoes, coyotes, and wolves) being definitive hosts and certain animals as intermediate hosts (including cows, sheep, horses, pigs, and deer). *N caninum* cysts have been retrieved from dogs, cattle, water buffalos, sheep, and white-tailed deer (*Odocoileus virginianus*). Experimental infection of a rhesus macaque has been described in the literature.

The life cycle of the parasite involves 3 stages: tachyzoites that infect the tissues extracellularly in between cells or through the bloodstream, bradyzoites within the dormant cysts present in the intermediate host, and the oocysts containing sporozoites shed in feces by definitive hosts. The bradyzoite-containing cysts are found within the CNS. There are 2 transmission routes: horizontally by the ingestion of sporulated oocysts and vertical transmission through the placenta. The canids are infected by ingestion of tissue cysts; this differs from dairy cattle, in which there is not transmission between adult animals and the transplacental transmission is the most important source of infection. Transplacental infection can be endogenous when tissue cysts within a previously infected animal are
reactivated and reach the fetus, causing abortion and commonly seen in endemic patterns of reproductive failure.\textsuperscript{2} Exogenous transplacental infection occurs when the pregnant animal ingests the sporulated cysts, and this infection is seen with an epidemic pattern of abortion.\textsuperscript{3}

The lesions observed in fetuses are related to the age of gestation when infection occurs. Abortion caused by \textit{N caninum} occurs between 3 months of gestation until near full term; however, most of the cases are reported between 5 and 6 months of gestation.\textsuperscript{2} The majority of the abortion occurs at the second trimester because the fetal immune system is immature.\textsuperscript{3} The fetuses can be grossly unremarkable or have several changes, including dying in utero, being resorbed, being mummified, being autolyzed, being stillborn, and being born alive without clinical signs or born clinical normally with chronic infection, especially if the infection occurs in the last trimester of gestation.\textsuperscript{2,3} \textit{N caninum} abortions occur throughout the year.\textsuperscript{2} Seropositive cows are more prompt to abort than seronegative ones.\textsuperscript{2,5} However, 95\% of the calves born from seropositive cows are clinically normal.\textsuperscript{2,5} Cattle younger than 2 months have several clinical signs, including ataxia, being underweight, flexion of hyperextension of limbs, exophthalmia, and proprioception deficit.\textsuperscript{2} The macroscopic evaluation in this case was unremarkable; however, abortion caused by \textit{N caninum} can lack gross lesions.\textsuperscript{1} In some cases, congenital defects including hydrocephalus or narrow spinal cord are seen.\textsuperscript{2} Affected placentas present cotyledon necrosis with unremarkable intercotyledonary areas.\textsuperscript{4}

Diagnosis of neosporosis is achieved by the combination of necropsy, histopathology, PCR, and immunohistochemistry.\textsuperscript{1} The best diagnostic recommended specimens are brain tissue, heart, liver, placenta, and body cavity fluids.\textsuperscript{1} The histopathologic lesions observed in this case correspond to the ones described in the literature. In abortions, \textit{N caninum} infection causes foci of necrosis in the cerebral cortex surrounded by glial cells, lymphocytes, plasma cells, and macrophages; multifocal lymphoplasmacytic and histiocytic infiltrates in the myocardium; and aggregates of mononuclear inflammatory cells and macrophages in the skeletal muscle.\textsuperscript{1} Lymphoplasmacytic infiltrates in lungs, kidney, and liver are rarely seen and less frequent, and lymphoplasmacytic, histiocytic, and neutrophilic placentalis with cotyledon necrosis are additional findings not reported in this case.\textsuperscript{1,3} Only a few cysts are present within the brain and, in autolyzed specimens, are difficult to assess; thus, immunohistochemistry is necessary to demonstrate the presence of \textit{N caninum}.\textsuperscript{1} Asymptomatic calves and adult animals can present lesions related to the presence of cysts within the brain and, less frequently, heart, skeletal muscle, and liver.\textsuperscript{3}

The use of different diagnostic methods is supported by previous studies that demonstrated histopathologic lesions in only 30\% of the cases with a PCR positive for \textit{N caninum}.\textsuperscript{1} Additionally, only 28.57\% of the cases with characteristic histopathological lesions associated with \textit{N caninum} infection were PCR positive.\textsuperscript{1} Thus, the use of different diagnostic tools is highly encouraged for a definitive diagnosis. Abortions with advanced autolysis can test negative on the PCR due to DNA degradation.\textsuperscript{2,5} The \textit{N caninum} DNA can be detected in paraffin-embedded tissues, but PCR on fresh tissues is more sensitive.\textsuperscript{2} Detection of \textit{N caninum} antibodies is done through serological tests, including enzyme-linked immunosorbent assays (ELISAs), Neospora agglutination test, and indirect fluorescent antibody test.\textsuperscript{5} The presence of fetal antibodies against \textit{N caninum} is diagnostic; however, a negative serological test is not definitive since antibodies titers are affected by factors including gestational age, infection time, and abortion.\textsuperscript{2} Blood, serum, and body fluid including peritoneal effusion are useful for serological diagnosis.\textsuperscript{2} Seropositive animals are considered to remain positive for life, with increased antibody titers that can persist for years after exposure.\textsuperscript{5}

Several risk factors have a direct influence on the rate of infection in the individuals or the herd, leading to endemic recurrent abortions. These risk factors have been identified through large retrospective cross-sectional or case-control studies in bovine neosporosis.\textsuperscript{4} Infection risk is influenced by the age of the animals (older animals are at increased risk); number of gestations; presence of definitive hosts at the farm, including dogs, coyotes, and other carnivores like cats; and presence of other intermediate hosts, including mice and rats, that make the oocysts available for the definitive hosts as well.\textsuperscript{4} Other factors are presence of oocyst- contaminated grass, food containers, and drinking water; colostrum contaminated with tachyzoites; calving management at the spring; cattle density; size of farmland and herd; and source of replacement heifers.\textsuperscript{4} Lastly, additional, external environmental and human-related factors include the climate, with a higher temperature favoring sporulation of oocysts; the vegetation index; the human population density (since there is a positive correlation with dog population density); the antibody-positive status for other infectious agents like bovine viral diarrhea virus and bovine herpesvirus-1; certain breeds, including Holstein Friesian and Rubia Gallega; and the type of housing.\textsuperscript{4}

Culling seropositive cows, heifers, and calves from seropositive cows is the elective method to control the vertical transmission in cattle; however, in herds with high prevalence, culling is impractical.\textsuperscript{2} Other control measures include embryo transfer from seropositive cows to seronegative cows and selection of seronegative cows for breeding stock.\textsuperscript{2,5} Horizontal transmission can be avoided by maintaining domestic and wild canids feces out of the pastures, barns, water containers, and cattle feed storage units.\textsuperscript{2,4} The consumption of dead calves, abortions, and placental membranes by dogs or wild canids should be avoided.\textsuperscript{2} The only available commercial vaccine (NeoGuard) has been taken out of the market due to the inconsistent efficacy.\textsuperscript{3} A yearly serological screening for \textit{N caninum} in a herd is recommended to establish control measures.\textsuperscript{3}
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Disclosures

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