PEER REVIEWED

Investigation of an abortion epidemic due to *Neospora caninum* in a beef herd on pasture

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Abstract

An outbreak of epidemic abortion in a commercial cow-calf herd was investigated. Within 3 weeks of the index cases, 69 reproductive abnormalities were recorded consisting of abortions, stillbirths, and premature calves born alive but dying within 24 hours. By the conclusion of the calving season, 85 abnormal reproductive outcomes were reported out of 154 cows at risk. The epidemiologic pattern of abortion highly suggested a point-source exposure. Results of diagnostic tests implicated *Neospora caninum* as the causative agent of abortion. Eleven aborted fetuses were submitted for diagnostic evaluation, and all had lesions consistent with protozoal abortion. The diagnosis was confirmed by polymerase chain reaction assays and immunohistochemical tests performed on brain tissue from 2 aborted fetuses. Once the diagnosis was made, no treatment options were available. Serologic testing was performed to determine the seroprevalence in the herd and the statistical correlation of a positive serum test to an abnormal reproductive outcome. Control measures were instituted to decrease the presence of the organism in the herd and the risk of horizontal and vertical transmission of the organism. Clinically, *N. caninum* is widely recognized as an important infectious cause of reproductive loss in cattle, and vertical transmission of the protozoan from infected dams to their progeny is considered the major mode of infection and maintenance of the parasite within the population. However, the consequence of a point-source exposure and horizontal transmission in a susceptible population at or near the same vulnerable stage of gestation can be an abortion epidemic, as evidenced by this outbreak.

Key words: abortion, *Neospora caninum*

Résumé

Une flambée d'avortement épidémique dans un troupeau vaches-veaux (bovins allaitants) commercial a été investiguée. Moins de trois semaines suivant l'apparition des premiers cas, on a recensé 69 anormalités de reproduction incluant des avortements, des mort-nés et des veaux prématurés qui mourraient en moins de 24 heures. À la fin de la saison de vêlage, on a répertorié 85 événements reproducteurs anormaux sur un ensemble de 154 vaches à risque. La distribution épidémiologique des cas d'avortement suggérait une exposition ponctuelle. Les tests diagnostic suggéraient que l'agent responsable des avortements était Neospora caninum. Des lésions compatibles avec un avortement protozoaire ont été notées chez chacun des 11 fœtus avortés examinés. Le diagnostic a été confirmé avec le test basé sur la technologie d'amplification en chaîne de la polymérase de même qu'avec un test immunohistochimique sur des tissus du cerveau provenant de deux fœtus avortés. Il n'y avait pas d'options de traitement lorsque le diagnostic fut établi. Des tests sérologiques ont été faits afin de déterminer la séroprévalence à la ferme et d'établir une association statistique entre les événements reproducteurs anormaux et la séropositivité. Des mesures de contrôle ont été mises en place pour décroître la présence de l'organisme dans le troupeau et le risque de transmission horizontale et verticale de l'organisme. Neospora caninum, au niveau clinique, est reconnu comme un agent infectieux responsable de perte en reproduction chez les bovins. La transmission verticale du protozoaire d'une mère infectée à ses jeunes est considérée comme la voie d'infection et de maintien du parasite la plus importante dans une population. Toutefois, une exposition ponctuelle alliée à une transmission horizontale dans une population susceptible dans la même étape vulnérable de la gestation peut engendrer une épidémie d'avortement, comme ce fut le cas dans cette flambée.

Introduction

Neospora caninum is a protozoan parasite that has emerged as an important infectious cause of epidemic and endemic abortion in cattle.[8] Dogs (*Canis familiaris*) and coyotes (*Canis latrans*) are the only known definitive hosts for *N. caninum*.[14,19] Cattle are the most common

and economically important intermediate host for the organism.[12] There are 3 infectious stages of the parasite: tachyzoites and bradyzoites that are in tissues of chronically infected hosts, and sporozoites that are only present in oocysts excreted in feces of the definitive canine host. Once oocysts are ingested, sporozoites excyst and, as tachyzoites, invade and divide rapidly within many types of cells.[8] At this juncture, maternal immune mechanisms determine whether the tachyzoites are released to the bloodstream and disseminate throughout the body, or slow their replication within cells, differentiate into bradyzoites, and become encysted. The life cycle of the organism is complete when dogs or coyotes consume infected tissues and subsequently pass unsporulated oocysts in the feces[2,6,7] after sexual reproduction (schizogenic and gametogenic) phases in the intestinal epithelial cells of dogs occurs.[29]

Little is known regarding *N. caninum* oocysts in dogs, and research published to date includes only small numbers of animals. One research trial revealed that puppies shed significantly more oocysts (mean 166,400) compared with adult dogs following primary exposure (mean 2900); therefore, a dog's age is thought to influence oocyst production.[15] Shedding begins at approximately 7 days post-infection and continues for 1 to 18 days either continuously or intermittently. After the dog recovers, immunity is acquired but not absolute. Oocysts may be shed spontaneously from the first exposure or from a subsequent exposure, and immunity may wane over time.[15] Prevalence data is lacking, but one study indicated 0.03% of dogs in the pet population evaluated were shedding oocysts.[24] The infective dose necessary to cause transplacental infection has not been precisely determined because it depends on the dose of oocysts ingested and the time of pregnancy when this occurs. Experimentally, a dose of 70,000 oocysts administered on day 70 of gestation did not cause fetal infection, whereas 1500 oocyts induced fetal infection in cows after day 160 of gestation.[14] Widespread dissemination of the organism on pasture by a single dog or coyote is unlikely. This is in contrast to cats infected with tissue cysts of *Toxoplasma gondii* that may shed up to a billion oocysts,[9] and a dose of 2000 oocysts can infect and cause abortion in pregnant sheep.[23] Abortion due to *N. caninum* is thought to occur by direct fetal and placental damage by the parasite, as well as immunological mechanisms triggered in the dam and fetus.[8]

History

The outbreak occurred in the Upper Southern region of the United States and involved a herd consisting of 168 predominantly Angus beef cows; approximately 50% were registered stock and there were 2 purebred Angus bulls. No purchased cattle had been added to the herd since 1998. A controlled 90-day breeding system was in use, and this herd was due to begin calving August 30, 2011. Of the 168 cows, 14 were either known non-pregnant or scheduled to be culled on the basis of signs of estrus, leaving 154 pregnant cows at risk. A majority of the susceptible dams were in the third trimester of gestation when the outbreak began. The herd was maintained on fescue (*Festuca arundinacea*) pasture with some clover (*Trifolium repens*) and weeds present. Pastures were rotationally grazed on a regular basis and no burn piles, pits, barns or sheds were in any of the fields where the cattle were grazed. The water source included a pond and a concrete, above-ground stock tank gravity-fed from the pond. A commercial trace mineral mix[a] was offered *ad libitum* to the cows in unprotected feed troughs.

Cattle were annually vaccinated 6 weeks before breeding with a combination inactivated vaccine against bovine herpesvirus type 1, bovine viral diarrhea virus (types 1 and 2), parainfluenza virus type 3, bovine respiratory syncytial virus, *Campylobacter fetus, Leptospira canicola, L. grippotyphosa, L. hardjo-bovis, L. icterohaemorrhagiae,* and *L. pomona*.[b] A macrocyclic lactone anthelmintic[c] (an avermectin) was administered at the time of annual vaccination.

The presenting complaint was late-term abortion beginning with 3 index cases on July 25, 2011. By day 8 of the outbreak, 42 fetuses had been aborted. The fetuses weighed approximately 40 lb (18 kg), and were fully haired and moderately autolyzed on expulsion. The cows exhibited no clinical signs of illness, but many had retained fetal membranes. By August 17, 2011 (22 days after the index case), 69 reproductive abnormalities were recorded consisting of abortions, stillbirths, and premature calves born alive but dying within 24 hours. In addition, 6 low birth-weight calves were born prematurely but survived. At the conclusion of the calving season, 10 cows presumed pregnant did not calve and were thought to have experienced fetal death *in utero*. In total, 85 abnormal reproductive events had occurred out of 154 cows at risk (55%).

The diagnostic plan consisted of a complete history, physical examination, sample submissions to veterinary diagnostic laboratories, and an investigation of the premises. The history and observations from the herd owner revealed several areas of concern. The trace-mineral mix fed to the cows had been changed on June 11, 2011 to a mineral containing S-methoprene[d] for fly control. The cows reportedly consumed twice the recommended intake of 4 ounces (113.4 gm) per head per day (exact amount unknown) in the first week, prompting the herd manager to reduce the amount of free-choice mineral available and to offer an additional salt block. The owner described the weather the week the outbreak began as "exceptionally hot", and the pond

used as the water source had a "particular stench" and a "green scum". A soybean field near the pond had been sprayed with a chemical of unknown type on or about July 10, and a 2.5-inch (6.35 cm) rainfall occurred shortly thereafter, with runoff likely from the sprayed field to the pond. The chemical was presumed to be a herbicide as the weeds subsequently wilted and died throughout the soybean field.

The ration fed throughout the winter and spring was 3 lb (1.36 kg) per head per day of a custom grain mix of one-third corn gluten, one-third soy hulls, and one-third corn screenings with monensin[e] added to deliver 0.005 ounces (150 mg) per head per day. The herd manager frequently observed raccoons and their scat in the feed before offering it to the cows. Coyotes were intermittently observed on the farm, as well as a few dogs from adjacent farms. On June 2, 2011, 44 cows were moved from the sentinel herd to another farm within 5 miles and managed separately. All management practices were identical between the two herds except feed and water. The 44 cows remained on the aforementioned grain mix, but it was discontinued to the sentinel herd. The transported cattle also had a different water supply from the sentinel herd. The herd manager reported no abortions in any of the cattle moved to the new location.

Clinical Findings

At the time of the initial herd investigation visit, the cows showed no outward signs of illness except retained placentas in many of the aborting dams. The cows were in excellent body condition with an average score of 6.5 on a 9-point scale. Physical examination of an aborting dam was unremarkable.

Ten aborted fetuses, 2 placentas, 6 serum samples from aborting dams, and 2 water samples were submitted to a veterinary diagnostic laboratory for analysis.[f] The fetuses averaged 40 lb (18 kg) and were fully covered with fine hair, suggesting a gestational length of 224[21] to 240[13] days. A fixed and stained slide of the brain tissue containing a protozoal organism from the tenth fetus was submitted for parasite identification.[g] An eleventh fetus was submitted to a second veterinary diagnostic laboratory for molecular confirmation of the neosporosis diagnosis and a water sample for polymerase chain reaction (PCR) analysis.[h]

Laboratory Findings

Results of diagnostic tests confirmed *N. caninum* was the causative agent of abortion. All 11 fetuses submitted for diagnostic investigation had lesions consistent with protozoal abortion; the organism in the brain of fetus 10 was identified as *N. caninum* by immunohistochemical tests[g] and *N. caninum* nucleic acids were detected in the brain of fetus 11 by PCR analysis.[h] The fetal brain was the most consistently affected organ, and the most characteristic lesion was multifocal nonsuppurative meningoencephalitis. Fetal cardiac and skeletal muscle also contained foci of nonsuppurative inflammation, some with mineralization.

Neospora caninum competitive enzyme-linked immunosorbent assay (cELISA) performed on fetal heart blood yielded only 1 positive result from 10 samples. Finding *N. caninum* antibodies in fetal serum can establish the presence of infection in the fetus, but a false negative often occurs due to the stage of gestation and the time between infection and abortion.[11]

Titers to various leptospiral serovars could not be differentiated from vaccine-induced titers, and no evidence of leptospirosis was found utilizing PCR assays or fluorescent antibody tests on fetal tissues. Clinically significant bacteria were not isolated from fetal or placental tissue. No viruses were isolated, and fluorescent antibody tests for bovine herpesvirus type 1, bovine viral diarrhea virus, parainfluenza virus type 3, and bovine respiratory syncytial virus were negative.

Toxicological testing of 8 fetal ocular fluid samples for nitrate and nitrite were negative, and metal analyses of 4 fetal liver samples were negative for toxic elements. Analysis of water samples from the pond and stock tank for anions, pH, and metals yielded results within normal limits for livestock. An organic compound screen used to detect numerous pesticides, drugs, and industrial chemicals was negative for the water samples submitted. Attempts to identify the *Neospora* organism from the water supply by PCR assay were unsuccessful.

Serums from 6 cows that aborted were tested for *N. caninum* antibodies utilizing a commercial cELISA,[i] and 5 yielded positive results. Follow-up serologic testing of the affected herd utilizing cELISA[i] was conducted 2 months after the initial abortion case. Testing revealed 86 of the 154 cows at risk were seropositive for antibodies to *N. caninum* (56%). Chi-square analysis confirmed that seropositivity increased the likelihood of an abnormal reproductive event by 2.594 times among the population at risk (P=0.006). Serologic testing of all of the various groups of cattle on the affected farm demonstrated minimum *Neospora* exposure in yearling bulls and heifers, pregnant heifers, the spring calving herd, and the subset of the sentinel herd moved before the outbreak. Heifers born during the outbreak and kept for replacement stock were serologically tested at 1 year of age. Of the 33 heifers tested, 21 were seronegative and 12 were seropositive for *Neospora*.

Therapeutic Management

Before the cause of the abortion outbreak was identified, the owner prophylactically administered

long-acting tetracycline[j] and a booster dose of the killed vaccine[b] previously used on the advice of a consulting veterinarian. There is currently no medical therapy approved or deemed effective for treatment of *Neospora*-induced abortion in cattle, but control measures were recommended to decrease the presence of the organism in the herd and the risk of vertical transmission of the organism. The owner was advised to cull all females that abort a second time in subsequent years, fail to calve in more than one year, or are non-pregnant after removal of the bull. The owner was further advised to serologically screen all females older than 6 months of age and exclude the progeny of any seropositive female as a replacement heifer unless she is *Neospora* cELISA test-negative. The use of embryo transfer from infected dams to uninfected recipients was advised to preserve valuable genetic traits.

Control of horizontal transmission was addressed by decreasing the possibility of canine fecal contamination in sources of feed, water, and mineral. The owner was advised to use covered mineral feeders above ground level, to keep grain mixes in a storage bin before feeding, and to improve water availability by the addition of above-ground stock tanks, preferably using municipal or well water as the source. It was further recommended to fence ponds and not allow cattle to use them as a water source. Rapid disposal of any aborted fetus and/or placenta was encouraged to prevent consumption by domestic dogs or coyotes and to disrupt the life cycle of the organism; this practice would also decrease environmental contamination with infective oocysts.

Discussion

External or point-source infections from feed or water contaminated with oocysts are the most likely cause of an abortion outbreak[20,30] due to *Neospora*. An outbreak is defined as more than 10% of cows at risk aborting within 6 to 8 weeks.[8] Horizontal or postnatal transmission of the organism, as suspected in this case, occurs when a naïve cow becomes infected by consuming canine feces containing sporulated oocysts of *N. caninum*. The sporozoites then differentiate to tachyzoites, which can then spread to the uterus by the mononuclear phagocytic system.[29] This process results in "exogenous transplacental transmission"[25] of the organism to the fetus.

Experimental evidence suggests that the time during pregnancy when fetal infection occurs is critical in determining fetal survival.[3,10,22] Fetal immunocompetence begins to develop at 100 days gestation, but the fetus is unable to recognize and respond to antigens until 150 days gestation.[22] Infection in early pregnancy (less than 95 days) may lead to rapid fetal death without obvious signs of abortion, which may be confused with infertility.[17] In the middle third of pregnancy, the fetus may be able to mount an immune response sufficient to prevent death. During the third trimester of pregnancy, the fetus is capable of mounting an increasingly competent defense that results in a clinically normal but persistently infected calf.[28] Most infected cows abort between 3 and 8 months gestation, with a majority of abortions occurring between 4 and 6 months gestation due to a transitory immunosuppression in T lymphocytes and increased sensitivity of the animals to parasitemia at that time.[1] Moderate autolysis of the fetus is often observed on expulsion. Rarely, a congenitally infected calf may be born alive with neurologic signs, birth defects, and/or born weak and unable to stand.[8] The aborted fetuses in this outbreak were calculated to be 224[21] to 240[13] days of gestation when death occurred.

In a study by Almería et al, fetal death was observed within 6 weeks after inoculation of a naïve dam with *N. caninum* tachyzoites at 110 days of gestation.[1] If consistent with this research, this would place the aborting cows in this outbreak at 6 to 7 months gestation when exposed. Cows exposed at 8 months gestation gave birth to live calves; this was likely due to the fetus's ability to mount a competent immune response and survive. Of the 69 live, normal calves delivered in the fall of 2011, 52 were born in the first 6 weeks of the calving season. Of these 52 cows, 23 were seropositive for *N. caninum*, and an additional 6 were seropositive at a later date. Many seropositive cows gave birth to live, normal calves, likely due to exposure late in gestation that resulted in clinically normal calves that were persistently infected with *N. caninum*. A positive serologic test result from a calf bled before ingestion of colostrum is indicative of fetal infection, but this was not possible to perform in this case due to management issues. Conversely, of the 17 calves born in the last 6 weeks of the calving season, only 5 cows were seropositive. This reflects the vulnerability of the fetus when transplacentally infected at an earlier gestational age. Seronegative dams, on the other hand, followed a normal calving distribution throughout the calving season. It is important to recognize the creation of a large susceptible population of cows through the management practice of a controlled breeding season which placed most of the pregnant cows at the same vulnerable stage of gestation at the same time with no vaccine available to decrease risk to the herd.

Perhaps the most challenging aspects of this case are predicting this herd's reproductive prognosis and controlling risk of future infection. Little is known regarding the persistence of infection in cows infected by ingestion of oocysts or if adequate protective immunity develops after abortion and seroconversion to prevent future reproductive problems.[18] However, it is clear that a heifer calf infected *in utero* by exogenous transplacental transmission will be persistently infected, and can transmit the organism *in utero* to all of her offspring in

all pregnancies.[16] To interrupt vertical transmission, it is particularly important to identify these persistently infected females and not retain or sell these as herd replacements;[16] therefore, serologic testing at 6 months of age or older of all heifer calves born to seropositive dams should be performed and seropositive heifers removed. In this herd, 33 replacement females born during the outbreak were retained as replacement stock. Of these, 21 were seronegative and only 3 of these seronegative heifers had seropositive dams. Twelve heifers were seropositive for *Neospora* and all 12 had seropositive dams. These infected heifers were sold to a feedlot to finish for beef.

To control horizontal transmission, contamination of feed, water, and mineral sources must be minimized. All fetal membranes and aborted fetuses should be disposed of before dogs or coyotes can consume them. Lastly, inclusion of an ionophore or coccidiostat in either the feed or the mineral mix may have some inhibitory effect on the protozoal organism, although this remains to be scientifically demonstrated.[26] An IgG avidity test[5] to estimate the relative duration of *N. caninum* infection in a herd has been described. Low avidity values indicate exposure to the organism within the previous 6 months, which would be strong evidence of a point-source exposure. To the author's knowledge, the avidity analysis is not offered in a commercial laboratory in the United States, and therefore was not a viable option during this outbreak. Alternatively, serologic testing of the various groups of cattle on the affected farm demonstrated minimum *Neospora* exposure in yearling bulls and heifers, pregnant heifers, the spring calving herd, and the subset of the sentinel herd that was moved to another farm. This suggests the infection was most likely a recent point-source exposure rather than an endemic problem persistent within the herd, especially considering that this herd produces all of its replacement cattle and has been closed to new additions for 13 years. New research involving typing of *N. caninum* by multilocus-microsatellite analysis may help characterize future abortion outbreaks. A recent study found that a common microsatellite pattern prevailed in all fetuses from an epidemic abortion outbreak, and this pattern is unique to the herd in which it occurred. This common microsatellite pattern supports the hypothesis of infection from a common point-source.[4]

This outbreak raises questions as to the source of the organism and how it affected so many animals so quickly and catastrophically. Many questions were raised about the nature of the infective oocyst, such as how long it survives in the environment and what animals (in addition to the dog) may be able to serve as reservoirs and/or definitive hosts. An unusual similarity between this epidemic and a large abortion outbreak described in a beef herd in 1997[27] is the history of a change in the trace mineral mix approximately 6 weeks prior to the index case. Waldner et al hypothesized that contamination of the mineral mix before or after distribution to the herd could potentially explain a consistent level of infection in a herd on pasture. Dogs and coyotes are known to investigate ground-level mineral feeders as a source of salt, which may result in fecal contamination. Water is also a common point-source of contamination, but it is difficult to explain the level of infection in the herd given the dilution of the organism in an artificial stock tank or pond. Attempts to identify the organism from the water supply by PCR assay were unsuccessful in this case.

Conclusion

To the author's knowledge, this is the largest abortion outbreak described over a 3-week period of time that was caused by neosporosis. Many factors, including serologic status of the dam, route of transmission of the organism to the dam, stage of gestation when infected, fetal immunocompetence, and environmental and nutritional stressors all contribute to the outcome of this protozoal infection and ensuing parasitemia in a pregnant cow. Clearly, more research is needed to understand the immense complexity of *N. caninum,* including its survival in the environment and other possible definitive hosts, in order to reduce the risk of a point-source exposure.

Endnotes

[a]Purina® Wind & Rain® All Season 7.5 Comp AU2800 Altosid®, Purina Mills, St. Louis, MO
[b]Vira Shield® 6 + VL5 HB, Novartis Animal Health US Inc., Larchwood, IA
[c]Cydectin® Pour-On, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
[d]Altosid®, Wellmark International, Schaumburg, IL
[e]Rumensin®, Elanco Animal Health, Indianapolis, IN
[f]University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY
[g]United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Animal and Natural Resources Institute (ANRI) Animal Parasitic Disease Laboratory (APDL), Beltsville, MD
[h]Breathitt Veterinary Center, Hopkinsville, KY
[i]Neospora caninum Antibody Test Kit, cELISA, VMRD Inc., Pullman, WA
[j]Liquamycin® LA-200®, Pfizer Animal Health, Exton, PA

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