Introduction

Neospora caninum is an apicomplexan parasite, first identified as distinct from Toxoplasma gondii in 1988. The first reports of neosporosis came in 1984 from cases admitted to the Norwegian College of Veterinary Medicine in which an unidentified protozoan was found in dogs. Several years later, a retrospective study was performed of 23 dogs seen at Angell Memorial Hospital in Boston with a Toxoplasma like illness. Investigators determined, on closer examination, that this organism did not fit into any known genus and it was given the name Neospora caninum. N. caninum is now known to have a worldwide distribution and infects a number of mammalian hosts, ranging from livestock, including dairy cattle, to companion animals.

N. caninum has become recognized as a common infectious cause of abortion in dairy cattle and, as such, has become an economic concern to the dairy industry. The organism is associated with late term abortion outbreaks with rates exceeding 30% and endemic infections resulting in yearly average abortion rates of up to 12%. Abortion is the primary clinical sign associated with N. caninum infection and cows may abort repeatedly. This would suggest that protective immunity against abortion does not seem to develop. While N. caninum has been reported in other parts of the country, no assessment of this organism's prevalence has been described in the Southeastern United States.

A case control study was designed to assess N. caninum's impact on Virginia dairy cattle, using herd yearly abortion rate as the primary definition of a case. This study sampled commercial dairy herds to assess N. caninum seroprevalence and investigate this parasite's significance as an abortifacient agent in Virginia by testing the hypothesis, "N. caninum seroprevalence in Virginia dairy herds with a high abortion rate is greater than in herds with a low abortion rate."

Literature Review

N. caninum is an apicomplexan protozoan parasite, first identified as distinct from *Toxoplasma gondii* in 1988. The first reports of neosporosis was reported in 1984 from dogs admitted to the Norwegian College of Veterinary Medicine with an unidentified protozoan infection. Several years later, a retrospective study of 23 dogs seen at Angell Memorial Hospital in Boston with a *T. gondii* -like illness was performed. Investigators determined, on closer examination of this organism's structure in tissue samples, that it did not fit into any known genus and gave this protozoa the name *Neospora caninum* (Smith, 1993). *N. caninum* is now known to be distributed worldwide and infects a number of mammalian hosts ranging from livestock, including dairy cattle, to companion animals (Dubey et al., 1996a).

Life cycle

The Apicomplexa is a phylum of protozoan parasites containing common 1 host coccidia and other genera with facultative or obligatory 2 host cycles. *Toxoplasma gondii* is an important coccidian parasite that may have a life cycle similar to that of *N. caninum*. Sexual stages of *T. gondii* develop in the feline gastrointestinal tract and oocysts are passed in the feces. A variety of warm blooded animals may be used as intermediate hosts. For instance, sheep and goats can be infected by consuming feed or pasture grasses contaminated with cat feces containing the sporulated *T. gondii* oocysts. Small ruminants may also be infected, in rare instances, by ingesting *T. gondii* infected fetal tissues or fluid. Asexual stages of the life cycle (tachyzoites and tissue cysts containing bradyzoites) are found in the intermediate hosts. In the intermediate host, tachyzoites may localize in the pregnant uterus and pass directly to the placenta and into the fetus. Fetal toxoplasmosis produces clinical signs ranging from fetal death and abortion to mild abnormalities depending on the fetal dose, gestational age and fetal immune competence (Anderson et al., 1994).

Although *N. caninum's* complete life cycle remains unknown, two asexual stages of development, tachyzoites and tissue cysts containing bradyzoites, have been identified (Dubey,

1993). Tachyzoites measure 3-7 x 1-5 μm depending on the stage of division. *N. caninum.*

tachyzoites can be distinguished from *T. gondii* tachyzoites only by electron microscopy and

possess many of the ultrastructural characteristics of other cyst forming coccidia (Speer et al.,

1989). This stage of *N. caninum* is found in the cytoplasm of macrophages, neural cells

fibroblasts, hepatocytes, myocytes, and renal tubular epithelial cells (Dubey et al., 1988).

Tachyzoites penetrate these cells via active invasion and result in multifocal nonsuppurative

necrotizing myocarditis, hepatitis, myositis, and encephalitis (Hemphill et al., 1996).

Tissue cysts are found only in neural tissue and are usually not surrounded by a zone of

host reaction. Cysts are larger than tachyzoites (107μm in diameter) and have a 4μm wall (Lindsay

et al., 1994a). The bradyzoites within the tissue cysts contain organelles similar to those of

tachyzoites. Cysts can form in neural tissue as early as 17 days post-infection (Dubey et al.,

1996a). How long cysts remain viable in neural tissue is not known, although under experimental

conditions cysts have been shown to be viable in mouse brain for at least one year (Lindsay et al.,

1992).

Cyst forming coccidia are 2 host parasites and based on *N. caninum's* similarities to other

cyst forming coccidia, it is likely that dogs or other carnivorous animals serve as the definitive host

in which sexual stages and oocysts of *N. caninum* occur. *N. caninum* cysts are known to be

resistant to HCl-pepsin solution, which would allow them to survive transport through the stomach

of a carnivorous host. However, when potential carnivorous definitive hosts were fed infected

tissues, fecal oocysts were not found (Dubey et al., 1996a). Although infection via a definitive

host has been theorized, vertical transmission is the only recognized mode of infection in nature

and has been reported in a number of species (Dubey et al., 1996a).

Experimental Infection

Both adult and neonatal animals have been experimentally infected with culture derived *N.

caninum* tachyzoites via many different routes including subcutaneous, intramuscular,

intravenous, intraperitoneal and oral (Dubey et al., 1996a). Transplacental transmission has been

achieved in dogs (Dubey et al., 1989b), cats (Dubey et al., 1989a), sheep (Dubey, et al., 1990d), pygmy goats (Lindsay, et al., 1994c), nonhuman primates (Barr, et al., 1994a), mice (Lindsay, et al., 1995), and cattle (Dubey et al., 1992c). Bovine fetuses have been inoculated in utero resulting in fetal infection (Barr et al., 1994b).

Field Studies and Case Reports

Case reports and investigations of known infected populations of dogs, coyotes, cattle, sheep, goats, deer and horses are commonly found in the literature. Fewer studies have been performed to assess the prevalence of *N.caninum* in the general population of these species. Perhaps confusion with *Toxoplasma gondii* in some cases and lack of a widely available, reliable testing procedure have limited investigations assessing general prevalence.

Dogs

Both case reports and seroprevalence studies can be found in the literature documenting canine neosporosis. Four convenience sample surveys have been performed in the USA (1) (Lindsay et al., 1990), United Kingdom (2) (Trees et al., 1993, Lathe, 1994), and Sweden (1) (Bjorkman, et al., 1994). Seroprevalence, as reported in a review article documenting these surveys, ranged from 0.25% to 16.6% (Dubey et al., 1996a). Researchers in New Zealand randomly selected two hundred clinically normal dogs and determined *N. caninum* antibody levels via immunoflourescence antibody testing (IFA). Twenty two percent of these dogs had a positive reaction at the 1:40 dilution and 9% had a positive reaction at the 1:200 dilution (Reichel, 1998). *N. caninum* seroprevalence was found to be 5% (5 out of 52) in live trapped coyotes from various Texas counties (Lindsay, et al., 1996b).

Clinical case reports describing canine *N. caninum* infection have come from Australia, Belgium, Canada, Costa Rica, Denmark, UK, Finland, France, Germany, Hungary, Ireland, Japan, Netherlands, Norway, South Africa, Spain, Switzerland, and the USA (Dubey, et al., 1996a, Cochrane, et al., 1993, Ruehlman et al., 1995, Cuddon et al., 1992). These reports detail the typical clinical signs associated with neosporosis in dogs. The most common clinical sign associated with canine neosporosis is ascending paralysis in young, congenitally infected pups.

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Pups may also experience difficulty swallowing, muscle atrophy and heart failure. However, dogs of any age may be affected by neosporosis. Cases of nodular dermatitis, polymyositis, and focal or mulitifocal CNS disease due to neosporosis in dogs 18 months to 6 years old have been documented (Knowler et al., 1995, Patitucci, et al., 1997, Fritz, et al., 1997). Treatment protocols for neurologic deficits consisting of clindamycin or trimethoprim combined with sulfadiazine and pyrimethamine have been successful in some dogs with early neosporosis-induced limb weakness (Barber, et al, 1996).

Deer

Two cases of neosporosis have been reported in deer. *N. caninum* tissue cysts were observed in brain sections, taken during necropsy, from a full term stillborn deer of Eld (*Cervis eldi siamensis*) from a zoo in France (Dubey, et al., 1996d). The second documented case was a wild 2 month old black-tailed deer (*Odocoileus hemionus columbianus*) found dead in California (Woods et al., 1994). The definitive diagnosis in the second documented case was made via immunohistochemical techniques for tissue identification.

Small Ruminants

Most reports of small ruminant neosporosis have involved goats. *N. caninum* abortion has been reported in pygmy goats in California (Barr et al., 1992), Pennsylvania (Dubey et al, 1992a), and Costa Rica (Dubey et al., 1996c). Stillbirth and the birth of weak kids due to neosporosis have also been reported (Dubey et al., 1992a). Only one case of ovine congenital neosporosis has been reported. Myelitis due to *N. caninum* tissue cysts was found in a lamb which was born weak and died at one week of age (Dubey et al., 1990b).

Horses

Abortion in an eight year old Appaloosa mare associated with *N. caninum* has been reported (Dubey, et al., 1990f). In two other cases of neosporosis in adult horses, clinical signs included weight loss and anemia in one horse, and rear limb paralysis and bizarre behavior in the

other (Gray, et al., 1996, Marsh, et al, 1996). In an infected one month old Quarter horse foal, ataxia and compromised vision were observed (Lindsay, et al, 1996c).

Cattle

Bovine neosporosis has been reported from Australia, Canada, Denmark, UK, Ireland, Israel, Japan, Mexico, the Netherlands, South Africa, Sweden, and the USA (Dubey, et al., 1996a). Most reports of bovine neosporosis involve dairy cattle although clinical disease due to *N. caninum* has been documented in beef herds. Investigators are still uncertain why neosporosis seems to affect dairy more than beef cattle. Some researchers have suggested that dairy cow management, with its tendency to maintain cattle in dense populations, would increase exposure to potentially infective tissues (placenta/fetal tissue from infected cows). Breed has also been proposed as a risk factor, however neither hypothesis has been proven (Smith, 1993). Abortion storms, seroprevalence studies involving known infected herds, and individual case reports comprise the majority of reports on bovine neosporosis.

Beef

Neosporosis in beef cattle has been reported in Canada and Australia. One Canadian study involving two beef operations in British Columbia found 67% (10 of 15) of aborting cows in Herd A and 43% (6 of 14) of aborting cows in Herd B to be *N. caninum* positive using immunofluorescence antibody testing (Hoar et al., 1996). Researchers in Australia examined 729 aborted fetuses from 126 *N. caninum* affected herds, 41% of which were from beef herds (Boulton, et al., 1995). *N. caninum* seroprevalence has also recently been assessed in water buffalo in Vietnam (Huong, et al., 1998). Individual case reports of neosporosis in aborted fetuses, stillbirths and neonatal calves have come from dairy and beef herds in Australia, Canada, UK, South Africa, and the USA (Anderson et al., 1991, Anderson et al., 1995, Barr, et al., 1990, Barr, et al., 1991, Barr, et al. 1993, Bryan et al., 1994, Dubey et al., 1998, Dubey, et al. 1990a, Dubey et al., 1992b, Dubey, et al., 1996a, Dubey et al., 1992c, Dubey, et al., 1990e, Ogino, et al., 1992, Jardine et al., 1993, McIntosh, et al., 1994, Lindsay, et al., 1989, Otter, et al., 1995).

<u>Dairy</u>

Neosporosis in dairy cattle is more widely reported than in beef cattle. Results of investigations of herds known to be infected with *N. caninum* are more common than crossectional seroprevalence studies. A prospective cohort study involving 2 infected California dairy herds showed that the dam's *N. caninum* serostatus at freshening was not a significant risk factor for calf survivabilty. In fact, seropositive calves had a consistently greater survivorship to 90 days of age than their seronegative herdmates (Pare′ et al., 1996).

Another recent study in the UK compared histology of fetal brain and heart to maternal and fetal serology for the diagnosis of abortion due to bovine neosporosis. The study involved three groups of cattle: (A) 36 *N. caninum* infected cows (B) 100 cows that had aborted and the calves had no *N. caninum* lesions and (C) 128 normally calving cows. Results showed that while a definitive diagnosis using immunohistochemistry of fetal brain is preferred, maternal and fetal serology can be diagnostic if *N. caninum* abortions have recently been diagnosed on the same farm (Otter, et al., 1997).

Another study of four known infected herds with epidemic (Herds 1 and 2) and endemic (Herds 3 and 4) *N. caninum* abortion found that 58.8% (20 of 34) of aborting cows in Herds 1 and 2 were seropositive. Of the cows that did not abort in those two herds, 17.7% (8 of 45) were seropositive. This investigation also found that 83.3% (10 of 12) of cows in Herds 3 and 4 with a previous history of *N. caninum* abortion had seropositive heifers, whereas only 3.6% (1 of 28) of seronegative cows produced seropositive heifers. The authors suggested that cows aborting a fetus with *N. caninum* were likely to have been infected congenitally, and that abortion was not necessarily a consequence of recent environmental exposure (Thurmond et al., 1995b).

Researchers in New Zealand recently reported the results of a study evaluating the prevalence of *N. caninum* antibodies in serum from dairy cattle. This study tested samples from a bank of dairy cow serum collected over a ten year period and found that *N. caninum* seroprevalence ranged from 6.75% in 1985 to 8.5% in 1995 (Reichel, 1998).

<u>Clinical signs</u>

Infection of cattle with *N. caninum*, whether naturally or experimentally, may result in one of three reproductive outcomes: (1) abortion/stillbirth (2) neonatal neurologic disease or (3) birth of a full term calf that is congenitally infected with no clinical abnormalities. Cases of seropositive cows giving birth to seronegative calves and seronegative cows producing seropositive calves are rare (Thurmond et al., 1995b, Pare′ et al, 1996). The factors which determine the outcome of *N. caninum* infection in the cow are unknown. Investigators have suggested that exposure to other infectious agents, whether abortifacient or not, may cause a general immunosuppression resulting in clinical neosporosis (Thurmond et al., 1995a). At this time, however, there is no experimental evidence to support this hypothesis.

Abortion due to *N. caninum* infection typically occurs between 3 and 8 months of gestation (Lindsay et al., 1996a). While the pathophysiology of *N. caninum* abortion is not well understood, the fetus is often autolysed upon presentation, with placentitis and placental edema being consistent pathologic features. Abortions due to *N. caninum* rarely result in a retained placenta or metritis. Characteristic histopathologic findings in fetal tissue, include multifocal, nonsuppurative, necrotizing lesions of the brain, heart, skeletal muscle, and liver (Dubey, et al., 1996a). Definitive diagnosis of neosporosis is based on cyst identification in serial tissue sections using an avidin-biotin complex immunoperoxidase method for demonstrating antigens of *Toxoplasma gondii*, *N. caninum*, and *Sarcocytis* spp. The diagnosis of *N. caninum* is made if staining is noted only with the antiserum to *N. caninum*. (Otter et al., 1994, McIntosh et al., 1994).

Within and among dairy herds, abortions due to *N. caninum* infection occur as either epidemic or endemic events during any time of the year. Abortion storms, some resulting in greater than 30% fetal loss, have been documented (Yaeger, et al., 1994, Collery, 1995., Neitfeld, et al., 1992, McAllister, et al., 1996, Thilsted, et al., 1989, Cox, et al., 1998, Obendorf, et al., 1995). These reports suggest a point source exposure to a concentrated source of *N. caninum*, perhaps in water or feed, although this has never been confirmed in any of the reports.

While abortion storms are one possible outcome of *N. caninum* infection, recent research indicates that *N. caninum* may be overdiagnosed as the cause of abortion epidemics. One study found a strong association between herd seroprevalence and abortion for only 5 of 14 herds with a presumed diagnosis of *N. caninum* as the cause of an abortion storm (Thurmond et al., 1997c). Furthermore, no association was found between dam and daughter seropositivity for herds experiencing an epidemic, suggesting that most cows aborting during an epidemic were infected postnatally. Despite results of fetal histopathology and immunohistochemistry implicating *N. caninum*, seropositive cows that abort may do so because of another abortifacient agent or factor. Therefore, it is recommended that other abortifacient factors and agents such as *Leptospira* spp. and BVD be considered in the face of an abortion storm investigation.

Sporadic and endemic abortion are more commonly reported in the literature than abortion storms (Rogers, et al., 1993, Jardine et al., 1995, Trees, et al., 1994, Buxton et al., 1997, Boulton, et al., 1995). These events consist of gradually increasing abortion rates in individual herds, as well as an overall increased number of state laboratory diagnosed *N. caninum* abortions. A study of 26 selected dairy herds in California identified *N. caninum* as the causative agent in over 40% of all abortions in the herds with a history of neosporosis and in over 30% of abortions in herds without a history of *N. caninum* infection (Anderson, et al., 1995). In fact, California state diagnostic laboratories have identified *N. caninum* as the most diagnosed single cause of abortion among dairy cows in that state. This is supported by a survey in the San Joaquin Valley over a four year period which found 95 (20.3%) of 468 fetuses submitted for evaluation in which a protozoal infection was suspected based on the lesions observed (Anderson, et al., 1990). *N. caninum* was thought to be a likely cause of these abortions as the arrangement of protozoa within endothelial cells was not consistent with *Sarcocystis* and *Toxoplasma* is considered an unlikely cause of abortion in cattle. Not only is this abortifacient agent of increasing concern to dairy producers in California, one study identified *N. caninum* as the fourth most frequently identified cause of abortion in dairy cattle nationwide (Neitfeld, et al., 1992). This prevalence estimate was based on the infectious agents identified in 655 fetuses presented from six states over a one year

period to a state laboratory in South Dakota. It is important to note that in a recent study assessing

N. caninum antibodies as a predictor of congenital infection, 80% of the seropositive cows did not

abort (Pare′, et al., 1997b). These results suggest that maternal immune response and timing of

exposure during gestation may influence the risk of congenital infection and potential abortion.

Calves that survive in utero exposure to this pathogen may exhibit neurologic deficits.

Calves may be weak, underweight or unable to rise at birth. Typically though, neurologic deficits

appear gradually within the first 7 days of life (Dubey, et al., 1996a), except for one report of

clinical neosporosis that developed in a 4 week old calf (Dubey et al., 1992). Clinical signs

include, but are not limited to, ascending paralysis, ataxia, scoliosis, ocular deformities and loss of

conscious proprioception. Calves either die or are euthanized due to their neurologic deficits.

Lesions associated with deficits are usually confined to the central nervous system where both

tissue cysts and necrotizing encephalitis have been described (Dubey, et al., 1990e, Ogino, et al.,

1992). Although a treatment protocol for dogs with neurologic deficits has been established, the

prolonged treatment and extra label use of these antimicrobials would make this protocol

prohibitive for food animals. Low doses of anticoccidials and other anitmicrobials have proven to

be efficacious against tachyzoites in vitro, but have yet to be tested in vivo (Lindsay, et al., 1997,

Lindsay et al., 1994b, Lindsay, et al., 1989a).

Infection of the bovine fetus with *N. caninum* may also lead to the birth of a full term calf

with a high precolostral *N. caninum* titer, but with no clinical evidence of disease (Dubey et al.,

1996a). In fact, many seropositive full term calves develop normally, indicating that congenital

infection does not necessarily have a detrimental effect on calf health (Pare′ et al., 1996). Many

heifers from these seropositive populations are subsequently used as replacements. These

replacements have the same potential as their dams to abort, produce calves with neurologic

disease, or produce congenitally infected, normal calves. Furthermore, a recent study found that

these replacements have a 7.4 fold increased risk of abortion during their first gestation and a 1.7

fold increased risk of abortion during their first lactation compared to seronegative herdmates.

Also, the same study found that congenitally infected calves that had aborted previously had a 5.6 fold higher risk of abortion during their second lactation compared to seronegative cows or cows that had not previously aborted (Thurmond, et al., 1997b).

Seroconversion of dam and fetus

Seronegative cows, in some studies, have not been shown to seroconvert when housed with seropositive herdmates (Barr, et al., 1994b). Other investigators have found that cows seroconvert at a rate of 8.5/100 cows/year (Pare′ et al., 1997b). As stated previously, a hypothesized mechanism for horizontal transmission involves the ingestion of *N. caninum* oocysts via exposure to contaminated feed or water, although fecal oocysts have never been identified. Placenta or fetal tissue infected with tachyzoites has also been suggested as a source of horizontal transmission (Dubey, et al., 1996a). Two pregnant heifers infected experimentally with tachyzoites at approximately 120 days gestation, seroconverted within nine days with titers peaking in 32 days. The heifers' calves, one taken via cesarean section 32 days postinfection and the other which was allowed to develop to full term, were both congenitally infected. In the same study, 55 naturally infected cows were found to have parasite specific antibody titers which appeared to peak at the time of abortion and the titers of 6 of these cows monitored long term decreased significantly within 150 days of the abortion (Conrad, et al., 1993). Significantly higher titers in British cows were also found at the time of abortion compared to cattle from herds in which there had been no recent abortions (Trees, et al., 1994).

Serologic tests

Currently, two serologic tests are available to assess *N. caninum* infection in cattle: immunofluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA). The IFA was the first serologic test developed and is used to detect antibody in second and third trimester fetal fluids as well as cow serum (Dubey, et al., 1996b, Wouda, et al., 1997). While it is specific, the IFA's sensitivity is less than optimal as suggested by one study which found that only 75% of cows that gave birth to congenitally infected seropositive calves were seropositive by IFA (Pare′, et

al., 1995). Immunofluorescent antibody tests are also difficult to reproduce and since it is

manually read, the results can be quite subjective. Also, an optimal cutoff point has yet to be

established for the IFA. Therefore, an ELISA was developed to provide a serologic test which

detects antibodies to *N. caninum* with negligible crossreactivity (Pare', et al., 1995). The ELISA

is more sensitive (88.6%), specific (96.5%) and reproducible within and among diagnostic

laboratories compared to the IFA. The IFA was used as a reference to which ELISA values were

compared and determined. Since an optimum cutoff point has not yet been established for the IFA,

ELISA interpretation is limited. Positive and negative predictive values for the ELISA were

calculated assuming a seroprevalence of 40%, which resulted in high predictive values for a

positive test (94.4%) and a negative test (92.7%). If a lower seroprevalence exists in a population

of cattle being tested, it would reduce the probability that a cow with a positive result actually has

the infection, altering the confidence in test interpretation.

Another diagnostic method currently being investigated involves the detection of *N.*

caninum from infected tissues via polymerase chain reaction (PCR) and DNA probe hybridization.

Initial studies using these techniques have shown these methods to be quite sensitive, particularly

for parasites in central nervous system tissues and even when antibody titers fall below normal cut-

off values by the IFA test. The PCR is also thought to be useful in detecting whether *N. caninum*

parasites exist in the tissues of naturally or experimentally infected cows and determining the tissue

distribution of tachyzoites in these animals (Yo, et al., 1997). This diagnostic method is,

however, quite new and until further research is performed both the IFA and ELISA remain the

most available and consistently reliable tests.

Serologic status and economic impact

Serologic response in adult dairy cattle and its relationship to future performance is an area

of intense research. Cows do not appear to develop any immunity to *N. caninum* which prevents

repeated abortion. While the specific economic impact of *N. caninum* infection has not been

determined, one British study estimated that the cost of a single abortion occurring prior to day 250

of gestation, including the cost of lowered production, averages 910 dollars (Wright, et al., 1993).

N. caninum infection may also be associated with culling. In one herd, seropositive cows were culled 6.3 months earlier and had a 1.6 times greater risk of being culled than seronegative cows (Thurmond, et al., 1996). The risk of a cow being culled for abortion, however, was not related to her serologic status even though an abortion significantly increased the risk of being culled. The same investigators also found that serologically positive cows were also at a greater risk for decreased milk production (Thurmond, et al., 1997a). Seropositive cows were significantly more likely to produce congenitally infected calves than their seronegative herdmates. The risk of a congenital infection does not seem to be associated with cow age, lactation number, history of abortion, calf gender or length of lactation (Pare′, et al., 1997b).

Preventative measures

Current recommendations suggest that when the abortion rate exceeds 5%, it is economically sound to invest resources to determine the cause, in order to initiate appropriate control measures (Wright, et al., 1993). When *N. caninum* is diagnosed, estimating the herd seroprevalence and performing histopathologic examination of aborted fetuses in a population of dairy cows are important initial steps in developing a control strategy. Researchers have also recommended that seroprevalence within a herd of those cows that abort and those that do not be compared to see if cows that abort are at any greater risk of *N. caninum* infection than those that carry a calf to term. This guides both the producer and veterinarian concerning infection as a culling criterion. If seroprevalence is low, culling seropositive cows may be beneficial. If seroprevalence is high or very few seropositive cows abort and perform well otherwise, culling is not a practical option. As well as removing seropositive cows, infection in a herd may also be controlled by purchasing only seronegative replacements. Although transplacental infection is the primary mode of transmission, horizontal spread can be minimized by reducing the herd's exposure to theoretical definitive hosts via rodent control and restricting a farm's cat and dog population (Thurmond et al., 1995). A recent Canadian study found a strong association between the presence and number of dogs on each dairy farm studied and the proportion of seropositive

cows, suggesting that the dog may be a significant source of infection (Pare′ et al., 1997a).

Therefore, covering commodities and feed, plus maintaining feedbunks free of carnivore fecal matter may also help reduce the incidence of disease.

While *N. caninum* is a newly recognized disease with little known about its life cycle, it has emerged as a formidable abortifacient agent in California and throughout the United States. No treatment yet exists for seropositive or clinically affected food animals, however, some general recommendations to help decrease disease incidence can be employed based on our knowledge of other Apicomplexan parasites.

Materials and Methods

Experimental Design

Twenty four herds, twelve per group, participated in this case-control study. Herds were selected based on abortion rate and were divided into two groups: high abortion rate herds and low abortion rate herds. Each high abortion rate herd was matched to a low abortion rate herd in the same geographic area. All herds were visited once at which time blood samples were taken from cows between 90 and 240 days of pregnancy. The serum was frozen and sent to the California Veterinary Diagnostic Laboratory System in Davis, California. Samples were tested for the presence of *N. caninum* specific antibody by an enzyme-linked immunosorbent assay and seroprevalence for each group was determined.

Herd Selection

All herds participating in the study participated in monthly Dairy Herd Improvement Association (DHIA) testing and had at least monthly reproductive herd health visits by a veterinarian. Herds were selected based on abortion rate as calculated by DHIA for the previous year. The Dairy Herd Improvement Association includes in the abortion rate calculation producer reported abortions, cows diagnosed pregnant and then later diagnosed open, and cows the DHIA computer system assumes have aborted. The DHIA computer will assume a cow is pregnant if she is inseminated and no heat or other insemination is recorded for more than 90 days. If a subsequent breeding or heat is then recorded, she is assumed to have aborted. Monthly reproductive herd health visits should decrease the number of assumed abortions because the pregnancy status of most cows not returning to heat after an insemination would be determined within 60 days of insemination.

Twenty four Holstein herds were included in the study. Each herd was located in one of three regions of Virginia: the Piedmont area, Shenendoah Valley, or southwest Virginia (Figure 1). A herd profile was also obtained in the form of a survey completed during the farm visit. Each producer was asked to detail general management, housing, feeding and feed storage practices, as

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well as list DHIA calculated reproductive and production parameters. Also included in the survey were questions designed to establish the type and number of potential hosts of *N. caninum* consistently maintained or found on each dairy.

High Abortion Rate

High abortion rate (HAB) in this study was defined as ≥ 6% annually. This percentage was chosen as most literature suggests that abortion rates above 5% warrant diagnostic investigation (Wright, et al, 1993). A list of 29 herds whose DHIA records indicated that they met this criterion were identified using the Dairy Access Records by Telephone SAS (DART-SAS®, Clay, et al, 1993) program. Not only did this program identify herds meeting this criterion, but it also listed the herd code, remote access code, farm name and telephone number associated with each operation. All twenty nine producers were contacted by telephone. At that time the abortion rate generated for each operation was confirmed, the nature of the project was discussed and the producer was asked to participate. Twelve dairies agreed to participate in the study.

Low Abortion Rate

The primary care veterinarian for each HAB herd was contacted and asked to suggest another herd in that same region that would be willing to participate in the study as a control. Control herds, or low abortion rate (LAB) herds, were defined as herds meeting all of the same criteria as HAB herd, but with a DHIA reported abortion rate of ≤2%. The abortion rate of the twelve control herds was confirmed via the DART-SAS® program prior to participation in the study.

Cow Sampling

Within each herd, all cows sampled were confirmed to be 90 to 240 days pregnant via rectal palpation during a previous reproductive herd health visit by that dairy's primary care veterinarian. This gestational range was chosen because reports in the literature indicate that most *N. caninum* abortions occur within this range (Dubey, et al, 1996a). In herds containing more

than 30 cows between 90 and 240 days gestation, a list of all cows within this gestational age was collected prior to the farm visit. Thirty cows were randomly selected from the list and the producer was contacted concerning the sample group. Random selection was performed by writing cow numbers on individual pieces of paper and then drawing them from a hat. The first thirty numbers drawn were sampled for the study. No heifers were included in the study.

Serology

A single 10 ml blood sample was collected in a Vacutainer® tube from all cows via the sacrococcygeal vein. The blood was allowed to clot at room temperature, centrifuged, and then a minimum of 0.5 ml of serum was collected from each sample. Sera were frozen at -20° C for a period of 10 to 90 days and then were shipped on ice and delivered within 24 hours to the California Veterinary Diagnostic Laboratory in Davis, CA. *Neospora* antibody level was determined for each serum sample via an enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of neosporosis in cattle (Pare', et al, 1994). This assay was developed using the better established immunoflourescence antibody (IFA) technique as a reference. Briefly, logistic regression was used to establish an optimum cutoff point and then sensitivity, specificity and overall correct classification was determined and compared to various IFA dilutions. The ELISA procedure uses whole sonicated *Neospora* tachyzoites of a bovine isolate with antigen in 96 well polystyrene microtiter plates. Both positive and negative controls were measured in triplicate for each sample and test sera were measured in quadruplicate. Adjusted optical density (OD) was obtained by subtracting the mean reference well O.D. from that of the test and control wells. Each sample's optical density was then recorded and all samples with an O.D. of 0.45 and greater were considered positive.

Statistical Analysis

Seroprevalence within each herd was determined by dividing the number of seropositive samples by the total number of samples for each herd. Frequency distributions were determined for individual cow optical density and herd seroprevalence (Figures 2 and 4). A distribution of the

percentage of cows in each optical density range was also determined (Figure 5). Average herd seroprevalence and mean herd optical density of the HAB and LAB rate herds were compared using the Mann-Whitney Rank Sum test. The seroprevalence of herd pairs was evaluated via a paired t test. All cows in both groups were stratified according to gestational age and average optical densities between groups were compared within each category using the Mann-Whitney Rank Sum test (Figure 3). Then, using the Kruskal-Wallis one-way analysis of variance test, average optical densities within the HAB group were compared. The same test was then performed to compare the LAB rate groups. Finally, both HAB rate and LAB rate optical density data within each gestational age were combined, and an average was determined. These averages were then compared across gestational ages using the Kruskal-Wallis one-way analysis of variance test.

Basic descriptive statistics were used to summarize the survey results. More specifically, the mean, median and range of all quantitative data were calculated so that herd size and general levels of management between groups could be compared. Each mean and median in each category (rolling herd average, calving interval, services per conception for pregnant cows and services per conception for all cows) were then compared using the Mann-Whitney Rank Sum test and the Median Test. All statistical tests were performed using the Statistix® 4.1 analytical software (Tallahassee, FL).

Results

Seroprevalence

Mean seroprevalence for the HAB rate and LAB rate herds was 14.8% and 6.9%, respectively. The Mann-Whitney Rank Sum test comparing mean seroprevalence of neosporosis between HAB and LAB rate groups resulted in no significant difference (Table 1, p=0.56). The Median test used to compare these two groups also resulted in no significant difference (Table 1, p=0.41). The paired t test resulted in no significant difference (p=0.28). Similarly, no significant differences were found between average optical densities (Table 1, p=0.84) or when optical densities were stratified by gestational age and gestational average optical densities were compared (Table 3). In neither instance was a significant difference found, (p=0.79 and 0.16 respectively) when comparing average optical densities within abortion rate groups (Table 3) or comparing optical densities between gestational ages after an average of the two optical density values in each gestational age had been calculated, (Table 4, p=0.15) .

Survey

Mean and median values were calculated for herd size, rolling herd average, calving interval, services per conception for pregnant cows and services per conception for all cows and results are as listed in Table 5. The majority of dairies in this study milked in a parlor, with only 1 HAB rate dairy utilizing a stanchion barn. A total mixed ration (TMR) was the most frequently used system of feed presentation with 6 HAB rate operations and only 1 LAB rate operation presenting feed either in a bunk mix with concentrate in the parlor (n=5) or using computer feeders (n=1). A wide range of silos including bunker, trench, concrete upright, and Harvestore® were used equally among the participants. Ensiling corn was also accomplished by the use of AgBags® on several dairies. Concentrates and other feeds were stored either in commodity sheds or grain bins. Most hay was round baled and stored either in a barn or outside and protected by a plastic covering. Access to pasture varied with lactating cows in both groups averaging 15 acres of pasture for 8 hours per day. Dry cows in both groups usually had access to an average of 16 acres

of pasture for 19-24 hours per day. The number of dogs and cats found on each farm averaged 3

dogs and 5 cats for the HAB rate group and 3 dogs and 7 cats for the LAB rate group. Most

dairymen reported that all potential carnivorous hosts either maintained or found on the farm had

free access to feed, with half of each group reporting that occasionally feces of some kind are

found in the hay, mineral feeders, or feedbunks. The majority of dairies included in this study

utilized free stall housing, however only 3 housed cows in a drylot and 3 utilized loose housing.

All dairies used artificial insemination as the primary method of breeding. Seven dairies

maintained a bull on the property. Each dairy employed a vaccination protocol which included at

least yearly administration of a IBR/BVD/PI3/BRSV and *Leptospira* spp. vaccine to adult cows

and additional vaccinations to replacement heifers consisting of 7-way *Clostridia* spp. and *Brucella*

abortus. Comparison of the qualitative survey data in each category resulted in no significant

differences (Table 5).

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Discussion

Most of the literature assessing bovine *N. caninum* seroprevalence in the United States has originated from California dairies. Results of these studies would suggest that *N. caninum* is the cause of at least 30% of all bovine abortions in California and that the seroprevalence in dairy herds throughout that state ranges from 30-57.9% (Anderson, et al., 1995 and Pare', et al, 1996). There are few reports of bovine neosporosis documented from the eastern United States, and currently none from Virginia. Based on the current literature, average seroprevalence in Virginia dairies was anticipated to be at least 30%. Results of this investigation found, however, that the average *N. caninum* within herd seroprevalence in Virginia dairies studied may be only approximately 10%.

This study found herd seroprevalence rates ranging from 0 to 18.75% in the LAB rate herds and 0 to 90% in the HAB rate herds. Despite the difference seen in group means (HAB rate, 14.8% and LAB rate, 6.9%), this study did not demonstrate a significant difference between the average *N. caninum* seroprevalence of HAB rate Virginia dairy herds and their LAB rate counterparts, suggesting that *N. caninum* does not contribute significantly to the abortion rate of HAB rate Virginia dairy herds compared to LAB counterparts. If this study reflects the situation throughout Virginia, the overall impact of *N. caninum* as an abortafacient on these Virginia dairy herds' average abortion rate appears to be minor.

While comparison of the average *N. caninum* seroprevalence for high and low abortion rate groups was the major aim of this study, the data were also stratified and assessed in several ways. Firstly, both mean and median herd seroprevalences were calculated and compared, neither of which resulted in a significant difference. Medians as well as means were compared because the median is considered the better measure of central tendency for small groups since it is less influenced by extreme values (Ott, 1993). Stratifying data often helps to establish trends and parameters for groups within populations. Stratifying, therefore, may highlight abnormalities within groups that may be obscured by the overall population mean. However, neither trends nor parameters significantly different from the population mean were identified after stratifying the data

according to gestational age. Optical density, regardless of gestational age, was also assessed and no significant difference was found. If study participants are paired, considering this in one's statistical analysis is important and can often increase the likelihood that a difference will be found (Ott, 1993). However, the paired t test analysis resulted in no significant diference.

Nonparametric tests were chosen to compare means and the median as the data were not normally distributed. The Mann-Whitney rank sum test is an alternative to the two-sample t test and requires fewer assumptions. The Kruskal-Wallis test is an extension of the rank sum test used for comparison of means from more than two sample populations. Neither test requires normality for the populations being tested, only that they be identical under H_o. When the assumptions for tests comparing means of normal populations, such as the t test or analysis of variance, are violated, rank sum tests are more likely to declare a difference when it exists (Ott, 1993).

Several limitations exist to the interpretation of this study's results. The *N. caninum* ELISA was developed assuming a 40% seroprevalence, which is considered conservative in light of the current literature available showing *N. caninum's* high rate of seroprevalence in California dairies (up to 60% in some herds). According to the probabilities associated with the ELISA, any sample with an O.D. of 0.45 or greater is considered positive. Assuming a 40% seroprevalence and using 0.45 as the cutoff point, the positive predictive value and negative predictive value of the ELISA are 94.4% and 92.7%, respectively and has a sensitivity of 88.6% and a specificity of 96.5%. If a disease is common, as *N. caninum* infection is in California dairy cattle, it becomes more likely that a positive test represents a true positive and a highly sensitive test is desired as it is less likely to generate false negatives. The rarer the disease, the more clinically useful a specific test becomes as it is less likely to generate false positives. The results of this study suggest that *N. caninum* seroprevalence in Virginia dairy herds sampled may be much less than the mean seroprevalence in California dairy herds. While the *N. caninum* ELISA is quite specific and therefore desirable in rare disease situations, it is still possible that due to the high seroprevalence assumed in its development, some of the optical densities observed in this study could have resulted in false positives, resulting in a slightly inflated impression of what already seems to be an

uncommon infection in Virginia's dairy cattle. In fact, if the results of this study are a true reflection of *N. caninum* seroprevalence in Virginia dairy herds, it is estimated using EpiInfo® that over 300 herds in each category would need to be sampled in order to demonstrate a statistically significant difference between these groups. Originally, extrapolating from the data generated by the many studies performed in California, a much higher seroprevalence in the HAB rate group was assumed (60%) and therefore a much lower sample size to establish significance was calculated (17 per group) for this study. Only 12 of the 29 HAB rate herds agreed to volunteer and, as this was a matched case control study, an equal number of herds in the LAB rate category were chosen.

The temporal relationship between exposure and/or fetal loss and peak optical density may also have affected the results. Using immunoflourescent antibody testing, peak titers occur within 32 days after experimental infection and naturally infected cows aborting *N. caninum* infected fetuses return to low titers within 150 days after fetal loss (Conrad, et al, 1993). As only pregnant cows were sampled, the post fetal loss increase in titer subsequent to that event should not have been detected. Also, since only one sample was taken from all cows participating in this study, perhaps the peak period of O.D. was missed by this sampling method.

Another factor limiting the interpretation of these results may be the use of seropositivity as a predictor of abortion. Seropositivity as a predictor of abortion and, therefore, abortion rate has recently been quantified. In one study, seropositive cows were twice as likely to abort as seronegative cows, however, 80% of all seropositive cows never aborted, suggesting that infection per se is not sufficient to cause abortion (Pare', et al, 1997b). Use of abortion rate as the primary parameter by which herds were selected and compared for this study may, therefore, have been inappropriate. Perhaps parameters that have since been shown to be more consistently associated with *N. caninum* status such as milk production or culling rate should have been incorporated into the parameters established for herd selection. It should be considered, as mentioned previously,

that had a larger number of herds been sampled, a difference may have been found even if abortion rate was used as the primary definition of a case.

The survey results of dairies in the study indicate that the majority of farms in both groups were managed similarly. Conventional methods of nutritional management, feed presentation, and feed storage were used in every dairy sampled. While access to and amount of pasture varied substantially between operations, neither parameter seemed to correlate closely with herd seroprevalence. More specifically, the herd with the highest seroprevalence of either group (90%), which also milked the fewest cows, allowed both lactating and dry cows access to 150 acres of pasture for at least 18 hours per day. Conversely, of the dairies utilizing drylot facilities, only one had a seroprevalence greater than 17%. If population density, as has been suggested, is a risk factor for *N. caninum* seroprevalence (Smith, 1993), it would seem that those animals having access to the greatest amount of acreage should have decreased exposure and, therefore, decreased seroprevalence to *N. caninum*. Interestingly, the operation with the highest seroprevalence also maintained the largest population of dogs (n=15). Exposure to dogs has recently been suggested as a risk factor for *N. caninum* infection.

The reproductive management within and between each abortion group as measured by calving interval and services per conception appeared to be similar. Milk production between herds in the investigation, as measured by rolling herd average, varied considerably, however the mean values for each group were similar. One investigation found that lower production was associated with *N. caninum* seropositive cows within a herd (Thurmond, et al , 1997). In this study, the producer with the lowest rolling herd average (12,824 lbs.) had a seroprevalence of 17% while the producer with the highest rolling herd average (23,000 lbs.) had a seroprevalence of 10%. While many factors contribute to the difference in milk production between herds, a comparison of individual milk weights from seropositive and seronegative cows within one or more of these herds would be necessary to establish whether a true relationship between seropositivity and milk production exists.

Not all herds contacted, fulfilling the definition of a case, chose to participate, which may have introduced selection bias into the study. Most producers cited the time commitment involved and anxiety concerning positive results and their implications as reasons for not participating. These factors may have biased the selection toward either highly progressive dairymen who enjoy participating in the advancement of knowledge which affects their industry or those individuals concerned enough about the reproductive status of their herds to warrant investing the time and effort involved. Progressive dairymen may recognize reproductive or production weaknesses in individual cows quickly and may cull more aggressively. This may have underestimated *N. caninum* seroprevalence in these herds. Conversely, less progressive producers may have biased the study by maintaining cows in their herds that should have been culled. These biases may have misrepresented typical management and *N. caninum* seroprevalence of all LAB rate and HAB rate Virginia dairy herds on DHIA, the group this study was meant to represent. However, because herds selected were in various geographic locations and of various sizes, certain general conclusions can be drawn from the survey information.

Additional information could be obtained by selecting other parameters using individual cow records, further analysis, such as (average) milk production and (average) reproductive performance, including early reproductive parameters (days to first service, services per conception, days open) and number of abortions, could be calculated and compared between HAB and LAB rate herds. Also, cull rate could be evaluated and compared to see if it results in similar findings to a recent study which found that *N. caninum* positive dairy cows were culled an average of 6.3 months earlier than their seronegative counterparts (Thurmond, et al, 1996). Ideally, the serostatus of all cows participating in the study should be evaluated at calving or visible abortion, to assess trends in optical density. Also, herds that use bulls as either the primary method of insemination or in a secondary capacity should be evaluated in light of the influence bull management may have on services per conception indices recorded with DHIA and/or *N. caninum* herd serostatus.

While a better understanding of *N. caninum* infection as it affects dairy cattle and other animals has occurred since its discovery in 1988, a great deal of mystery still surrounds this organism. For instance, further studies are still needed to determine very basic information such as *N. caninum's* life cycle. With this knowledge, hopefully, the best treatment protocol and/or preventative strategies to control its prevalence could be developed. Other questions that remain unanswered include: Why do some seropositive cows abort while others do not? Is there a relationship between other abortifacient agents/immunosuppressive conditions and *N. caninum* abortions in seropositive cows? While agents such as mycotoxins and BVD have been proposed as the initiating immunosuppressive event that can lead to abortion in a seropositive cow, studies have yet to be performed to establish this relationship. Clearly, there is much to be learned concerning *N. caninum's* impact on not only dairy cattle, but other animals as well . Its worldwide distribution and effects on commercial food animal operations make further study critical to improved animal health and production.

Conclusion

N. caninum seroprevalence in high abortion rate Virginia dairy herds is not significantly different than *N. caninum* seroprevalence in low abortion rate Virginia dairy herds.

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Table 1. Seroprevalence and optical density for HAB rate and LAB rate herds.

	HAB Rate Herds	LAB Rate Herds
Mean seroprevalence	14.8%	6.9%
Median seroprevalence	10.7%	4.9%
Range	(0-90%)	(0-18.75%)
Mean Optical Density	0.32±0.27	0.29±0.26

Table 2. Optical density (OD) distribution for HAB rate and LAB rate herds.

O. D.	HAB Rate Herds	LAB Rate Herds
<0.2	72 cows	70 cows
0.2-0.44	155 cows	171 cows
0.45-0.7	12 cows	4 cows
>0.7	20 cows	15 cows
Total	259 cows	260 cows

Table 3. Mean optical density stratified by gestational age for HAB and LAB rate herds.

Gest. Age(days)	Mean O.D.±SD HAB		Mean O.D.±SD LAB		p value
90-119	0.31±0.23	(n=81)	0.31±0.27	(n=64)	0.38
120-149	0.34±0.32	(n=66)	0.29±0.19	(n=79)	0.79
150-179	0.31±0.25	(n=36)	0.25±0.14	(n=47)	0.76
180-209	0.34±0.30	(n=42)	0.30±0.25	(n=43)	0.84
210-240	0.28±0.28	(n=34)	0.34±0.36	(n=27)	0.57
	Total n=259 cows		Total n=260 cows		

Table 4. Mean optical density of cows in HAB and LAB rate herds within each gestational age.

Gestational Age(days)	Mean O.D.±SD	n
90-119	0.31±0.25	145
120-149	0.31±0.26	145
150-179	0.29±0.22	83
180-209	0.32±0.27	85
210-240	0.30±0.32	61

Table 5. Mean and median DHIA calculated production and reproductive values of HAB rate and LAB rate herds.

HAB Rate

	Herd size (# cows)	RHA[*]	CI[**]	SPCPC[+]	SPCAC[++]
Mean	129	19,304	13.7	1.9	2.4
Median	111	18,800	14	1.9	2.4
Range	54-234	15,207-23,000	12.5-14.7	1.7-2.4	2-3.5

LAB Rate

Mean	151	19,352	13.9	2.0	2.5
Median	120	20,188	13.9	2.0	2.3
Range	68-430	12,824-21,500	12.7-15.6	1.7-2.4	2.0-3.5

p values

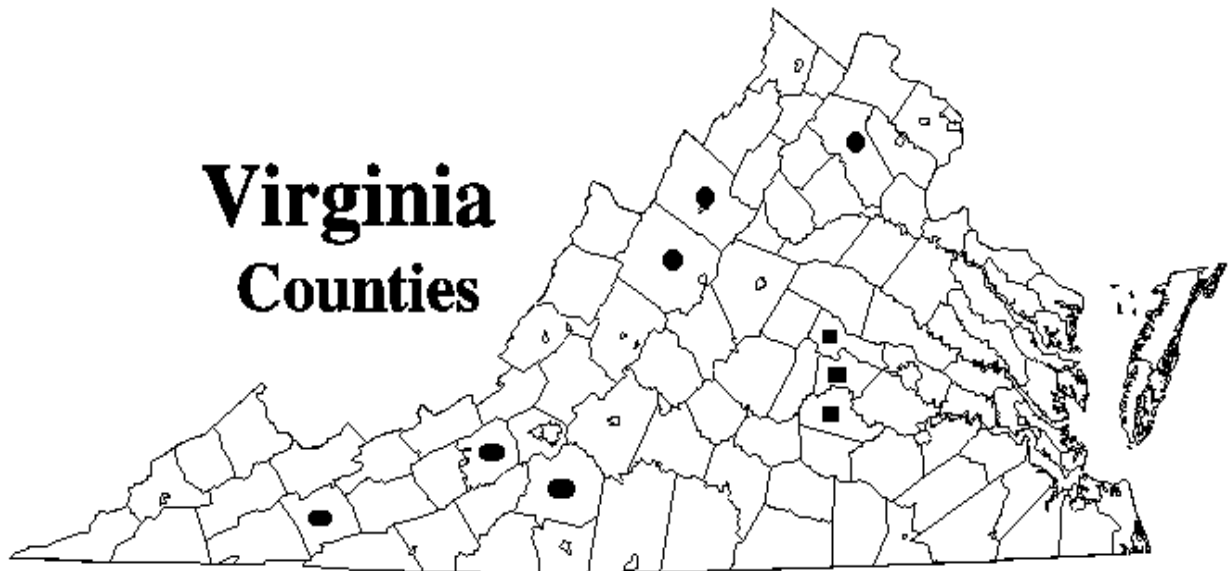|					
Rank Sum	0.71	0.72	0.95	0.88	0.71
Median Test	0.41	0.41	0.65	1.00	0.41

[*]Rolling Herd Average

[**]Calving Interval

[+]Services per conception for pregnant cows

[++]Services per conception for all cows

Table 6. Seroprevalence of high and low abortion rate herds (percent positive and number positive/number of cows sampled)

Pair #	HAB Rate	LAB Rate
1	90%(9/10)	3.7%(1/27)
2	6.9%(2/29)	0%(0/26)
3	4%(1/25)	0%(0/8)
4	10.3%(3/29)	8.7%(2/23)
5	11.5% (3/23)	16.7%(4/24)
6	3.8%(1/26)	18.75%(3/16)
7	13%(3/23)	0%(0/27)
8	0%(0/10)	13.8%(4/29)
9	15%(3/20)	6.25%(1/16)
10	11.5% (3/26)	0%(0/20)
11	0%(0/8)	0%(0/17)
12	11.1%(3/27)	14.8%(4/27)

Figure 1. Regions of Virginia in which dairy herds participating in the study are located.

⬤   Southwest Virginia
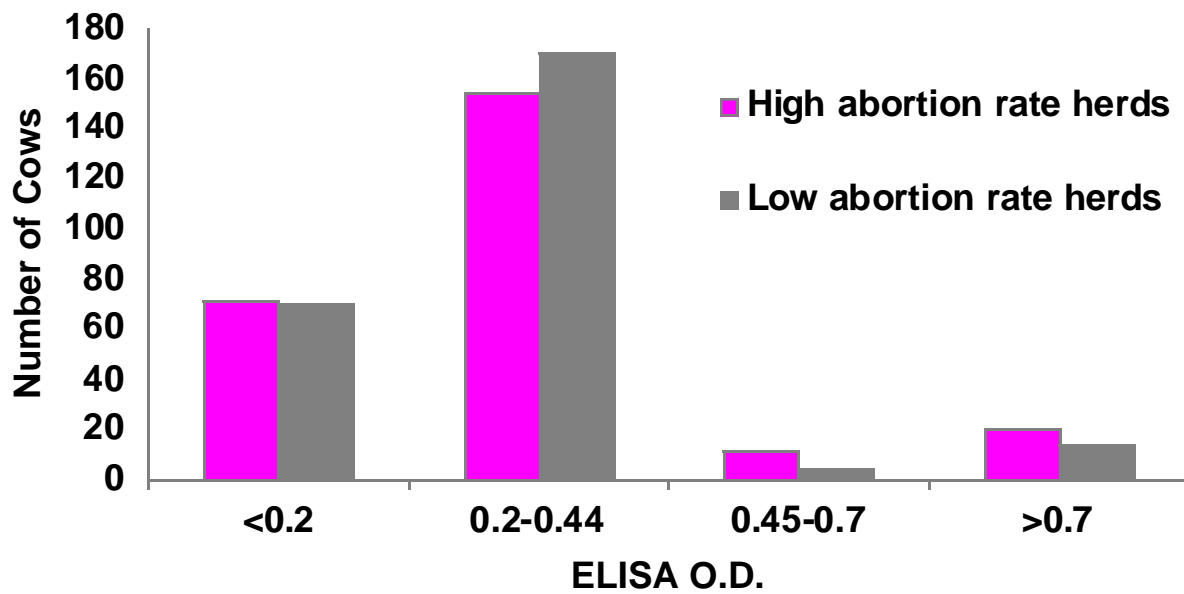
▪   Piedmont area

●   Shenendoah Valley

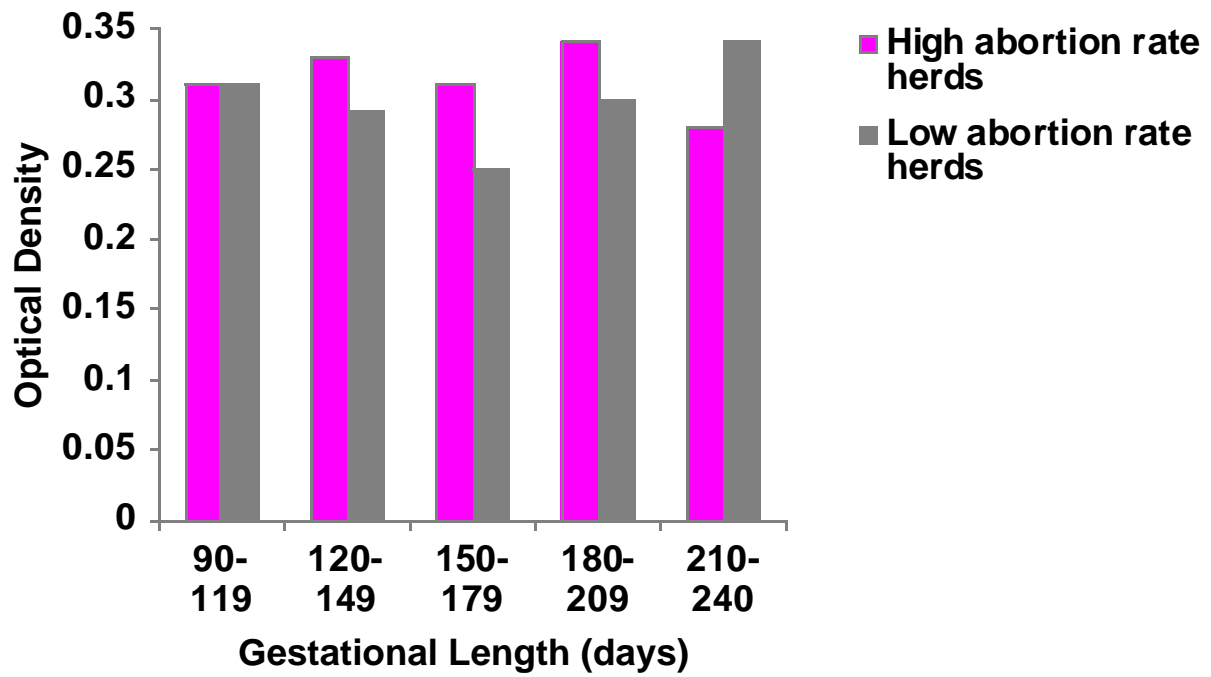**Figure 2.** Distribution of *N. caninum* ELISA optical densities.

Figure 3. Mean *N. caninum* ELISA optical densities stratified by gestational length.
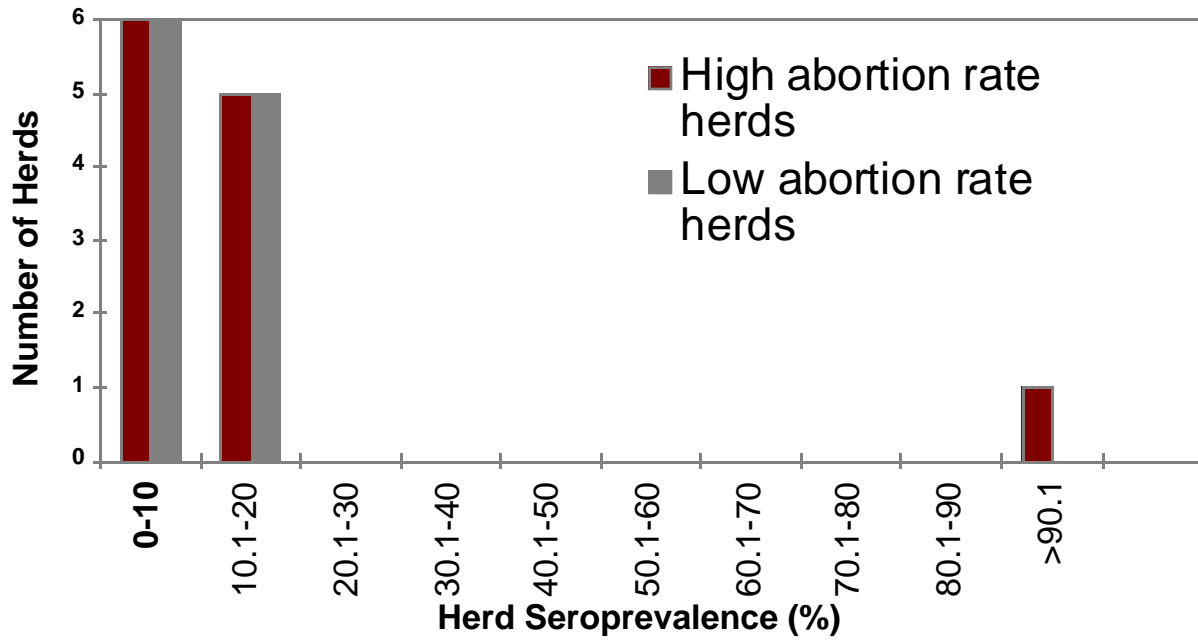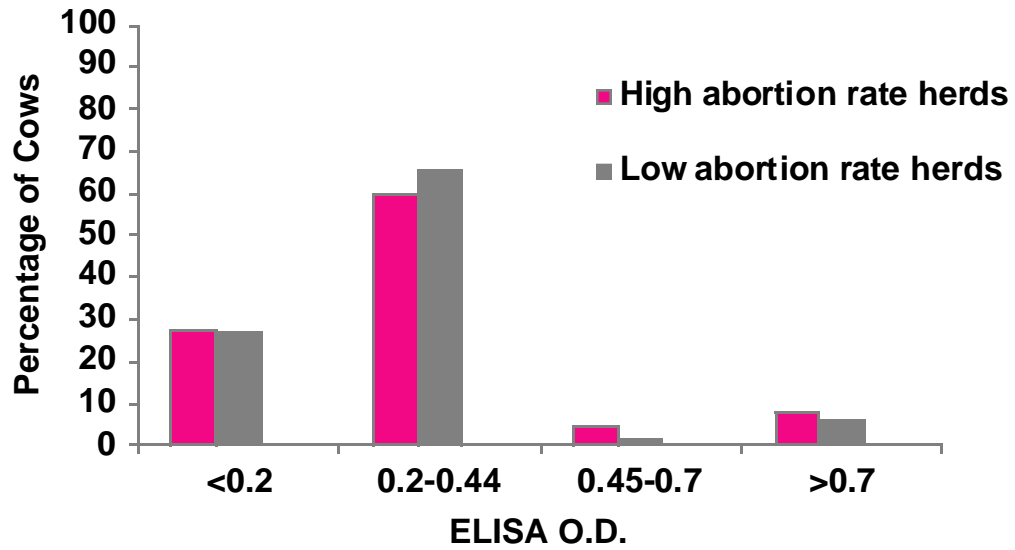
Figure 4. Distribution of *N. caninum* seroprevalence.

Figure 5. Percentage of cows within ELISA O.D. ranges.

Appendix I

Neospora Survey

Name: Veterinarian's name:
Address: Address:

Telephone #:   Telephone #:

County:

<u>Milking area:</u> (Please circle one)   Stanchion Barn   Parlor Type:

        If cows are fed in the parlor, please describe the type of feedstuff
and how much is offered.

        Are cows milked 2X or 3X per day?

<u>Feed:</u>   Please list the components of your lactating ration:

How is the ration presented to the cows?
TMR?   Bunk Mix?   Silage top dressed with grain?

How is the feed stored?

        On average, how many hours per day do lactating cows have access
to pasture? What type and number of acres?

        Please list the components of your dry cow ration, including any
transition ration you may feed in anticipation of the next lactation:

How is the dry cow feed stored?

On average, how many hours per day do dry cows have access to pasture? What type and how many acres?

Carnivores: Please estimate the number of dogs and cats on your farm:

Do these animals have free access to feed?

Do you ever find fecal material in the bunk, hay racks, silos or other areas where feed is stored or placed for consumption?

Housing: What type of housing do you have? (Please circle one)
Free stall   Dry lot   Tie stall

Other or a combination   (Please describe)

Cows: On average, how many cows are in milk at any one time?

How many cows are in your herd currently? (include dry cows)

What is your rolling herd average?

What is your calving interval?

Do you use artificial insemination or natural service or both?

How many services per conception do you average per pregnant cow? for all cows?

Please describe your vaccination program for both calves and cows. Please list the products used and the age at which they are administered. If vaccinations are seasonal, please list the times during the year when they are administered.

VITA

A native of rural Maryland and unable to resist the allure of the big city, Julia first set off to seek adventure and excitement by attending an institution in Baltimore of which her parents, John and Loretta, approved: a small, Catholic, women's college, with a nun to student ratio of 1:5, from which she earned a Bachelor's degree in Biology in 1988. Assuming that the sum total of excitement experienced during her college years was not a representative sample, she pursued her professional degree at the Virginia-Maryland Regional College of Veterinary Medicine, at which, after finally mastering the acronym, she earned her DVM in 1992. Several years of private practice, first in the wilds of southwest Virginia and then among the Amish of Lancaster county Pennsylvania, then followed before returning the VMRCVM to pursue a residency under the auspices of the Production Management Medicine department in 1995. After completion of this advanced training, Dr. Murphy's career options include employment within the pharmaceutical industry and governmental positions emphasizing regulatory and epidemiologic aspects of veterinary medicine.