PEER REVIEWED

A Probable Source of *Neospora caninum* Infection in an Abortion Outbreak in Dairy Cows

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Abstract

A small dairy had an outbreak of neosporosis. One-third of pregnant cows aborted or gave birth to a weak, premature calf over a period of 11 weeks. Serologic results of a *Neospora caninum* avidity enzyme-linked immunosorbent assay (ELISA) test were indicative of widespread, recently acquired infections in the cows and also in the farm dog. This dog was accustomed to sleeping and defecating in a pile of chopped hay used in the total mixed dairy ration, so it was a possible vector of infection in this outbreak. Replacement heifers, which did not consume the mixed ration or the chopped hay, had a low seroprevalence to *N. caninum* and did not have abortions. A new isolate of *N. caninum*, designated NC-Illinois, was obtained from a premature calf.

The circumstances at this dairy illustrate a specific management factor that could help prevent outbreaks of neosporosis. To reduce the risk of infecting cattle with *N. caninum*, dogs and coyotes, which are definitive hosts of this parasite, should be prevented from defecating in stored feedstuffs. The small dairy in this study might have prevented the outbreak by simply keeping the door to the hay room closed. Large dairies could prevent contamination of stored feedstuffs by erecting a chain-link fence with automatic gates around the area where piles of silage and commodities are kept.

Résumé

Une flambée de néosporose est survenue dans une petite ferme laitière. Sur une période de 11 semaines, près d'un tiers des vaches gestantes ont avorté ou donné naissance à des veaux prématurés affaiblis. Les résultats d'un test ELISA pour détecter *Neospora caninum* indiquaient la présence d'une infection récemment acquise chez plusieurs vaches de même que chez le chien de la ferme. Il arrivait souvent à ce chien de dormir et de déféquer dans un tas de foin coupé utilisé dans la ration totale mélangée des vaches et il est donc probable que le chien ait été un vecteur de l'infection dans cette flambée. Les taures de remplacement, qui n'avaient pas mangé cette ration mélangée ou le foin coupé, avaient une séroprévalence moins élevée contre *N. caninum* et n'avaient pas d'avortements. Un nouvel isolat de *N. caninum*, dénommé NC-Illinois, a été obtenu à partir d'un veau prématuré.

Les évènements entourant cette flambée dans la ferme laitière mettent en lumière une pratique de gestion qui pourrait prévenir l'apparition de la néosporose. Il faudrait empêcher les chiens et les coyotes, qui sont des hôtes définitifs du parasite, de déféquer dans les aliments du bétail entreposés afin de réduire le risque d'infection du bétail avec *N. caninum*. Cette petite ferme laitière aurait pu empêcher la flambée simplement en tenant fermée la porte du bâtiment contenant le foin. Les plus grandes fermes laitières pourraient prévenir la contamination des aliments du bétail entreposés en érigeant une clôture en maille de chaîne avec portes automatiques autour des zones où l'ensilage et les suppléments alimentaires sont gardés.

Introduction

In North America, outbreaks of bovine abortion are most often attributed to neosporosis, leptospirosis, or infection with bovine viral diarrhea virus.[2] Other differential diagnoses of abortion epidemics include epizootic bovine abortion ("foothills abortion"), which occurs in California and neighboring states and is transmitted by soft shelled *Ornithodorus* ticks; toxicosis from grazing needles of the Ponderosa Pine, which is found in Western States; and nitrate toxicosis. Because of widespread use of effective vaccines, infectious bovine rhinotracheitis virus is an uncommon cause of bovine abortion, and brucellosis is now rare in the US and Canada because of eradication efforts; nevertheless, the possibility of these diseases should not be discounted in outbreaks of bovine abortion.

Although neosporosis is a cause of bovine abortion throughout the US and around the world,[17,22] it is important to realize that many infected cows do not have abortion or other clinical signs of disease.[17,18] Cow herds with high prevalence of infection 1) may suffer no visible consequence; 2) may have an endemic disease pattern, with a mild or moderate increase in annual abortion rates; or 3) less commonly, a herd may have an outbreak of abortion. The reasons why some previously uninfected herds have abortion epidemics following point-source exposure to the causative organism,[9,20] while others suffer no visible effect,[8,18] have not been elucidated.

Neospora caninum is a protozoal parasite that is transmitted between dogs and cattle.[12] Coyotes and white-tailed deer are other definitive and intermediate hosts, respectively.[15,16] Cattle are believed to develop permanent, although usually subclinical, infections when they consume oocysts. Congenital infection is also very common following transplacental transmission of the organism from a chronically infected[1,4] or an acutely infected[14] cow to the fetus. Dogs become infected when they consume tissues[12] or placenta[7] from infected cattle, and then shed the oocysts in feces for approximately two weeks.

Here we report an outbreak of abortion in a small dairy herd in Illinois, with evidence of point-source exposure to *N. caninum*. A management practice on this dairy is highlighted that increased the risk of parasite transmission throughout the herd, and protective measures are suggested. A new isolate of the organism was obtained in cell culture.

Materials and Methods

Herd Investigation

An outbreak of bovine abortion on a small dairy in Illinois was investigated. At the onset of the outbreak, the dairy had 60 cows and 51 nulliparous replacement heifers. The index case of abortion occurred on June 22, 1999 (day 1). The owners had not noted any abortions in the preceding months. The investigators visited the farm during the abortion outbreak on July 24 (day 33), and again on September 15 (day 86), shortly after the outbreak had ceased. Farm records for each cow and replacement heifer were examined for breeding dates, results of pregnancy palpations performed during regularly scheduled veterinary examinations, and calving dates and abortions. The premises were examined and management practices reviewed. During both visits, serum samples were obtained from all cows and replacement heifers. During the first visit, serum samples were also obtained from a farm dog and from a neighbor's dog that frequently visited the farm.

Three aborted fetuses were submitted to the University of Illinois Veterinary Diagnostic Laboratory in Urbana, and to the Illinois Department of Agriculture's Bureau of Animal Diseases Laboratory in Centralia. Diagnostic work-ups of the aborted fetuses included necropsy and histopathology, virus isolation and/or fluorescent antibody detection of bovine viral diarrhea virus, bacterial culture of abomasal content and lung for aerobic and microaerophilic bacteria, fluorescent antibody tests for infectious bovine rhinotracheitis and *Leptospira* spp, and dam serology for seven serovars of *Leptospira* spp.

Serology

All bovine sera were analyzed in Dr. Björkman's laboratory in the Swedish University of Agricultural Sciences, Uppsala, Sweden, for antibodies to *N. caninum*, using a previously described iscom ELISA.[5] This serologic test has an estimated sensitivity and specificity of 99 and 96%, respectively.[11] Sera with absorbance values >0.20 were considered positive, and these samples were further analyzed using the same underlying ELISA procedure, but with added steps to also assess the avidity of the reactive antibodies.[6,11] Avidity serology, which is a useful research tool but is seldom available for routine diagnostics, measures not only the amount of antibodies against a pathogen, but also determines the binding strength, or avidity, that these antibodies have for the organism. The binding strength of antibodies produced against an organism will increase over time, so that high avidity is indicative that an infection is chronic, while low avidity indicates that an infection was acquired recently.[6] In the present study, sera that were positive for *N. caninum*, but that had low avidity (values <50), were considered to be early antibody responses (i.e. evidence of acute infections). Positive sera with avidity ≥50 were considered to be evidence of chronic infections.[6] Dog sera were also analyzed for *N. caninum* antibodies using iscom ELISA,[3] with avidity testing as above.

Statistics

Absorbance values of the *N. caninum* ELISA on day 33 of the abortion outbreak were compared between pregnant cows that aborted or gave birth to a premature (i.e. <264 days gestation) weak calf, and cows that did not abort, using a two-tailed Student's t-test. ELISA absorbance values and avidity were each compared between first and second serum samples for all cows, again using a two-tailed Student's t-test.

Isolation and Characterization of Parasite

A 39-day-old ill-thrift calf, born prematurely during the abortion outbreak, was euthanized. Mixed chunks of fresh brain totaling 15 g were homogenized using a glass tissue grinder, suspended in Phosphate Buffered Saline (PBS), filtered through cheesecloth, centrifuged at 350 g for five minutes, and the pellet was resuspended in PBS to a total volume of 5 ml. Four gamma-interferon knockout mice[a] and four Mongolian Gerbils[b] were each inoculated intraperitoneally with 0.5 ml of the brain suspension and observed daily for evidence of illness. Rodents that became ill were promptly euthanized; separate specimens of brain, liver and spleen were homogenized using a blender, and the homogenates were suspended in PBS, filtered through cheesecloth, washed twice using PBS and centrifuged at 350 g, and added to Vero cell monolayers in 25 ml flasks containing RPMI-1640 medium supplemented with 10% horse serum; the medium was changed after four hours. The cells were observed daily for protozoal tachyzoites, using phase contrast microscopy on an inverted microscope. Tachyzoites recovered from cell culture were examined using a previously described *N. caninum*-specific PCR assay,[24] including a positive control of cultured *N. caninum* (Nc-SweB1)[21] and a negative control of uninfected Vero cells. A young adult New Zealand White rabbit[c] was inoculated subcutaneously with 8 x 10^5 tachyzoites; serum was collected six weeks later and examined using a previously described *N. caninum* indirect fluorescent antibody test (IFAT).[10]

Results

Herd Investigation

At the time of the abortion outbreak, which lasted from June 22 to September 6, 1999 (77 days), 41 of 60 cows were pregnant. During this period, 13 of these 41 cows suffered abortions, and a 14th cow had a weak, premature calf. Thirteen of the 14 cows that aborted or had premature calves were seropositive for *N. caninum*, while one seronegative cow aborted twins (a possible cause of that abortion). There were also 51 replacement heifers, 15 of which were pregnant in or near the second or third trimester of gestation. Abortions did not occur in the heifers.

Three aborted fetuses were examined. Mild lesions, compatible with but not diagnostic of neosporosis, were observed in two fetuses, and the third fetus had typical histological lesions of neosporosis (nonsuppurative endomyocarditis, encephalitis, hepatitis and placentitis, with a large protozoal pseudocyst containing tachyzoites in the placenta). Fetal tissues were negative for IBR and BVD viruses, aerobic or microaerophilic bacterial pathogens and leptospires. Their dams were seronegative for seven serovars of *Leptospira* spp, but were seropositive for neosporosis.

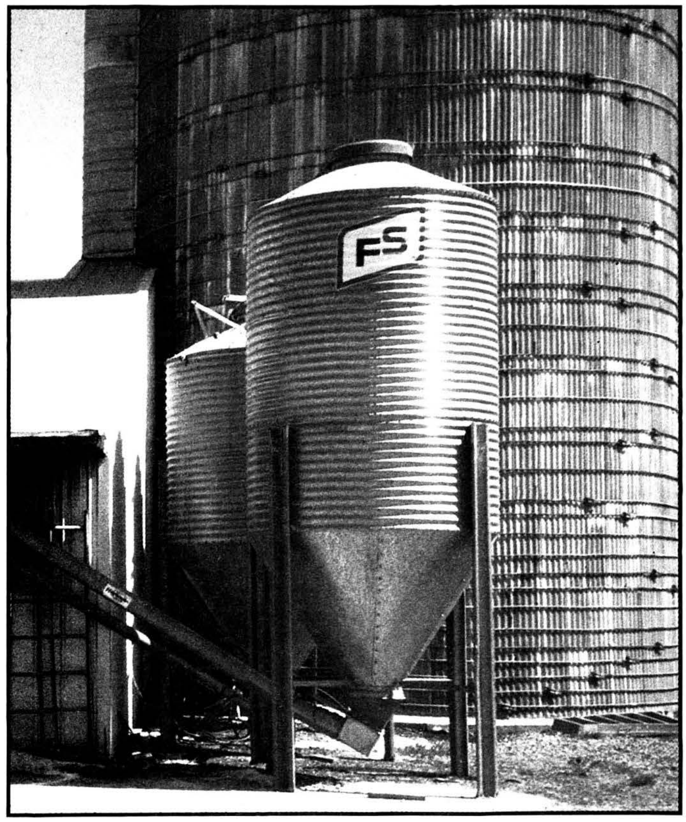

Figure 1. Silage and grain were stored in secure containers that ensured these feedstuffs could not become contaminated by canine feces. Although enclosed containers are a good option for small traditional dairies, they may be impractical to use on modern large-scale dairies because of the sheer volume of feedstuffs that must be stored and moved on a daily basis.

All cows were fed a total mixed ration (TMR). The diet consisted of corn silage stored in an upright silo, a grain mix stored in an upright grain bin (Figure 1), whole fuzzy cotton seed stored in a gravity wagon and chopped alfalfa hay. Round bales of alfalfa hay were chopped every three weeks and stored on the floor of a storage room attached to the dairy barn. The door to this room remained ajar to allow the single farm dog (a mixed breed adult male) and several barn cats to sleep on the chopped hay (Figure 2). Canine and feline feces were observed in this room, and the owner stated that the dog would defecate in the hay. Dead stock (calves and the occasional cow) were disposed of by moving the carcasses to the edge of a wooded area on the farm. The owner stated that the farm dog frequently ate offal from slaughtered cattle, placentas and portions of dead carcasses. The owner also said that a neighbor's dog also frequented the premises, and that coyotes were observed in the area.

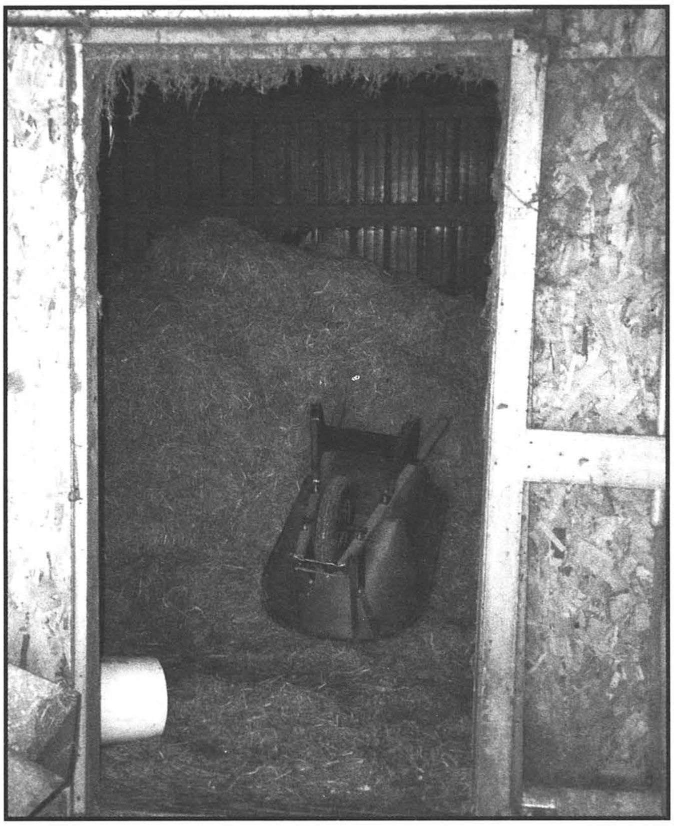

Figure 2. Chopped hay was stored on the floor of an open room in the barn, where the farm dog would often sleep and defecate. This hay was mixed into the cows' ration. Contamination of any ingredient of a mixed ration with *Neospora caninum* oocysts has the potential to cause rapid widespread transmission to a large proportion of the herd. Closing the door to the hay room might have prevented the abortion outbreak in this dairy.

The replacement heifers were kept on pasture and supplemented with large round bales of wheat hay. The heifers were not fed the TMR that was provided to the cows, nor the chopped alfalfa hay that was part of the TMR.

Serology

On day 33 of the abortion outbreak, 49 of 60 cows were seropositive for *N. caninum*; ELISA absorbance values were higher ($P < 0.002$) in cows that aborted or that gave birth to a weak premature calf (mean = 0.764) than in cows that did not have these conditions (mean = 0.461). Mean ELISA values dropped between the first (0.531) and second (0.310) serum samples ($P < 0.001$). Of the 49 seropositive cows, antibody avidity could be determined in 41 of them; 39 of these 41 cows had avidity values below 50, which is evidence of recently acquired infections in the great majority of infected cows. In the cow herd, mean avidity values rose from 27 to 34 between the first and second serum samples ($P < 0.04$), again consistent with early maturation of antibodies in recently acquired infections.

During the outbreak of abortion in the cows, five of 51 heifers were seropositive for *N. caninum* antibodies, and two of the positive heifers had avidity values >50. Thus, two of the five infected heifers likely had chronic infections (possibly congenitally acquired), while the other three infections likely were recently acquired. On day 86, when a second set of sera were obtained, four of 50 heifers were seropositive, and the same two continued to have high avidity values. One heifer with a low positive absorbance in the first sample had converted to seronegative status in the second sample.

The farmer's dog was *N. caninum* seropositive at the time of the outbreak, with an ELISA absorbance value of 0.432 and an avidity below 39, which is consistent with a recently acquired infection. The neighbor's dog was seronegative.

Isolation of Parasite

The calf that was born prematurely (251 days of gestation) was given nursing care to overcome weakness and ill-thrift for several weeks, but the owner observed it to have seizures at 34 days of age. The calf was euthanized during the second farm visit, when it was 39 days old, at which time it was seropositive with an ELISA absorbance of 1.098. Four gamma-interferon knockout mice and four gerbils were inoculated with samples of homogenized brain from the euthanized calf. After 35 days, one of the mice became ill, euthanasia was performed and various organs were processed as described for inoculation of Vero cell cultures. Protozoal tachyzoites were observed after six days in cultures that had been inoculated with spleen, and after seven days in cultures that had been inoculated with liver; cell cultures that were inoculated with brain were destroyed by fungal contamination. Two other mice became ill at 39 and 41 days after inoculation; culture attempts from the tissues from these mice were aborted after tachyzoites had been recovered from the first mouse. The gerbils remained healthy, and were euthanized 82 days after inoculation. Attempts to isolate organisms from their tissues were unsuccessful. The result of a *N. caninum*-specific polymerase chain reaction (PCR) test of the cultured tachyzoites was positive. A rabbit inoculated with this organism developed an IFAT *N. caninum* antibody titer of 1:6,400, without showing any evidence of illness. Confirmatory genetic sequencing of the organism's Internal Transcribed Spacer-1 region was reported previously.[13] This new isolate of *N. caninum* was designated NC-Illinois, and a culture was deposited[d] in the American Type Culture Collection.[e]

Discussion

Serologic and epidemiologic evidence indicates the majority of cows in this herd were acutely infected with *N. caninum*. The abortion pattern (33% of pregnant cows aborted within 11 weeks) is typical of a point-source exposure to this parasite. Although it was not possible to prove the source of the infections, circumstantial evidence was consistent with the possibility that the TMR had been contaminated, and the farm dog may have been the vector of contamination. The abortion outbreak was confined to the cows, which had 82% seroprevalence, and the great majority had low antibody avidities (indicative of recently acquired infections). In contrast, seroprevalence in replacement heifers was <10%, and abortions did not occur.

The cows, but not the replacement heifers, were fed a TMR containing alfalfa hay that was chopped every three weeks and piled on the floor of a room in the barn. The farm dog slept on the hay pile, and also frequently defecated in it. If the dog was recently infected with *N. caninum*, it would be expected to shed protozoal oocysts in its feces for approximately two weeks.[12] In support of this possibility, the dog was seropositive for *N. caninum*, and its antibody avidity was low, which is consistent with a recently acquired infection.

Oocysts are infectious to cattle of all ages.[12,14] In a recent study, *N. caninum* infections were induced in pregnant cows by administration of oocysts.[14] Transplacental transmission of the infection from dam to fetus occurred with greater frequency when cows ingested oocysts later than the 130th day of gestation. In that experiment, abortion occurred in only one of 19 infected cows, which is far less than occurred under natural circumstances in this herd, as well as in other previously reported outbreaks.[9,19,20,23] It is not clear what conglomeration of factors will result in neosporosis abortion outbreaks in some herds following widespread exposure, while others may have few or no abortions. Strain virulence, numbers of oocysts ingested, stage of gestation at the time of exposure and previous infection history probably each influence the outcome of newly acquired *N. caninum* infections in pregnant cows.

Conclusions

The circumstances in this dairy illustrate a manageable factor that influences the probability of rapid dissemination of *Neospora* infection in a herd. Because dogs and coyotes are definitive hosts of this parasite, it is important to hinder them from defecating in stored feedstuffs. If a feedstuff becomes contaminated, then a large percentage of cattle could become infected in a short period when they consume a mixed ration with the contaminated ingredient. On this particular farm, the most likely contaminated feedstuff was the alfalfa hay, which was chopped into a pile every three weeks and used by the farm dog as both a bed and a latrine.

In this small dairy, the risk of feed contamination could have been reduced by simply keeping the door closed to the room that contained the hay pile. An additional management technique would be to dispose of bovine carcasses, offal and (when possible) placentas in a way that prevents their consumption by dogs or coyotes. Dumping of carcasses next to a woods, as was practiced on this farm, increases the odds that dogs or coyotes will consume tissues of any chronically infected cattle, and then shed oocysts.

For the largest dairies, which usually store vast quantities of feedstuffs outdoors or in open-faced sheds, preventing canine fecal contamination takes more effort than simply closing a door in the barn. Nevertheless, there are practical solutions, such as erecting a chain-link fence around the entire area in which the feedstuffs are kept; large gates can be designed to open and close automatically whenever a tractor approaches, thus keeping dogs and coyotes out, but not hindering the loading and unloading of feeds.

Footnotes

[a] BALB/c-Ifngtm1Ts, Jackson Laboratories, Bar Harbor, Maine

[b] Harlan Sprague Dawley, Indianapolis, Indiana

[c] Myrtle's Rabbitry, Thompson Station, Tennessee

[d] catalog number PRA-139

[e] Manassas, VA 20108, USA, www.atcc.org

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References

1. Anderson ML, Reynolds JP, Rowe JD, Sverlow KW, Packham AE, Barr BC, Conrad PA: Evidence of vertical transmission of *Neospora* sp infection in dairy cattle. *J Am Vet Med Assoc* 210:1169-1172, 1997.
2. Barr BC, Anderson ML: Infectious diseases causing bovine abortion and fetal loss. *Vet Clin North Am Food Anim Pract* 9:343-368, 1993.
3. Björkman C, Lunden A, Holmdahl J, Barber J, Trees AJ, Uggla A: *Neospora caninum* in dogs: detection of antibodies by ELISA using an iscom antigen. *Parasite Immunol* 16:643-648, 1994.
4. Björkman C, Johansson O, Stenlund S, Holmdahl OJ, Uggla A: *Neospora* species infection in a herd of dairy cattle. *J Am Vet Med Assoc* 208:1441-1444, 1996.

5. Björkman C, Holmdahl OJ, Uggla A: An indirect enzyme-linked immunoassay (ELISA) for demonstration of antibodies to *Neospora caninum* in serum and milk of cattle. *Vet Parasitol* 68:251-260, 1997.
6. Björkman C, Nåslund K, Stenlund S, Maley SW, Buxton D, Uggla A: An IgG avidity ELISA to discriminate between recent and chronic *Neospora caninum* infection. *J Vet Diagn Invest* 11:41-44, 1999.
7. Dijkstra T, Eysker M, Schares G, Conraths FJ, Wouda W, Barkema HW: Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. *Int J Parasitol* 31:747-752, 2001.
8. Dijkstra T, Barkema HW, Björkman C, Wouda W: A high rate of seroconversion for *Neospora caninum* in a dairy herd without an obvious increased incidence of abortions. *Vet Parasitol* 109:203-211, 2002.
9. Dijkstra T, Barkema HW, Hesselink JW, Wouda W: Point source exposure of cattle to *Neospora caninum* consistent with periods of common housing and feeding and related to the introduction of a dog. *Vet Parasitol* 105:89-98, 2002.
10. Dubey JP, Hattel AL, Lindsay DS, Topper MJ: Neonatal *Neospora caninum* infection in dogs: isolation of the causative agent and experimental transmission. *J Am Vet Med Assoc* 193:1259-1263, 1988.
11. Frössling J, Bonnett B, Lindberg A, Bjorkman C: Validation of a *Neospora caninum* iscom ELISA without a gold standard. *Prev Vet Med* 57:141-153, 2003.
12. Gondim LFP, Gao L, McAllister MM: Improved production of *Neospora caninum* oocysts, cyclical oral transmission between dogs and cattle, and in vitro isolation from oocysts. *J Parasitol* 88:1159-1163, 2002.
13. Gondim LFP, Laski P, Gao L, McAllister MM: Variation of the internal transcribed spacer 1 sequence within individual strains and among different strains of *Neospora caninum*. *J Parasitol* 90:119-122, 2004.
14. Gondim LFP, McAllister MM, Anderson-Sprecher RC, Björkman C, Lock TF, Firkins LD, Gao L, Fischer WR: Transplacental transmission and abortion in cows administered *Neospora caninum* oocysts. *J Parasitol* 90:1394-1400, 2004.
15. Gondim LFP, McAllister MM, Mateus-Pinilla NE, Pitt WC, Mech LD, Nelson ME: Transmission of *Neospora caninum* between wild and domestic animals. *J Parasitol* 90:1361-1365, 2004.
16. Gondim LFP, McAllister MM, Pitt WC, Zemlicka DE: Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int J Parasitol* 34:159-161, 2004.
17. Larson RL, Hardin DK: Review: *Neospora caninum*-induced abortion in cattle. *Bov Pract* 37(2):121-126, 2003.
18. Mainar-Jaime RC, Thurmond MC, Berzal-Herranz B, Hietala SK: Seroprevalence of *Neospora caninum* and abortion in dairy cows in northern Spain. *Vet Rec* 145:72-75, 1999.
19. McAllister MM, Huffman EM, Hietala SK, Conrad PA, Anderson ML, Salman MD: Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. *J Vet Diagn Invest* 8:355-357, 1996.
20. McAllister MM, Björkman C, Anderson-Sprecher R, Rogers DG: Evidence of point-source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. *J Am Vet Med Assoc* 217:881-887, 2000.
21. Stenlund S, Björkman C, Holmdahl OJ, Kindahl H, Uggla A: Characterization of a Swedish bovine isolate of *Neospora caninum*. *Parasitol Res* 83:214-219, 1997.
22. Wouda W: Diagnosis and epidemiology of bovine neosporosis: a review. *Vet Quart* 22:71-74, 2000.
23. Yaeger MJ, Shawd-Wessels S, Leslie-Steen P: Neospora abortion storm in a midwestern dairy. *J Vet Diagn Invest* 6:506-508, 1994.
24. Yamage M, Flechtner O, Gottstein B: *Neospora caninum*: specific oligonucleotide primers for the detection of brain "cyst" DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). *J Parasitol* 82:272-279, 1996.

Abstract

Frequencies of PrP Genotypes in 38 Breeds of Sheep Sampled in the National Scrapie Plan for Great Britain

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Between October 2001 and January 2003 the prion protein (PrP) genotypes of over 250,000 sheep were determined through the operation of the National Scrapie Plan (NSP); the results for 38 breeds were analysed to provide an estimate of the underlying PrP genotype distribution of the British sheep population. Although there was marked variability among the genotype profiles of the different breeds, several trends emerged. A comparison of the allele frequencies demonstrated that the breeds could be grouped into three categories: breeds dominated by ARR and ARQ in which the frequency of ARR exceeded the frequency of ARQ; breeds dominated by ARR and ARQ in which the frequency of ARQ exceeded the frequency of ARR; and breeds with significant levels of either AHQ, ARH or VRQ. Hill breeds were more likely to have a lower proportion of animals at low risk of scrapie (NSP type 1) and a higher proportion of animals at an intermediate risk of scrapie (NSP type 3) than other breeds. Most breeds had a small proportion of animals at high risk of scrapie (NSP type 5). The frequency of ARR/VRQ (NSP type 4) was variable.