

BOVINE ABORTION DUE TO A NEOSPORA-LIKE PROTOZOAN

Jerome Nietfeld,¹ Michael Yaeger,¹ J. P. Dubey,² Mark Anderson,³
Melissa Libal,¹ Regg Neiger¹

¹Department of Veterinary Science, Box 2175, South Dakota State University, Brookings, SD 57007. ²Zoonotic Disease Laboratory, Livestock and Poultry Sciences Institute, USDA, ARS, Beltsville MD 20705. ³California Veterinary Diagnostic Laboratory System, P. O. Box 1770, University of California, Davis, CA 95617

A protozoan closely related to Neospora caninum, which until more fully characterized is usually referred to as a Neospora-like protozoan, was recently demonstrated to be the most common cause of abortion in dairy breed bovine fetuses submitted to the South Dakota Animal Disease Research and Diagnostic Laboratory (SDADRDL).¹⁰ The purpose of this report is to briefly review Neospora-like protozoan as a cause of bovine abortion and to discuss the cases submitted to the SDADRDL.

Neospora caninum is a cyst-forming coccidium in the phylum Apicomplexa which was first identified in 1988 as a cause of fatal meningoencephalitis and myositis in dogs.⁶ Previously, N. caninum infections were often misdiagnosed as being due to Toxoplasma gondii. However, N. caninum is structurally and antigenically distinct from T. gondii. In 1989 a Neospora-like protozoan was identified as the cause of an abortion storm in a New Mexico dairy.¹² Isolated cases of bovine abortion or congenital infection by a Neospora-like protozoan have been reported from Washington,^{7,11} California,⁴ and Maryland.⁸ In 1991 a Neospora-like protozoan was shown to be the most common cause of abortion in California dairy cattle.¹⁻³ Approximately 20% of the bovine fetuses submitted to the California Veterinary Diagnostic Laboratory System are infected with this parasite. California researchers have demonstrated that while this protozoan closely resembles N. caninum in structure and reacts with N. caninum antisera, there are distinct ultrastructural and antigenic differences between the canine and bovine parasites.⁴ More recently the parasite has been isolated from two aborted bovine fetuses and one congenitally infected calf.⁵ Preliminary studies indicate that the protozoan is a Neospora species.

Twenty-two cases of Neospora-like protozoan infection in bovine fetuses have been confirmed by the SDADRDL. In each fetus the diagnosis was based on the presence of histopathologic lesions and positive immunohistochemical staining using N. caninum antisera.^{1,3,9} Immunohistochemical staining was done by the California Veterinary Diagnostic Laboratory System, Davis, California or by the Zoonotic Disease Laboratory, USDA, Beltsville, Maryland. One of the fetuses was submitted to the SDADRDL in 1989. The remaining 21 fetuses were part of a group of 733 bovine abortion submissions received between December 17, 1990 and December 31, 1991. The fetuses originated from 17 herds located in South Dakota (6), Minnesota (3), Wisconsin (3), Texas (2), Arizona (2), and Iowa (1). Bovine viral diarrhea virus and bovine herpesvirus-4 were isolated from one fetus each. Evidence of infection by an agent other than the Neospora-like protozoan was not found in the other 20 fetuses.

At the time of abortion the gestational age of the 17 fetuses whose age was available ranged from 3 to 8.5 months with 76% of the fetuses being between 4 and 6 months of gestation. Fourteen of the 17 herds had multiple abortions during the preceding few weeks or months. In some instances there were several abortions in a few weeks, while in other herds sporadic abortions occurred over several months. For example, at least 7 abortions occurred over a 6 week period in a Minnesota herd of 100 cows, and in an Arizona herd of 1300 cows approximately 40 abortions were detected in a 2 week period. The Wisconsin herd from which the 1989 case originated consists of 60 to 70 Holsteins in the milking herd. In each of the past 4 to 5 years 20% to 30% of the cows have aborted. In the first 5 months of 1991 eight fetuses were received from this herd. Four fetuses were mummified unsuitable for accurate evaluation. Neospora-like protozoa were identified in the remaining four fetuses.

All fetuses necropsied at the SDADRDL were autolyzed and did not have diagnostic gross lesions. The brain proved to be by far the most important tissue to examine histologically for both lesions and protozoa. Multifocal, nonsuppurative encephalitis, often associated with focal necrosis, was characteristic of Neospora-like protozoan infection (Fig. 1). This lesion was present in all 18 protozoan infected fetuses in which the brain was examined and was found only in fetuses infected by the Neospora-like agent. Nonsuppurative myositis and myocarditis were also consistent lesions in fetuses with Neospora-like protozoan infection; but these lesions were also occasionally found in fetuses without positive evidence of protozoal infection. Nonsuppurative hepatitis, nephritis, pneumonia and/or placentitis, occasionally accompanied by necrosis, were present in approximately one-half of the fetuses (Fig. 4). Like myocarditis and myositis, these lesions were suggestive of, but not specific, for protozoal infection.

Protozoa were present in low numbers and found in hematoxylin and eosin stained slides from the brains of four fetuses and the kidney of one fetus (Fig. 2). Protozoa were detected by immunohistochemical staining in the brain of 17 of the 22 fetuses (Fig. 3). In four cases the brain was either too autolyzed for histopathology or not submitted for examination. In the fifth case the brain contained lesions characteristic of protozoal infection but organisms were found only in the placenta. In addition to brain, immunohistochemical staining revealed Neospora-like protozoa in skeletal muscle (3 fetuses), kidney (2 fetuses), heart (1 fetus), and placenta (1 fetus). Histologic lesions, especially those in the brain, provide a presumptive diagnosis of infection by the Neospora-like organism. Presently, the only way to confirm the presence of the protozoan is by immunohistochemical staining of formalin fixed tissues using N. caninum antisera.

The problem appears to be almost exclusively limited to dairy cattle. Between December 17, 1990 and December 31, 1991, 37% of the bovine fetuses (whose breed was specified) submitted to the SDADRDL were from dairy cattle. During this period 95% (20 of 21) of the Neospora-like protozoan infected fetuses were of a dairy breed (19 Holstein, 1 Brown Swiss, 1 White Park). Similarly, the protozoal abortions and congenital infections reported from California, New Mexico and Washington were in dairy cattle.^{1-4,7,11,12} An abortion in a Hereford from Maryland along with the White Park in this survey are the only Neospora-like protozoan abortions reported from beef cattle

from the United States.^{8,10} During 1991 the Neospora-like protozoan was the agent most often identified in aborted dairy fetuses submitted to the SDADRDL. Overall the parasite was the third most prevalent cause of bovine abortion with only bovine viral diarrhea virus and Actinomyces pyogenes diagnosed more often.

Currently the parasite's life cycle and the source of infection for cattle are unknown. Researchers at the University of California are in the process of better characterizing the organism and are attempting to identify the host species and thus the source of infection for dairy cattle.⁵ Most likely, cattle are infected by ingesting feed contaminated by fecal material from another animal species, similar to sheep which are infected with T. gondii by ingesting feed contaminated by cat feces containing T. gondii oocysts. This hypothesis has not been proven and until the source of infection and the parasite's life cycle are known it is impossible to make sound recommendations concerning prevention and control of this emerging bovine disease.

Summary

Twenty-two aborted bovine fetuses submitted to the South Dakota Animal Disease Research and Diagnostic Laboratory (SDADRDL), were found to be infected with a Neospora-like protozoan. Twenty-one of the 22 fetuses were of dairy breeds. In 1991, this protozoan was the most commonly diagnosed cause of abortion in dairy cattle at the SDADRDL. The 22 fetuses originated from 17 herds located in South Dakota, Minnesota, Wisconsin, Iowa, Texas, and Arizona. Multiple abortions were reported in 14 of the 17 herds. Seventy-six percent of the fetuses were between 4 and 6 months of gestation, most were autolyzed, and diagnostic gross lesions were not present. In each fetus, the diagnosis was based on the presence of histologic lesions and positive immunohistochemical staining using N. caninum antisera. Nonsuppurative encephalitis with multifocal necrosis was specific for protozoal infection and was present in all 18 fetuses in which the brain was examined. Nonsuppurative myocarditis and myositis were also consistent but somewhat less specific lesions in affected fetuses. By far, the best tissue to detect the Neospora-like protozoan was brain (17 of 22). Protozoa were also found in skeletal muscle (3), kidney (2), heart (1), and placenta (1).

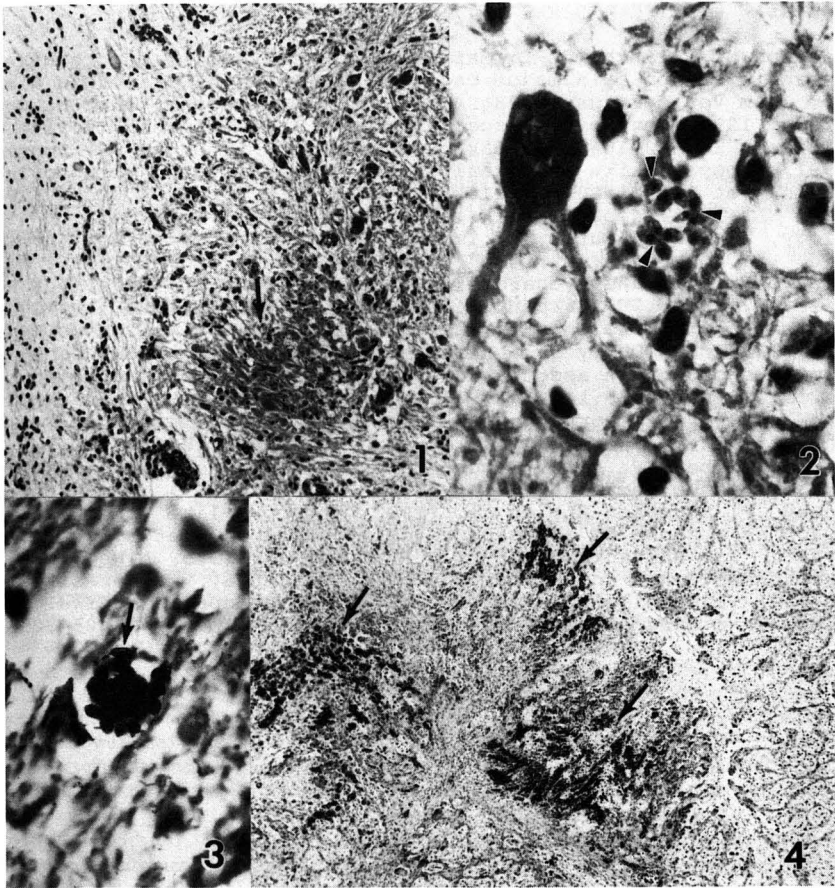
Resumen

Un protozoario semejante a Neosporum sp., fué identificado en veintidos fetos de vacas que abortaron, al ser examinados en el Laboratorio de Investigaciones sobre Enfermedades Animales y Diagnóstico de Dakota del Sur (SDADRDL). Veintiuno de los 22 fetos pertenecían a razas lecheras. En 1991, este protozoario fué la causa más frecuentemente diagnosticada de abortos en vacas lecheras, en el SDADRDL. Los 22 fetos provenían de 17 rebaños localizados en los Estados de Dakota del Sur, Minnesota, Wisconsin, Iowa, Texas y Arizona. Abortos múltiples fueron reportados en 14 de los 17 rebaños. El 76% de los fetos se encontraban entre 4 y 6 meses de gestación, la mayoría estaban autolizados, y no se encontraron lesiones a simple vista. El diagnóstico en cada feto estuvo basado en la presencia de lesiones histológicas y una prueba positiva de tinción inmunohistoquímica usando antisuero contra Neosporum caninum.

Encefalitis no supurativa, con necrosis multifocal, fué típica en infección de protozoarios, y estuvo presente en todos los 18 fetos cuyo cerebro fué examinado. Miocarditis no supurativa y miocitis fueron también observadas en todos los fetos pero las lesiones eran algo menos específicas. El cerebro fué el mejor tejido para la identificación de protozoarios semejantes a Neosporum sp. pues 17 de los 22 fetos fueron positivos aunque también se encontró protozoarios en músculo del esqueleto (en 3 fetos), riñón (en 2 fetos), corazón (1 feto) y placenta (1 feto).

Resume

Vingt deux foetus provenant de vaches ayant avorte, ont ete examines au Laboratoire de Recherches sur Maladies Animales et Diagnostique du Dakota du Sud (SDADRDL). Ils etaient infectes avec un protozoaire semblable a Neosporum sp. Vingt et un foetus appartenait a des races laitieres. Ce protozoaire a ete la principale cause d'avortement en betail laitier, diagnostique au SDADRDL en 1991. Les vingt deux foetus parvenaient de dix sept cheptels dans les provinces du Dakota du Sud, Minnesota, Wisconsin, Iowa, Texas et Arizone. Avortements multiples ont ete trouves dans 14 des 17 cheptels. Le 76% des foetus etaient dans les 4 a 6 mois de gestation, la plupart etant autolyses, et des lesions a l'oeil n'ont pas ete trouves. Le daignostique pour chaque foetus a ete fait en vue de la presence de lesions histologiques ainsi que des epreuves positives de coloration immunohistochemique en employant de l'antiserum pour Neosporum caninum. Encephalite non supurative avec necrose multifocale fut caracteristique chez l'infection a protozoaire, ayant ete trouve sur tous les 18 foetus dont leurs cerveaux furent examines. On a trouve aussi des miocardites non supuratives et des miocitoses chez tous les foetus, mais les lesions etaient moins specifiques. Le meilleur tissu pour isoler des protozoaires semblables au Neosporum sp. a ete le cerveau, car 17 echantillons sur 22 etaient positifs, bien que le meme protozoaire a ete trouve aussi dans le muscle du squelette (en 3 foetus), reins (2 foetus), coeur (1), et placenta (1).



Legends:

Figure 1 Photomicrograph of bovine calf brain with a focus of necrosis (arrow) and infiltration of glial cells and mononuclear cells. HE stain.

Figure 2 Higher Magnification of brain from the calf in Fig 1 showing a cluster of 12 extracellular Neospora-like tachyzoites (arrowheads). HE stain.

Figure 3 Higher magnification of the brain in Fig 1 showing a cluster of tachyzoites (arrow) that stain immunohistochemically positive using antiserum to N. caninum. Avidin-biotin immunohistochemical stain.

Figure 4 Photomicrograph of placental cotyledon from the calf in Fig 1. Note necrosis and mineralization of placental villi (arrows). HE stain.

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References

1. Anderson ML, Blanchard PC, Barr BC, et al.: 1991, Neospora-like protozoan infection as a major cause of abortion in California dairy cattle. *J Am Vet Med Assoc* 198:241-244.
2. Anderson M, Barr B, Conrad P, et al.: 1991, Bovine protozoal abortions in California. *Bovine Practitioner* 26,102-104.
3. Barr BC, Anderson ML, Dubey JP, Conrad PA: 1991, Neospora-like protozoal infections associated with bovine abortions. *Vet Pathol* 28:110-116.
4. Barr BC, Conrad PA, Dubey JP, Anderson ML: 1991, Neospora-like encephalomyelitis in a calf: pathology, ultrastructure, and immunoreactivity. *J Vet Diagn Invest* 3:39-46.
5. Conrad PA, Barr BC, Sverlow KW, Anderson ML: 1992, Bovine fetal encephalitis and myocarditis associated with protozoa. *Vet Rec* 130:147.
6. Dubey JP, Carpenter JL, Speer CA, et al.: 1988, Newly recognized fatal protozoan disease of dogs. *J Am Vet Med Assoc* 192:1269-1285.
7. Dubey JP, Leathers CW, Lindsay DS: 1989, Neospora caninum-like protozoan associated with fatal myelitis in newborn calves. *J Parasitol* 75:146-148.
8. Dubey JP, Miller S, Lindsay DS, Topper WJ: 1990, Neospora caninum-associated myocarditis and encephalitis in an aborted calf. *J Vet Diagn Invest* 2:66-69.
9. Lindsay DS, Dubey JP: 1989, Immunohistochemical diagnosis of Neospora caninum in tissue sections. *Am J Vet Res* 50:1981-1983.
10. Nietfeld JC, Dubey JP, Anderson ML, et al.: 1992, Neospora-like protozoan infection as a cause of abortion in dairy cattle. *J Vet Diagn Invest* 4:223-226.
11. Shivaprasad HL, Ely R, Dubey JP: 1989, A Neospora-like protozoan found in an aborted bovine placenta. *Vet Parasitol* 34:145-148.
12. Thilsted JP, Dubey JP: 1989, Neosporosis-like abortions in a herd of dairy cattle. *J Vet Diagn Invest* 1:205-209.