

RESEARCH COMMUNICATION

The prevalence of neosporosis in aborted bovine foetuses submitted to the Allerton Regional Veterinary Laboratory

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ABSTRACT

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Combined histological examination and avidin-biotin immunohistochemical staining of formalin-fixed brain and myocardium from aborted bovine foetuses presented to the Allerton Regional Veterinary Laboratory were utilized for the diagnosis of bovine neosporosis. Two out of 144 cases were diagnosed positive for neosporosis, indicating a low prevalence of infection and confirming *Neospora caninum* to be a cause of sporadic abortion in the population surveyed.

Keywords: Neosporosis, prevalence, aborted bovine foetus, *Neospora caninum*

INTRODUCTION

Neosporosis, the infection with the parasitic cyst-forming protozoan, *Neospora caninum*, is recognized as a significant cause of bovine abortion and neonatal death with cases reported from countries in Europe, North America, Australasia, Asia and Africa (Lindsay, Dubey, Cole, Nuehring & Blagburn 1993). On morphological and phylogenetic grounds, *Neospora* is closely related to *Toxoplasma gondii*, and is presumed to have a similar life cycle, although this has yet to be elucidated and the definitive host in the presumed two-host life cycle has yet to be identified (Ellis, Luton, Baverstock, Brindley, Nimmo & Johnson 1994; Lindsay *et al.* 1993).

The only known natural route of transmission is transplacental. Adult cattle show no clinical signs of infection and little is known of the incubation period, factors affecting susceptibility, or seasonal occurrence of abortions (Thornton, Gajadhar & Evans 1994). Infection has been associated with sporadic abortion, enzootic abortion and abortion storms (Lindsay *et al.* 1993; Thornton *et al.* 1994; Yaeger, Shawd-Wessels & Leslie-Steen 1994). Foetuses may be aborted between 3 and 9 months of gestational age with a mean of 5.6 months (Lindsay *et al.* 1993). Repeated transmissions may occur from infected dams to their progeny (Barr, Conrad, Breitmeyer, Sverlow, Anderson, Reynolds, Chauvet, Dubey & Ardans 1993), although repeated abortion does not appear to be a consistent finding in an infected herd (Thornton *et al.* 1994). Gross examination of the abortus shows no significant lesions and diagnosis is typically based on characteristic light-microscopic lesions and the specific identification of the causative organism by means of immunohistochemical techniques on formalin-fixed, wax-embedded tissue sections (Lindsay *et al.* 1993; Yaeger *et al.* 1994). Lesions are characterized by

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multifocal necrosis and non-suppurative inflammation and are most commonly encountered in the brain, but also in the myocardium, placenta, adrenals, liver, lungs and kidneys (Jardine & Last 1993; Lindsay *et al.* 1993; Yaeger *et al.* 1994).

An immunofluorescent antibody (IFA) technique in which cultured-tachyzoite antigens are utilized, has been developed to detect anti-*Neospora* antibodies in the tissue fluids of the aborted foetus (Barr, Rowe, Sverlow, BonDurant, Ardans, Oliver & Conrad 1994; Conrad, Sverlow, Anderson, Rowe, BonDurant, Tuter, Breitmeyer, Palmer, Thurmond, Ardans, Dubey, Duhamel & Barr 1993). This technique has the advantage of much more rapid diagnosis than the rather slow and costly histological examination, but is considered unreliable in foetuses aborted at or after 7 months gestation owing to false-negative results (Yaeger *et al.* 1994). IFA-based serological tests on the dam failed to consistently provide diagnostic results, owing to the rapid degradation of the high post-abortion antibody levels, but can be reliable if utilized within 1 month of the abortion (Thornton *et al.* 1994). *Neospora* organisms can be isolated in tissue cultures although this method is expensive, demanding, generally ineffective and better suited to the research environment than it is suited for routine diagnosis (Lindsay *et al.* 1993). Currently, the most reliable technique for the diagnosis of neosporosis in aborted bovine foetuses is the combined use of histopathological examination of formalin-fixed and haematoxylin- and eosin- (HE-) stained samples of the brain, the diagnosis then being confirmed by the avidin-biotin immunohistochemical techniques as used in this survey (Lindsay *et al.* 1993; Yaeger *et al.* 1994).

MATERIALS AND METHODS

All bovine foetuses submitted to the Allerton Regional Veterinary Laboratory between May 1991 and April 1993 were included in the survey. In each case (both foetuses from a twin pregnancy were considered as a single case) a full necropsy examination, and routine bacteriologic, mycologic and protozoologic examinations were performed (Jardine & Last 1993). Samples consisting of either a longitudinally divided half of the brain and the apex of the myocardium, or the halved brain alone, were fixed in 10% buffered formalin.

These specimens were submitted to the Department of Pathology, Faculty of Veterinary Science, University of Pretoria, where they were routinely embedded in paraffin wax, sectioned and stained with HE for histopathologic examination. Duplicate sections were stained by means of an avidin-biotin immunohistochemical procedure for antigens to *Neospora caninum*. Briefly, the procedure was performed as follows: sections were affixed to poly-L-lysine-coated

glass slides (Sigma Chemical Company, P.O. Box 14508, St Louis, Missouri, USA) and dried overnight in an incubator at 37°C. The tissue sections were then dewaxed in xylene for 5 min, rehydrated through graded alcohols and washed in distilled water for 3 min. Endogenous peroxidases were then quenched by immersing sections in 3% hydrogen peroxide in methanol for 30 min, whereafter the sections were rinsed twice consecutively for 5 min in phosphate-buffered saline (PBS, pH 7.6) containing 0.1% bovine-serum albumin. Sections were then treated with 0.05% Pronase E (Sigma Chemical Company, P.O. Box 14508, St Louis, Missouri, USA) for 15 min at 37°C to expose sequestered antigens. Non-specific antibody binding was blocked with 10% normal goat serum (Labotec, P.O. Box 5103, Durban 4000, South Africa) for 20 min. The sections were then blotted and the slides covered in a 1:500 dilution of primary rabbit anti-*Neospora caninum* antibody (supplied by Dr J.P. Dubey, USDA, 10300 Baltimore Ave, Beltsville, MD 20705-2350, USA) for 60 min at room temperature. Following two further consecutive 5-min washes in PBS, the sections were incubated for 30 min at room temperature, with a 1:500 dilution of biotinylated goat anti-rabbit secondary antibody (Dako A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark). Two further washes in PBS were followed by covering the sections with avidin-biotin-complex (Dako A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark) for 30 min according to the manufacturer's recommendations. The sections were again rinsed in PBS and treated for 8 min with the peroxidase substrate diaminobenzamine tetrahydrochloride (DAB) (BDH Laboratory Supplies, Poole, Dorset, England). Slides were finally rinsed in distilled water, counterstained with Mayer's haematoxylin, dehydrated, mounted and examined with a standard light microscope.

RESULTS

A total of 144 cases of bovine abortion were examined, of which 105 were dairy breeds, 30 were beef breeds and nine were not specified. In only two cases was *Neospora caninum* positively identified, both being in dairy breeds, and one of them including a pair of twins (Jardine & Last 1993). All of these cases showed characteristic histopathologic lesions which consisted of multiple foci of necrosis and gliosis with an associated, often perivascular, inflammatory infiltrate characterized by macrophages, lymphocytes and plasma cells, with occasional neutrophils. Tachyzoites were difficult to discern in HE-stained sections, but stained strongly-positive with the avidin-biotin immunohistochemical technique.

CONCLUSIONS

A low prevalence of neosporosis was detected in this study of aborted bovine foetuses submitted to the

Allerton Regional Veterinary Laboratory, indicating that *Neospora caninum* is a cause of sporadic abortion in the surveyed cattle population and further suggests that no cases of enzootic abortion or abortion storms were encountered during the study period. Previous reports showed the predominance of infections in dairy herds, with an ostensibly far lower occurrence of reported cases in beef cattle (Lindsay *et al.* 1993). This survey corroborates these findings with diagnosis of infection only in dairy breeds.

The failure to identify cases of enzootic *Neospora* abortion or abortion storms in this survey, does not preclude these scenarios from developing at a later stage or being a current problem in other areas in southern Africa. A case of enzootic *Neospora* abortion has recently been diagnosed in a dairy in the eastern highlands of Zimbabwe, where four of the six fetuses submitted for examination were positive for neosporosis (Jardine & Wells 1995). The disease is increasingly being reported as a major cause of bovine abortion in especially the United States of America and New Zealand (Lindsay *et al.* 1993; Yaeger *et al.* 1994) and it should be considered a possible cause of abortion in all geographical localities of southern Africa.

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