First Confirmed Case of Neospora caninum-associated Abortion Outbreak in Portugal

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Contents
In a dairy herd experiencing an abortion outbreak, 49% of the 119 cows were positive for Neospora caninum antibodies and the remaining 51% were seronegative. Using immunohistochemical analysis of foetal brain tissue, Neospora cysts and bradyzoite clusters were identified in two of the 15 aborted foetuses submitted for testing. Other agents that commonly cause abortions were not found. These results, coupled with neurological clinical symptoms in new-born calves, implicated N. caninum as the major cause of abortions in this dairy herd. This is the first report that associates N. caninum infection with bovine abortions in Portugal.

Introduction
Neospora caninum is a tissue cyst-forming coccidian parasite that can infect and cause disease in a variety of mammalian genera (Dubey and Lindsay 1996). Neosporosis is an emergent disease, and has been recognized in the last decade as an important cause of bovine abortion throughout the world (Dubey et al. 1989; Anderson et al. 1991; Dubey and Lindsay 1996). The infection has been associated with sporadic, endemic and epidemic abortions in dairy herds (Anderson et al. 1997; McAllister et al. 1998).

It has been estimated that 20 to 40% of all bovine abortions in California (Anderson et al. 1991, 1995) and 15 to 20% in the Netherlands (Wouda et al. 1999) are attributable to neosporosis. Significant economical losses due to reduced milk production, higher culling rates, abortions and stillbirths has been reported (Thurmond and Hietala 1996, 1997a). It is estimated that neosporosis costs the dairy industry US$ 85 million/year in Australia and US$ 35 million/year in California (Ellis 1997).

Vertical transmission of Neospora sp. in dairy cattle has been established (Dubey and Lindsay 1996; Anderson et al. 1997). It can contribute to the persistence of the infection in the herd (Thurmond and Hietala 1996; Bjorkman et al. 1996) and may be responsible for cerebral, spinal and heart lesions in the foetus. Calves younger than 2 months can show nervous symptoms such as ataxia, decreased patellar reflex, exophtalm or have asymmetrically looking eyes (Barr et al. 1993). Transmission via milk ingestion has also been proven experimentally (Uggla et al. 1998).

In this article serological, histological and immunocytochemical evidence is presented which implicates N. caninum, for the first time in Portugal, as the main aetiological agent of an abortion outbreak in a dairy herd.

Materials and Methods
Dairy herd
The affected animals belonged to a dairy farm in Northern Portugal, with 119 milking cows producing an average yield of 8600 kg of milk/cow per year.

In the dairy herd, abortions had been observed since 1996, and showed a pattern of increase over time (Table 1). The highest rate (27%) of abortions were registered during the period of the present study (1999). No other signs of disease were detected in the aborting cows and their herd mates. Abortions occurred around 3 months of gestation (31% of total abortions), between 3 and 6 (63%) and at over 6 months of gestation (6%); most were observed by the farmer and a few were from confirmed pregnant animals that were found to be empty during routine veterinary work.

From a total of 119 breeding cows, 27% aborted between January and September 1999. Other more common causes of abortion (Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhoea (BVD), Brucella, Leptospira, Campylobacter, Salmonella, fungi, etc.) were ruled out by standard laboratory methods that also included serologic testing. Some cases of cows aborting in consecutive gestations (32 cows out of the 50 that had a previous abortion record) as well as abortions in the progeny of previously aborted cows were also reported. Some calves born in the herd showed neurological clinical symptoms.

Sampling and diagnostic procedures
In September 1999, blood samples were collected by tail venipuncture from the 119 breeding cows, and reproductive data was collected. Serum from each cow was separated and stored at −20°C until tested. Fifteen foetuses from among the 23 abortions that occurred between March and September of 1999 were submitted to the authors’ laboratory for histopathological and immunohistochemical analysis. Identification of the dam and the gestational age of the foetus were recorded.

All sera were assayed for anti-N. caninum antibodies using an enzyme-linked immunosorbent assay (ELISA; HerdCheck Anti-Neospora; IDEXX Laboratories, Inc., Westbrook, Maine, USA) with a cut-off value of ≥ 0.5 OD0.20.

Table 1. Prevalence of abortions between 1996 and 1999

<table>
<thead>
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<th>Year</th>
<th>1996</th>
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<td>% abortion (no. adult cows)</td>
<td>14 (80)</td>
<td>16 (90)</td>
<td>18 (90)</td>
<td>27 (119)</td>
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Fig. 1. Photomicrographs of sections of brain of aborted foetuses.  
(a) Cluster of intensely positive tachyzoites. Immunoperoxidase, stained with anti-*N. caninum* antiserum, formalin-fixed tissue section; magnification, 100x.  
(b) Tissue cyst stained with anti-*N. caninum* antiserum (Immunoperoxidase), formalin-fixed tissue section; magnification, 100x.  
(c) A large group of bradyzoites. Immunoperoxidase stain of formalin-fixed section; magnification, 100x.
The 15 aborted foetuses were necropsied and histological examination of haematoxylin and eosin-stained sections of tissues of the brain, heart, liver and skeletal muscle were performed. Additional sections from brain and heart with histological lesions suggestive of protozoa infections (non-suppurative encephalitis, myocarditis) were also examined using anti-N. caninum rabbit antisera on paraflin-embedded sections (Barr et al. 1991).

Results

The serological results showed that 51% of the 119 cows sampled were seronegative and 49% were seropositive to N. caninum. Sixty-six per cent of the cows with abortion history were seropositive to Neospora and 64% had aborted for the second or third time. Ten of the 50 cows with abortion history were progeny of cows that had aborted. Of the cows without abortion history, 38% were seropositive to N. caninum.

Histological examination of heart and liver sections revealed focal areas of necrosis in only 35% of the foetuses. Immunohistochemical testing of brain tissues revealed Neospora cysts and clusters of bradyzoites in two of the foetuses (Fig. 1).

Discussion

Analysis of the data on abortion history, clinical signs on neonatal calves, together with preliminary serologic evaluation, strongly suggested infection with Neospora as the main cause of the abortions. Most of the abortions occurred between 3 and 6 months of gestation, which is in agreement with the findings of Thurmond and Hietala (1997b), who concluded from a cohort study of congenitally infected cows that the highest risk for N. caninum abortion is between 90 and 180 days of gestation.

The presence of specific antibodies in serum is only indicative that the cows had been exposed to Neospora. Analysis of seroprevalence in this herd indicated that for the (specific) cut-off value used, a high percentage (66%) of cows with abortion history were seropositive to N. caninum, whereas only 38% of non-abortion cows were seropositive. This is in agreement with data from Thurmond and Hietala (1997a) showing that cows with N. caninum antibodies are more likely to abort than seronegative cows.

The examination of aborted foetuses is of major importance in establishing a conclusive diagnosis, which can be achieved by histopathological examination and immunocytochemistry of tissues (Dubey 1999). In this study, typical lesions attributed to Neospora infection were evident in five of the 15 submitted and analysed foetuses. The immunohistochemical analysis of the five foetuses positively demonstrated N. caninum cysts and bradyzoite clusters in tissues of two of the aborted foetuses (Fig. 1). These results were expected because infected tissues often have very few organisms. The existence (in this herd) of cows that had aborted more than once, 64% (32 of a total of 50 cows that aborted), and the progeny of aborting cows that also aborted, 20% (10 of 50), supports the results by Anderson et al. (1997) indicating that congenitally infected cows remain infected and also infect their offspring, which therefore carry an increased risk of abortion. The laboratory results obtained in this study, coupled with the information on abortion patterns and clinical signs in newborn calves, are strongly suggestive of a Neospora-associated abortion outbreak.

To the authors’ knowledge this is the first report that implicates N. caninum as a cause of bovine abortions in Portugal. There have been speculations on Neospora-induced abortions in northern Portugal, solely based on serology by ELISA. Further studies are needed to determine the prevalence of this disease in the country and to assess the degree to which neosporosis contributes to economic losses in the dairy farming sector.

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References


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