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Reproductive Losses due to *Neospora caninum* in a Beef Herd in Argentina

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Summary

Reproductive losses in a beef herd of 857 heifers with a pregnancy rate of 86.3% are described. After pregnancy testing, 69 abortions were seen during a 3 month period. Before calving season, three heifers had delivered pre-mature non-viable calves. Serum samples from 58 of 69 aborted heifers were available for serological tests. In order to compare the seroprevalence in non-aborted vs. aborted heifers, 214 pregnant animals were bleed during the abortion storm. In addition, blood samples were collected from two heifers with pre-mature calves and from 16 heifers with their calves prior to colostrum intake. All available serum samples were tested for *Neospora caninum* antibodies using an indirect fluorescence antibody test (IFAT). Fifty-nine of 290 (20.3%) evaluated heifers were seropositive. Heifers that aborted and heifers with pre-mature calves were more likely to be seropositive than pregnant heifers and heifers with normal calves [odds ratio (OR), 12.01; 95% CI, 6.18–23.30]. Vaginal mucus from four aborted heifers, and samples from two aborted foetuses and three pre-mature calves were available. Laboratory tests for *Tritrichomonas foetus*, bacterial and viral isolation, and histological examination were performed. Culture from vaginal mucus and foetal samples were negative. Histological lesions consistent with neosporosis and positive immunohistochemistry (IHC) to *N. caninum* were found in one aborted foetus and in one pre-mature calf. It is the first description of reproductive losses because of *N. caninum* in beef herds in Argentina.

Introduction

The protozoon *Neospora caninum* is recognized worldwide as cause of abortion in cattle (Dubey, 1999). Several reports of abortions have been widely documented in dairy herds (Dubey, 1999; Anderson et al., 2000). *Neospora caninum* abortion was also recognized in beef cattle in Australia (Boulton et al., 1995), Belgium (De Meerschman et al., 2002), Canada (Bryan et al., 1994; McIntosh and Haines, 1994; Hoar et al., 1996; Waldner et al., 1998, 1999, 2001), and the USA (McAllister et al., 2000). Nevertheless, neosporosis outbreaks in beef herds are not reported as commonly as in dairy herds (Waldner et al., 1998, 1999, 2001; McAllister et al., 2000).

Argentina has an important beef cattle industry and reproductive failure because of infectious diseases produces important economic losses (Campero et al., 1994). *Neospora caninum* infection has been identified in beef and dairy herds of Argentina in the past decade (Venturini et al., 1995, 1999; Campero et al., 1998). In addition, *N. caninum* was involved as a cause of abortions in dairy cattle (Campero et al., 1998).

Recently, Moore et al. (2002) have described that the prevalence of *N. caninum* in beef cattle was lower than in dairy cattle in Argentina. The disease was also confirmed by immunohistochemistry (IHC) in sporadic aborted beef foetuses submitted for diagnosis purposes (Moore et al., 2002). However, the presence of *N. caninum* in a single aborted foetus simply may be a reflection of infection in the herd (Thurmond and Hietala, 1995). Therefore, abortion storms caused by *N. caninum* have not been reported in Argentinean beef herds yet. The objective of the present work was to describe reproductive losses associated with *N. caninum* infection in a beef herd where a high abortion rate occurred.

Materials and Methods

Cattle and herd management

The farm had approximately 4000 head of cattle housed on 2000 ha of pasture. The heifers involved in this study were born during the winter of 1999 and raised under extensive grazing conditions on cultured pastures. Animals were bought as weanings from four different herds located in the breeding area of the Buenos Aires province. Reproductive losses were observed on 857 Aberdeen Angus cross-breed heifers during the autumn of 2001. For that time, the stock present in the farm was constituted by weanings, yearling steers and open heifers.

Animal nutrition management during the pre-breeding period was adequate to reach 65% of the expected mature weight and a good body condition by the time of the first mating (15 months of age). Heifers were grazing on pasture with a stocking rate of two heads per hectare. During the breeding time to the end of calving season, all heifers maintained an adequate to good body condition (score 6).

Clinical and genital tract examination, body condition and pelvic area measurements and vaccination against bovine campylobacteriosis were performed on the heifers 1 month before breeding time. Breeding was carried out by artificial insemination (AI) with daily heat detection during 2 months (November and December, 2000) and by natural service for 1 month with bulls (January, 2001). Five months after the beginning of the breeding season, pregnancy testing was performed by rectal examination. The herd was under a brucellosis control programme (seroprevalence of 0.8%). No cattle-working dogs were present in the property.

Table 1. Protocol sampling for herd of beef cattle with an outbreak of abortions during the autumn of 2001

Months	Events	Available specimens for analysis	Control measures
March	Pregnancy test: 111 open heifers and six heifers with mummified foetuses	None	Culling
April to June	Sixty-nine abortions were recorded	Necropsy: two aborted foetus; vaginal mucus from four aborted heifers; sera from 58 aborted heifers and 214 pregnant heifers	Heifers that had aborted were culled
July	Four pre-mature calves were delivered. Only one of them was viable	Three pre-mature dead calves and serum from two dams	Three dams were culled
July to September	Calving season	Blood samples were collected from 16 dams and their calves before colostrum intake	None

Abortion was defined as foetal loss occurred between 42 and 260 days of gestation, pre-mature deliveries as those occurred in the period from 260 days to term, and stillbirth as those deaths occurred during birth (Hubbert, 1972). Foetal age was estimated from the crown-rump length (Hubbert, 1972) or, when available, from AI or breeding data.

Sampling and laboratory procedures

All specimens and samples were processed by Veterinary Diagnostic Laboratories at INTA, Balcarce. Due to extensive management conditions, only some animals or specimens were involved in the study. Animals that had aborted were identified and examined by private veterinarians.

Serum samples were obtained from aborted heifers between 15 and 90 days after the first abortion. In order to compare if the proportion of seropositivity in aborted heifers was larger than the proportion in non-aborted heifers, at least an equal number of non-aborted heifers as aborted heifers were bleed as was mentioned by Thurmond and Hietala (1995). Therefore, 214 pregnant heifers were randomly sampled 4 weeks after the first abortion according to availability and usefulness of the herd owner. The total number of animals in the herd, specimens available for sampling, and control management are shown in the Table 1.

Thoracic fluid samples were also obtained from foetuses or pre-mature calves when it was possible. Pre-mature calves were easily recognized by referring to AI breeding records.

Vaginal mucus from four aborted heifers, and samples from two aborted foetuses and three pre-mature calves including abomasal content, lung and spleen were cultured for *Tritrichomonas foetus*, bacteria and viruses (Campero et al., 1986, 1994). Isolation procedures for fungi, *Leptospira* sp., *Mycoplasma*, and *Chlamydia* were not performed.

Tissue samples (brain cortex, midbrain, medulla and cerebellum, heart, lung and liver) from aborted foetuses and pre-mature calves were taken for histological examination. Tissues were fixed in 10% buffered neutral formalin, embedded in paraffin and sectioned at 4 μm, stained with haematoxylin and eosin (H & E), and examined by light microscopy (Olympus Optical, Japan). A commercially available kit system based on avidin–biotin complex was used for IHC procedure (ABC Vector Laboratories, Burlingame, CA, USA) (Lindsay and Dubey, 1989) in those specimens with histological lesions of non-suppurative necrotizing meningoencephalitis compatible with *N. caninum* as was suggested (Anderson et al., 2000).

Sixteen heifers required assistance during calving. When the dams were restrained, blood samples were obtained. Their calves were also bled prior to colostrum intake.

Serological tests

Serum samples were tested for *N. caninum*-specific IgG by using an indirect fluorescence antibody test (IFAT) on multiwell slides coated with *N. caninum*-infected Vero cells (Dubey et al., 1988). Fluids from aborted foetuses and pre-mature calves were also tested by IFAT. A serological titre of $\geq 1:200$ and $\geq 1:25$ were considered as positive in serum samples and fluids, respectively (Reichel and Drake, 1996; Wouda et al., 1997). In addition, serological dilutions were examined up to 1 : 3200. The IFAT was considered to be positive when typical peripheral staining pattern of the tachyzoites was observed.

Serological tests for *Brucella abortus*, bovine viral diarrhoea virus (BVDV), and bovine herpes virus type 1 (BHV-1) were performed on sera of aborted heifers. Paired serum samples were collected for antibodies to BVDV and BHV-1 with an interval of 21 days during the outbreak of abortions. Antibodies to *B. abortus* were identified by plate agglutination (Alton et al., 1988). Sera were heat-inactivated at 56°C for 60 min and 2-fold dilutions were tested for end-point neutralizing antibodies to National Animal Disease Laboratory (NADL; USA) reference isolate of BVDV and LA (Los Angeles) reference strain of BHV-1 using a standard microtitration procedure (Rossi and Kiesel, 1971).

Statistical analysis

The association between serological status and reproductive performance was determined using odds ratio (OR) (Thurmond and Hietala, 1995). *P*-values ≤ 0.05 were required to demonstrate statistical significance. Data were processed by the use of MED CALC programme (MED CALC, 1993).

Results

Reproductive performance

After pregnancy test was performed (March, 2001), 740 (86.3%) heifers were pregnant, 111 heifers were found open, and six animals had a mummified foetus. All open heifers and females with a mummified foetus were culled. Unfortunately, serum samples were not obtained from these animals.

Table 2. Serological results in heifers from a beef herd with reproductive losses because of neosporosis

Reproductive performance (*n*)	Seropositive (%)	Antibody titre[a]				
		200	400	800	1600	≥3200
Aborted heifers (58)	34 (58.6)	14	11	5	2	2
Heifers with pre-mature calf (2)	1 (50.0)	–	–	1	–	–
Pregnant heifers (214)	22 (10.3)	4	5	5	3	5
Dams with normal calf (16)	2 (12.5)	–	–	–	2	–
Total evaluated heifers (290)	59 (20.3)	18	16	11	7	7

[a]Reciprocal of dilution.

Table 3. Findings in foetal and pre-mature calves in a beef herd with neosporosis

Specimen	Sex	Months of age	Necropsy findings	Antibody titre[a]	Histology	IHC
Foetus	Male	4	SF	100	MNE, E, PH	+
Foetus	Male	5	WAL	(–)	WAL	ND
Pre-mature calf	Female	8	SO	800	MNE, E, PH	+
Pre-mature calf	Male	8	HF	(–)	WAL	ND
Pre-mature calf	Male	8	WAL	(–)	WAL	ND

[a]Reciprocal of dilution; SF, presence of serohaemorrhagic fluid in cavities; MNE, multifocal necrotizing encephalitis; E, epicarditis; PH, periportal hepatitis; WAL, without apparent lesion; ND, not done; SO, subcutaneous oedema; HF, hyperflexion of hind and forelimbs.

In a 3 month period after pregnancy examination, 69 heifers aborted (9.3%). Before the beginning of the calving season, four heifers had calved prematurely, but only one calf was viable. Aborted heifers and three pre-mature dams were also culled (Table 1). Therefore, a total of 72 pregnancy losses (9.7%) were recorded. There were also 39 stillbirths, however, these were associated with dystocia according to private practitioners. Unfortunately, serum samples for analysis were not available from stillbirths and their dams.

Serology

Reproductive performance and serological status are shown in Table 2. Serological status was associated with reproductive performance; aborted heifers, and heifers with pre-mature calves were more likely to be seropositive than heifers that had normal calves (OR, 12.01; 95% CI, 6.18–23.30). On the contrary, two of 16 calves bled prior to colostrum intake had antibodies to *N. caninum* (dilution titre: ≥1 : 3200); both of these calves had seropositive dams.

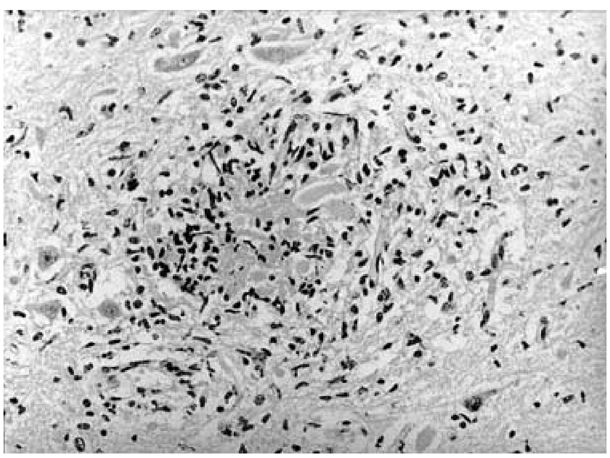

Fig. 1. Photomicrographs of section of brain from a bovine foetus with necrotizing encephalitis characterized by mononuclear cell infiltration and glial proliferation. Haematoxylin and eosin stain, 200×.

Serological test to *B. abortus* was negative in all the aborted heifers. Similarly, there were no substantial changes in the BVDV, and BHV-1 antibodies titres.

Laboratory examination

Findings in foetal and pre-mature calves are presented in Table 3. All vaginal mucus and foetal specimens were negative for *T. foetus*, and bacterial and viral organisms. Histological examination of both an aborted foetus and a pre-mature calf revealed epicarditis, periportal hepatitis and multifocal necrotizing encephalitis with mononuclear cell infiltration and glial proliferation (Fig. 1 and Table 3). No protozoal organisms were seen in routine H & E stains. However, in the central

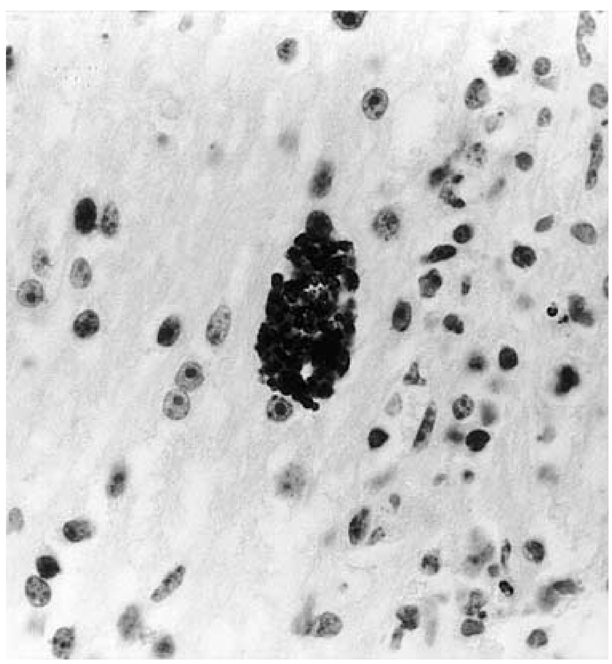

Fig. 2. Clusters of *Neospora caninum* tachyzoites detected by immunohistochemistry (IHC) in the brain from a bovine foetus. 400×.

nervous system of those specimens with multifocal necrotizing encephalitis, *N. caninum* tachyzoites were detected by IHC (Fig. 2).

Discussion

In the present study, evidence is given of reproductive losses because of *N. caninum* in a beef herd in Argentina. The expected reproductive losses during pregnancy and parturition in local beef herds with controlled animal health and nutritional programmes were estimated between 3.1 and 5.4% (Campero, 1998). The diagnosis of bovine neosporosis was based on positive serology, the presence of microscopic lesions consistent with the disease in aborted or pre-mature calves, the presence of tachyzoites of *N. caninum* revealed by IHC, and the absence of other infectious agents (Bryan et al., 1994; Anderson et al., 2000).

The reproductive losses found in this study could be associated with a recrudescence of chronic infection (Anderson et al., 1994; Agerholm et al., 1997) or primary infection (McAllister et al., 2000). To discriminate between recent and chronic *Neospora*-infections an avidity ISCOM-ELISA is available (Björkman et al., 1999). However, a low antibody avidity was not necessarily associated with recent *Neospora*-infection and low avidities could be only an indicator of increasing abortion risk (Sager et al., 2003).

The prevalence found in all evaluated heifers (20.3%) was lower than the 80% mentioned by others (Waldner et al., 1999; McAllister et al., 2000). In addition, the percentage of abortions because of *N. caninum* reported in this study was lower than the cases mentioned by others in beef herds (Hoar et al., 1996; Waldner et al., 1998, 1999, 2001; McAllister et al., 2000). Bovine exposure to oocysts of *N. caninum* may occur more frequently in intensive beef and dairy production systems (McAllister et al., 1996, 2000). In Argentina, beef herds are raised under extensive conditions. However, changes of management to intensive production systems might increase the prevalence of *N. caninum* in beef herds. This circumstance was shown in Texas, USA, where the seroprevalence to *N. caninum* in beef calves was increased when stocking rate was more than a cow/calf unit per 2.2 ha (Barling et al., 2001). The high stocking rate (two heifers per hectare) maintained from breeding to the end of calving season in the present study might have contributed to a common point-source exposure which produced reproductive losses because of neosporosis.

The passage of protozoa through the placenta from the infected dam is a well known mode of natural infection of neosporosis in cattle (Dubey, 1999). The facts that both an aborted foetus and a pre-mature calf were positive by IHC, and two calves bled prior to colostrum intake were seropositive by IFAT, are evidence of transplacental transmission.

Several economic losses because of *N. caninum* have been identified in the beef industry, including increased risk of foetal loss by abortion or cows being culled for reproductive failure (Waldner et al., 1998). In addition, low post-weaning weight gain and poor carcass quality were associated with seropositive beef calves (Barling et al., 2000). Moreover, different seroepidemiological studies have demonstrated a high seroprevalence to *N. caninum* in beef livestock. *Neospora caninum* seroprevalence in 2585 cows of 55 beef herds in the north-western USA was 24%, and all herds presented at less one seropositive animal (Sanderson et al., 2000). Another study in beef cattle in Spain showed that *N. caninum* seroprevalence was 17.9 and 55.1% of herds were positive (Quintanilla-Gozalo et al., 1999). The mentioned studies and the results obtained here indicate the importance of neosporosis in beef cattle as a factor of economic losses and the need for control programmes.

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