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Seroepidemiology of beef and dairy herds and fetal study of *Neospora caninum* in Argentina

Dadín Prando Moore [a], Carlos Manuel Campero [b,*], Anselmo Carlos Odeón [b], María Adela Posso [b], Dora Cano [b], María Rosa Leunda [b], Walter Basso [c], María Cecilia Venturini [c], Ernesto Späth [c]

[a] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[b] *Estación Experimental Agropecuaria, Patología Veterinaria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 276, 7620 Balcarce, Argentina*
[c] *Facultad de Ciencias Veterinarias de la Universidad Nacional de La Plata (UNLP), 1900 La Plata, Argentina*

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Abstract

The purpose of the present work was to study the epidemiology of *Neospora caninum* in beef and dairy herds in the Humid Pampas of Argentina. The seroprevalence of *N. caninum* was evaluated in 2414 serum samples of cows from beef and dairy farms. An indirect fluorescent antibody test (IFAT) was used to determine specific antibodies. The sera was screened at a dilution ≥1:200 and ≥1:600 in cows with reproductive disease antecedents and without them, respectively. Cows without history of reproductive diseases from nine beef and fifteen dairy farms were grouped according to the percentage (> or ≤ to 50%) of seropositive dogs. Additionally, the seroprevalence in beef and dairy cattle cohabiting in the same farm with these dogs was compared. Microscopic studies were performed in 188 aborted fetuses and/or their placentas. Formalin-fixed fetal tissues with microscopic lesions compatible with *N. caninum* were processed by immunohistochemistry (IHC). The seroprevalence in cows without reproductive diseases was 4.7% (19/400) for beef cattle and 16.6% (174/1048) for dairy cattle. The seroprevalence of *N. caninum* in dairy cattle was higher ($P < 0.05$) in farms grouped according to the percentage (> or ≤ to 50%) of seropositive dogs. The analysis of 966 serum samples from aborted cows, demonstrated positive 18.9% (41/216) and 43.1% (323/750) from beef and dairy herds, respectively. Microscopic lesions compatible with *N. caninum* were observed in 43 of 188 (22.8%) fetuses and/or placentas evaluated. The protozoan was identified in 29 of 43 (67.4%) aborted specimens, being the largest number of positive results

* Corresponding author. Tel.: +54-2266-439100; fax: +54-2266-439101.
E-mail address: ccampero@balcarce.inta.gov.ar (C.M. Campero).

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in dairy fetuses. The results obtained demonstrate a high association between neosporosis and dairy herds, however, our data also reveals that *N. caninum* is an important risk factor for reproductive losses in the extensively farmed beef cattle in the Humid Pampas of Argentina.

Keywords: Epidemiology; Cattle-protozoa; *Neospora caninum*

1. Introduction

Neospora caninum is a protozoon that causes abortions and economic losses in cattle worldwide (Dubey, 1999a; Trees et al., 1999; Anderson et al., 2000). In cattle, transplacental transmission is the main mechanism by which the parasite persists in a herd, (Anderson et al., 1997, 2000). After recognizing the dog as the definitive host of the parasite (McAllister et al., 1998), epidemiological work established the association between the presence of dogs and the disease in cattle (Wouda et al., 1999). Additionally, the association of canids with cattle on their premises, has been postulated as a risk factor for the disease (Paré et al., 1998; McAllister et al., 2000). Similarly, it was established that intensive herd management was associated with increased seroprevalence to *N. caninum* (Sanderson et al., 2000). The presence of wild canids and have also been related with high prevalence of seroreactive cattle (Barling et al., 2000).

In Argentina, beef and dairy herds are managed in different production systems. Beef cattle are usually raised in extensive grazing systems (stocking rate, 0.8 head/ha), whereas, dairies are intensively exploited (stocking rate 1.5 head/ha) (Carrillo and Schiersmann, 1992; Buelink et al., 1996).

Although, in Argentina the disease was identified by serology in aborted cows (Venturini et al., 1995), and its presence was later confirmed by immunohistochemistry (IHC) in aborted bovine fetuses (Campero et al., 1998), its importance as a cause of abortions in cattle in Argentina has not been established yet.

Considering, the differences in management between these production systems, the objectives of this work were to characterize the epidemiological situation of neosporosis in beef and dairy herds, and to establish the importance of *N. caninum* as cause of abortion.

2. Materials and methods

2.1. Seroepidemiological study

2.1.1. Animals and samples from beef and dairy farms

The animals evaluated were located in the humid pampas, the main agricultural and cattle area of Argentina that embraces the provinces of Buenos Aires, north of the Río Negro, east of La Pampa, south of Santa Fe, Córdoba, and south of Entre Ríos. Within this region is the depressed basin of the Salado river with an approximate area of 7 million ha. In this basin, cattle raising constitutes the most important agricultural activity with 5 million cattle managed under pastoral systems (Dillon et al., 2000). In the humid pampas there are also important dairy areas: Abasto Norte, Abasto Sur, Mar y Sierra, Pampa and south of Córdoba and Santa Fe. The Abasto Norte and Sur area surround the Federal District and have 214 525 cows under production. The Mar y Sierras area is located to the southeast of the province of Buenos Aires and has 66 699 cows. The Oeste basin embraces the northwest of the province of Buenos Aires and has 316500 head of cattle approximately. The Pampa basin is located to the northeast of the province with the same name and has 10763 cows. Finally, it is estimated that in the south of Córdoba and Santa Fe there are 166 285 head of cattle (Buelink et al., 1996).

Serum samples were obtained with the support of producers, private veterinarians, regional research institutes and specimens sent to the Service of Specialized Veterinary Diagnosis (SSVD) of INTA Balcarce.

One thousand four hundred and forty eight multiparous bovine females without a history of abortions belonging to 69 commercial beef and dairy farms were evaluated. Of these, 400 animals belonged to 17 commercial beef farms located in the humid pampas (minimum: 16, maximum: 35 animals per farm). The remaining 1048 animals of similar characteristics belonged to 52 commercial farms located in the Abasto Norte area (1 farm), Mar y Sierras area (26 farms), Oeste area (17 farms), Pampa area (1 farm) and south of Córdoba and Santa Fe (7 farms; minimum: 14, maximum: 33 animals per farm).

Nine of the 17 beef farms and 15 of 52 dairy farms mentioned previously, were grouped according to the percentage ($>$ or $\leq$ to 50%) of seropositive dogs detected by the indirect fluorescent antibody test (IFAT) at a dilution of 1:50 (Barber et al., 1997). The serological results to *N. caninum* of 103 dogs belonging to 9 of the 17 beef farms and to 15 of 52 dairy farms mentioned previously correspond to those published by Basso et al. (2001). In this analysis the seroprevalence in beef and dairy cattle cohabiting in the same farm with these dogs was compared.

2.1.2. Females with reproductive losses

Nine hundred and sixty-six samples of blood serum belonging to aborted bovine females were obtained, with the age of the dam and month of gestation at the moment of the reproductive loss recorded. Two hundred and sixteen of 966 samples (22.3%) were obtained from beef cattle belonging to 39 farms located in the province of Buenos Aires (34), Córdoba (2), La Pampa (2) and Río Negro (1). The number of samples varied from 1 to 15 (mean 5.4 ± 0.7). Seven hundred and fifty of 966 samples (77.6%) belonged to 49 commercial dairy farms located in the province of Buenos Aires (38), Santa Fe (3), La Pampa (3), Córdoba (2), Río Negro (2), and Jujuy (1). The number of animals per herd varied from 1 to 172 (mean 15.3 ± 4.3). Sera were collected and stored at $-20\,^{\circ}$C until analysis.

2.1.3. Serological test

IFAT was used as previously described (Dubey et al., 1988) using in all cases positive and negative control sera. Slides were examined with an epifluorescence microscope (Nikon Fluophot, 40×1.3). A serological titer of $\geq$1:200 in cows without history of reproductive disease (Reichel and Drake, 1996) and a titer of $\geq$1:600 in aborted or open cows (Paré et al., 1995) were considered positive.

2.2. *Exam of fetuses and placentas*

2.2.1. *Antecedents, necropsy, and collection of samples*

Two hundred and forty aborted specimens processed by the SSVD, INTA Balcarce, from 1997 to 2000 were analyzed. The specimens were submitted from 130 farms located in the province of Buenos Aires (116), La Pampa (6), Santa Fe (3), Córdoba (2), Entre Ríos (1), Río Negro (1), and Jujuy (1). Necropsy of the aborted fetuses and/or macroscopical analysis of the organs sent were made, identifying lesions. Simultaneously, samples of lung, spleen and abomasal fluid were collected for microbiological and virological studies to establish the etiology (Campero et al., 1994). Also, samples of different organs (CNS, heart, kidney, lung, liver, suprarenal glands, spleen, thymus, skeletal muscle) were obtained and were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned, mounted, and routinely stained with hematoxylin and eosin (H&E).

2.2.2. *Serological test*

The thoracic–abdominal fluid of the aborted fetuses was analyzed by IFAT. A titer of ≥1:25 (Wouda et al., 1997a) was considered positive. In mummified fetuses, where the fluid was not available, several washes of both cavities with 10 ml of sterile physiologic solution were made.

2.2.3. *Microscopic analysis and immunohistochemistry*

Routine microscopic evaluation of H&E stained fetal tissues was made. The tissues of fetuses and placentas fixed in 10% buffered formaldehyde with lesions of non-suppurative multifocal necrotizing meningoencephalitis, myocarditis, hepatitis, and placentitis were processed by the technique of Avidin Biotin Complex (Vector, Peroxidasa Elite ABC PK-601) (Lindsay and Dubey, 1989).

In IHC positive cases the severity of the lesions of fetal organs was analyzed (CNS, liver, heart, lung, striated muscle, periorbital muscle, adrenal gland, kidney, small and/or large intestine, spleen, and placenta). The CNS was arbitrarily divided into three regions: 1 (cervical cord, spinal bulb, cerebral protuberance and peduncles), 2 (frontal and occipital cortex), 3 (cerebellum). Similarly, were established 4 degrees of lesions according to the severity: 0 (absence of lesion), 1 (less than 3 focuses of hemorrhage or gliosis), 2 (presence more than 3 focuses of hemorrhage or gliosis and light non-suppurative meningitis) and finally, 3 (with presence inflammatory infiltrate and necrosis).

2.3. *Analysis of data*

Bovine herds where at least one seropositive animal to *N. caninum* was detected were considered positive for the seroepidemiological analysis. The analysis of data was carried out by the t and χ^2 tests.

In those cases where the serum of the aborted female and its fetus was obtained, as well as in those cases where fluid and fetal organs were obtained agreement tests were performed.

Statistical analysis for histopathologic lesions was performed by Kruskal–Wallis' test for non-parametric data and means were analyzed by Duncan's test.

Agreement between maternal and fetal results was carried out by concordance test and kappa statistic was performed (Thrusfield, 1995). The statistical programs SAS/STAT (SAS, 1987) and Win Episcope (Win Episcope, 2000) were used. For all the tests, a 5% significance level was used.

3. Results

3.1. Seroepidemiological study

3.1.1. Seroepidemiology in cattle from beef and dairy farms

Antibodies to *N. caninum* were detected in 13.3% of 1448 bovine females without antecedents of reproductive disease. Considering beef and dairy farms, the proportion of seropositive dairy cattle was higher (16.6% of 1048) than seropositive beef cattle (4.7% of 400; $P = 0.001$; Table 1). On the other hand, 52.9% of 17 beef herds and 92.3% of 52 dairy herds were positive (Table 1). Nine of the 17 beef farms and 15 of the 52 dairy farms were grouped according to the percentage of seropositive dogs. In such farms, seroprevalence in beef and dairy cattle had significant differences ($P < 0.05$) according to the percentage of seropositive dogs present (Table 2).

3.1.2. Females with reproductive losses

When analyzing 216 serum samples from aborted beef cattle, 18.9% were positive to *N. caninum*. Also, 51.2% of 39 farms had at least one animal seropositive to the disease (Table 3). In dairy females with similar reproductive histories, 43.1% of 750 were positive to *N. caninum*, whereas 91.8% of 49 dairies evaluated were positive (Table 3). The serological findings and origin of aborted females had positive statistical associations, with a higher

Table 1
Serology of beef and dairy herds without *N. caninum* antecedents (IFAT ≥1:200)

	Herds (n)	Positive (%)	Cow sera (n)	Positive (%)
Beef	17	52.9	400	4.7*
Dairy	52	92.3	1048	16.6*
Total	69	82.6	1448	13.3

* $P = 0.001$.

Table 2
Seroprevalence to *N. caninum* in 9 beef herds and 15 dairy herds grouped at random according to the percentage (≤ or > to 50%) of seropositive dogs present in each herd

Percentage of seropositive dogs	Beef cattle		Dairy cattle	
	Seropositive (%)*	Herds (n)	Seropositive (%)	Herds (n)
≤ 50	6.5 ± 4.4	3	20.1 ± 4.1	7
> 50	6.7 ± 2.5	6	23.0 ± 3.2	8

* Average percentage ± S.E.

Table 3
Beef and dairy herds with seropositive *N. caninum* aborted cows (IFAT ≥1:600)

	Herds (*n*)	Positive (%)	Cow sera (*n*)	Positive (%)
Beef	39	51.2	216	18.9*
Dairy	49	91.8	750	43.1*
Total	88	73.8	966	37.7

* $P = 0.001$.

proportion of dairy seroreactive cows ($P = 0.001$; Table 3). The mean age in seropositive aborted cows was 4.75 ± 0.24 and 3.56 ± 0.15 years for beef and dairy cattle, respectively. The month of gestation at the moment of abortion was of 6.41 ± 0.43 and 4.85 ± 0.18 months in dairy cows and beef cows, respectively.

3.2. Examination of aborted fetuses and placentas

3.2.1. Disease histories and necropsy

Of 240 fetuses processed, 134 (55.8%) were obtained from 87 beef herds and 96 (40.0%) from 38 dairy herds. In 10 fetuses from 10 herds, information was not available. Of 240 abortion cases, 86 (35.8%) were females; 90 (37.5%) were males; sex was not determined in 59 cases and in 5 cases only placentas were sent. The mean gestational age at the moment of abortion was of 6.9 months $\pm$ 0.12. The mean weight of the fetuses was 13.3 ± 0.8 kg and the length 60.7 ± 1.8 cm. The mean gestational age showed significant differences ($P < 0.05$) between fetuses from beef herds and fetuses from dairy herds, being 7.6 ± 0.1 and 6.1 ± 0.2 months, respectively. Fetuses with moderate to severe degree of autolysis were processed. The macroscopic lesions observed were scarce.

Eight (3.3%) abortion cases corresponded to mummified fetuses from 3 dairy herds. The mean gestational age of these fetuses was of 4.5 months (range 1.5–7 months).

3.2.2. Serology to N. caninum

Fluid from cavities was obtained from 122 (50.8%) fetuses to determine the presence of *N. caninum* specific antibodies. Thirty specimens (24.6%) were seropositive, 12 and 18 from beef and dairy fetuses, respectively. There were significant differences in the number of fetuses seropositive when their origin was considerate ($P = 0.001$).

3.2.3. Histopathology and immunohistochemistry

Histopathological studies were done in 188 of the 240 (78.3%) specimens. In 155 (82.4%) there were lesions compatible with an infectious cause (data not shown). Additionally, in 43 of the 155 (27.7%) fetuses and/or placentas there were microscopic lesions of non-suppurative multifocal necrotizing meningoencephalitis, epicarditis, (Figs. 1 and 2) myocarditis, hepatitis, myositis, and placentitis. Whereas 34 of 72 (47.2%) fetuses from dairy herds had histopathological lesions compatible with *N. caninum*, only 9 of 83 (10.8%) fetuses from beef herds showed these lesions ($P = 0.001$).

N. caninum was identified by IHC in 29 of 240 fetuses (12.1%), also, IHC detected positive fetuses in 67.4% of the 43 aborted fetuses with compatible histopathological lesions. In the

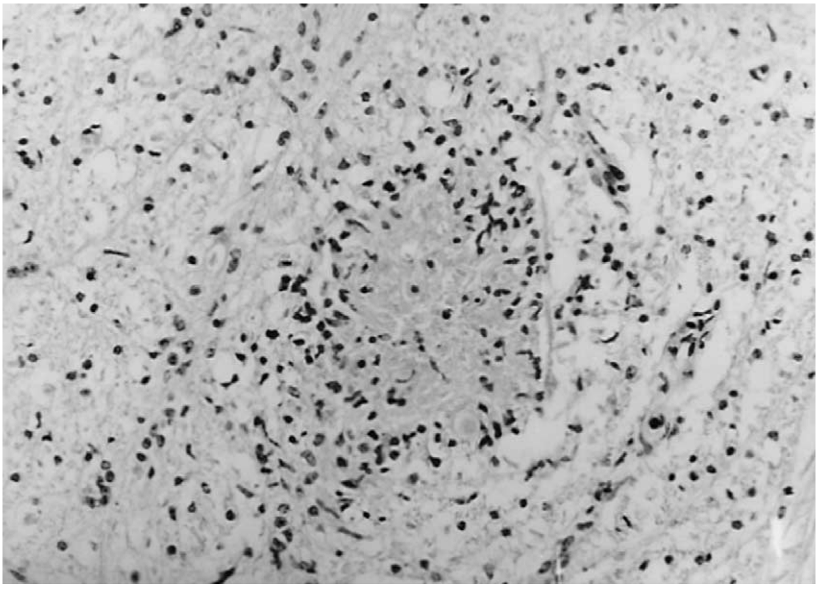

Fig. 1. Photomicrographs of focus of central necrosis surrounded by mononuclear inflammatory cells and glial cells in sections of brain from naturally aborted fetus to *N. caninum* (H&E stain).

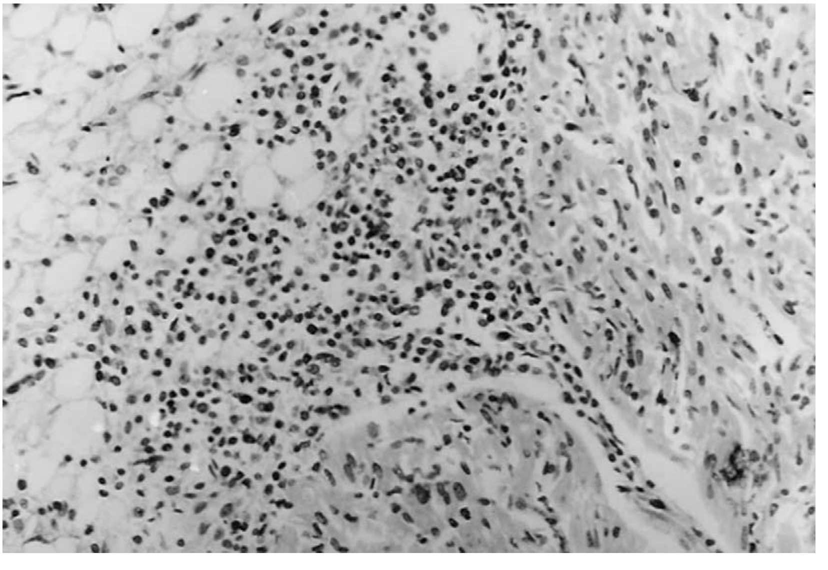

Fig. 2. Photomicrographs of epycarditis with mononuclear inflammatory cells from naturally aborted fetus to *N. caninum* (H&E stain).

Table 4
Beef and dairy herds with aborted fetuses positives to *N. caninum* by IHC

	Herds (*n*)	Total	Percentage
Beef	6	87	6.9*
Dairy	15	38	39.5*
Total	21	125	16.8

* $P = 0.001$.

CNS, tachyzoites associated to focal necrosis and gliosis were identified. These parasitic stages were also observed grouped in form of clusters among the myocardioocytes or in the placenta. *N. caninum* cysts identified by IHC in 5 of 29 (17.2%) aborted fetuses and were located in the CNS not associated with any inflammatory reaction. Considering the origin of the bovine aborted fetuses, the proportion of positive dairy specimens (23.9% of 96) was higher than positive beef fetuses (4.5% of 134; $P = 0.001$). Fetuses aborted due to *N. caninum* were identified in 6 of 87 (6.8%) in and 15 of 38 (39.4%) beef and dairy farms, respectively ($P = 0.001$; Table 4).

In the 29 specimens positive by IHC the severity of the inflammatory lesions in the organs was analyzed. One hundred and fifty-seven sections were observed, that is CNS (21), liver (18), heart (24), lung (16), striated muscle (17), periorbital muscle (9), adrenal gland (10), kidney (17), small and/or large intestine (8), spleen (12), and placenta (5). Organs more frequently and severely affected were the CNS, liver, heart, and lung ($P = 0.0001$). On the other hand, the adrenal gland, kidney, intestine, and spleen were less affected ($P = 0.0001$). Finally, the organs and/or tissues with intermediate frequency and severity were the striated muscle, periorbital muscle, and placenta ($P = 0.0001$).

It was possible to analyze the localization of the histopathological lesions in the CNS in 21 of the 29 (72.4%) aborted bovine fetuses positive by IHC. Fifty-two histopathological sections were analyzed (region 1: cervical cord, bulb, cerebral protuberance and peduncles (21), region 2: frontal and occipital cortex (17) and region 3: cerebellum (14). Lesions of multifocal necrotizing encephalitis were more frequent and more severe in region 1 ($P = 0.0007$).

3.2.4. *Other diagnoses*

Other possible causes of abortion were determined in 75 (31.3%) cases; in the remaining 131 (54.5%) cases the causal agent was not found. Finally, 5 (2.1%) cases were mummified fetuses without definitive diagnoses (data not shown).

3.2.5. *Mummified fetuses*

In four of eight mummified fetuses viral isolation was negative. It was also possible to analyze the fluid of fetal cavities in two opportunities. In one case there was a neutralizing titer to the DVB virus. Moreover, in five cases bacteriological cultures were negative. Maternal serum was obtained for analysis in six cases. In two cases where the fluid was available, the samples were positive by the IFAT test. The three cases processed by histological and IHC methods were positive to *N. caninum*.

3.2.6. Relationship between maternal and fetal results

In 42 cases of bovine abortion, the relationship between the maternal and fetal serological results to *N. caninum* was moderate ($P < 0.05$). Dam and fetus were seropositive to the serum dilutions used in 12 cases. On the other hand, there were six seronegative fetuses, born to seropositive dams. In three cases dams without *N. caninum* antibodies detectable at the ≥1:600 dilution aborted fetuses with *N. caninum* antibodies and in 21 cases, dams and fetuses were seronegative.

The significance was also statistically moderate ($P < 0.05$) when comparing the serological maternal antibodies and the presence of *N. caninum* compatible lesions in fetal tissues of 49 cases of abortion. In 15 cases the dam was seropositive (titer ≥1:600), showing lesions compatible with *N. caninum* in fetal tissues. The dam was seronegative in 21 cases, without lesions in the fetus. On the other hand, 12 fetuses without lesions due to *N. caninum* were delivered by seropositive dams (titer ≥1:600). Only in one fetus which had lesions of *N. caninum* the dam did not show detectable antibodies at the ≥1:600 dilution.

Concordance between the results of fetal serology and microscopic lesions was moderate in 97 cases of bovine abortion ($P < 0.05$). In 17 opportunities, fetuses were seropositive and they showed lesions due to *N. caninum*. On the other hand, 11 seropositive fetuses did not have histopathological lesions and 7 seronegative fetuses had neosporosis lesions. Finally, 62 fetuses were seronegative without microscopicc lesions.

4. Discussion

Considering the scarce seroepidemiological data on *N. caninum* in herds in Argentina, the results of this study contribute to the characterization of the importance of this disease in herds of this country. Previous data in beef cattle in 1001 calves of 6–12 months of age in 120 beef farms located in seven districts of Corrientes evidenced that the prevalence to *N. caninum* was 16.8%, with 86 (67.5%) herds exposed to the parasite (Moore et al., 2000). The results of the present work confirm the presence of *N. caninum* in national beef herds. However, the percentage of seropositive animals (4.7%) found in this analysis is lower to that described previously for beef herds (Moore et al., 2000).

In this work, the presence of positive cows in 92.3% of the dairies analyzed without antecedents of the disease is significant. Also, the proportion of seropositive cows to *N. caninum* (16.6%) indicates a high prevalence. In coincidence with the data obtained in this study, other authors detected a prevalence of 15–27.5% in 320 dairy cattle of 8 herds of the provinces of Santa Fe and Córdoba, finding seropositive cows in all the herds studied (Echaide et al., 1998).

The seroepidemiologic study in 52 dairy herds located in the Mar y Sierras area, west area and south of Córdoba and Santa Fe allowed to establish a prevalence to *N. caninum* higher than that found in the 17 beef herds of the humid pampas. On the other hand, considering a sensitivity and specificity of 89.7 and 87.85% for the IFAT to serum dilutions between 1:64 and 1:640 (Atkinson et al., 2000), the proportions of seropositive animals to *N. caninum* were lower than those mentioned in beef and dairy herds of Spain, although there the indirect ELISA test was used (sensitivity 100%, specificity 93.8%) (Quintanilla-Gozalo et al., 1999). These authors mentioned that 306/1712 (17.9%) and 402/1121 (35.9%) beef and dairy animals were positive, respectively. Although in the present work, the seropositive percentage was lower in beef cattle and in dairy cattle, the fact of finding higher sero-prevalences to *N. caninum* in dairy herds coincides with the results obtained in the Spanish herds.

The number of beef herds without clinical antecedents of the disease where was detected at least one seropositive animal was lower than that described by Quintanilla-Gozalo et al. (1999) where 55.1% herds were positive. The result of this survey were also lower than those reported by other authors in Canada or in the northwest of US, where the 100% of beef herds were exposed to *N. caninum* (Waldner et al., 1998; Sanderson et al., 2000).

Even though seropositive dogs seems to be a high epidemic risk (Wouda et al., 1999), seronegative dogs would also eliminate oocysts in their feces (McAllister et al., 1998; Lindsay et al., 1999; Dijkstra et al., 2001b). Although, there was a significant lower proportion of seropositive beef cattle than dairy cattle where dogs were allowed in the premises, the proportion of seropositive dogs was statistically similar between the two types of exploitation system (Basso et al., 2001). Even though this finding is difficult to explain with the present knowledge of the disease (Dubey, 1999b), the ethology and carnivorous habits of dogs make this species susceptible regardless the serologic status of the bovine population (Basso et al., 2001; Peters et al., 2001). A fact that may explain differences between dairy and beef farms and dog population is the postnatal exposure (Dijkstra et al., 2001a) from oocysts that contaminate water and food sources of cattle (Dubey, 1999b; Anderson et al., 2000). This is more likely in dairy cattle due to risk factors not yet identified and inherent to intensive management (Paré et al., 1998; McAllister et al., 2000). Also, this possibility of postnatal infection in beef cattle could be diminished when considering the extensive management of beef herds in our country.

Beef cattle raising is carried out extensively in regions of alkaline and flooded soils, achieving forage resources from natural pastures. In the basin of the Salado river, main cattle raising area in the country and place of origin of more than 80% of beef aborted fetuses in this study; 93% of the farms have most of their grazing areas with natural pasture (Dillon et al., 2000). With this production system of beef calves, oral oocyst infection through forage seems unlikely. However, this postnatal exposure in beef cattle could be more frequent if the stocking rate per ha is increased, or if intensive rotating pasturing or strategic supplementations are implemented. These last practices have increased since 1990 in Argentina.

The mean gestational age at the moment of abortion in seropositive beef and dairy cows is in agreement with that previously described in natural cases (Wouda et al., 1998; Dubey, 1999b). Additionally, data presented in this study indicate that 5% of seropositive dairy cows aborted in the first third of gestation (data not shown). Although abortions due to *N. caninum* seem to be more frequent in the second third of the gestation (Anderson et al., 2000), *N. caninum* pathogenicity during the first 3 months of pregnancy is unknown (Williams et al., 2000; Waldner et al., 2001).

Aseptic dehydration is a common finding in bovine fetuses naturally aborted (Moen et al., 1998), or in experimental cases of *N. caninum* (Barr et al., 1994). The finding of mummified fetuses born to dams that were positive by the IFAT in this work, allows to associate with *N. caninum* infection, coinciding with the results of other authors (Barr et al., 1994).

The presence of seropositive fetuses observed in the present study demonstrates that transplacental transmission of *N. caninum* is a common fact. The fact that six IFAT negative fetuses were aborted by seropositive dams suggests an inadequate fetal humoral response (Barr et al., 1994; Anderson et al., 2000).

Although the relationship between microscopic fetal lesions and maternal serology was moderate, in 73.5% of the cases there was coincidence in the parameters evaluated. The circumstance that 12 aborted fetuses born to seropositive dams did not have microscopic lesions it is possibly related to the low sensitivity of the histopathological techniques (Reichel and Drake, 1996) or due to the prevention of fetal infection by the maternal immune response (Paré et al., 1997; Anderson et al., 2000). On the other hand, considering that seropositive dams do not always abort (Stenlund et al., 1999), reproductive losses have not possibly been of infectious origin or of an infectious cause not evaluated (data not shown).

The relationship found between fetal antibodies and microscopic lesions partially coincides with that previously mentioned by other authors (Barr et al., 1995; Wouda et al., 1997a). Barr et al. (1995) have observed only one seropositive fetus, of 64 evaluated without *N. caninum* infection or any other cause of abortion established. In comparison, the results of the present work determined that 11 of 73 fetuses without histopathological lesion compatible with *N. caninum* were positive by IFAT at a dilution of 1:25. Due to the transplacental transmission of *N. caninum*, it is reasonable to assume that fetuses congenitally infected might develop antibodies without presenting tissue damage and born clinically normal (Dubey, 1999b).

The IHC technique could help to clarify diagnoses of possibly infectious etiology. The results of this study revealed that 12.1% of the bovine abortions analyzed were due to *N. caninum*. This finding coincides with those reported by other investigators (Anderson et al., 1991; Otter et al., 1995; Buxton et al., 1997). The use of IHC in those tissues, CNS, heart, and placenta that showed lesions compatible with *N. caninum* (Campero et al., 1998) determined the high efficacy of this technique. This fact is interesting and coincides with that mentioned by other authors (Wouda et al., 1998; Anderson et al., 2000) who describe a good performance of IHC with a previous histopathological study, although its diagnostic value has been questioned (Thurmond et al., 1999). In cases of spontaneous bovine abortion, 100% of 13 cases with regular lesions due to *N. caninum* and positive by IHC were positive by *N. caninum*–PCR, however, PCR–*N. caninum* was equally positive in six of eight cases with typical lesions due to *N. caninum* but negative by IHC (Baszler et al., 1999).

The localization of the histopathological lesions determined by IHC in the positive cases, coincidently with similar studies (Wouda et al., 1997b), indicate that the CNS, liver, and myocardium were the organs more frequently and severely affected. In our study, the CNS sections most commonly affected were diencephalon and mesencephalon. This finding contrasts with data reported by Helman et al. (1998) who described microscopic lesions located in the cerebral cortex. These differences may be explained by alternate tropism for the CNS of regional strains. This hypothesis points out to the need of additional studies to understand the pathogenesis of the bovine abortion caused by *N. caninum*.

In conclusion results of this study showed that *N. caninum* is a frequent cause of abortions in dairy herds in Argentina. The presence of *N. caninum* in beef herds also suggest the potential risk for this type of cattle exploitation system. Epidemiological risk factors such as intensive production system is though to be associated to the transmission and the clinical presentation of the disease in dairy herds. These hypotheses were verified in animals with or without antecedents of reproductive disease and in aborted bovine fetuses.

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