PREVALENCE OF ANTI-*Neospora caninum* ANTIBODIES IN CATTLE FROM THE STATE OF MATO GROSSO DO SUL, BRAZIL[1]

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ABSTRACT: OSHIRO, L.M.; MATOS, M.F.C.; OLIVEIRA, J.M.; MONTEIRO, L.A. R. C.; ANDREOTTI, R. **Prevalence of anti-*Neospora caninum* antibodies in cattle from the state of Mato Grosso do Sul, Brazil**. [Prevalência de anticorpos anti-*Neospora caninum* em rebanhos bovinos de Mato Grosso do Sul, Brasil.] *Revista Brasileira de Parasitologia Veterinária*, v. 16, n. 3, p. 133-138, 2007. Embrapa Beef Cattle, BR 262, km 4, Caixa Postal 154, Campo Grande, MS 79002-970, Brazil. E-mail: andreott@cnpgc.embrapa.br

Neospora caninum is an obligate intracellular parasite that can infect domestic and wild canids, as well as ruminants and equines. It was described in 1988 and has been known as a major cause of abortion in bovines and neuromuscular alterations and death in dogs. To estimate the prevalence of bovine neosporosis in the 22 municipalities of the so-called Estrato 1 subregion of the Brazilian state of Mato Grosso do Sul, blood samples were collected from cows aged 24 months and older, from December 2003 to March 2004. During sample collection, a questionnaire was used to gather data of epidemiological interest. The samples were subjected to serological diagnosis (indirect fluorescence antibody test – IFAT). Prevalences of 14.9% (449/2488) and 69.8% (143/205) were found for the animals and herds sampled, respectively. The variable found to be associated with seropositivity to *N. caninum* was abortion (OR 2.52; CI 1.25-5.06). The results revealed the presence of infection by *N. caninum* in the herds investigated, drawing attention to its role as a potential cause of abortion in cattle in Mato Grosso do Sul.

KEYWORDS: *Neospora caninum*, bovines, indirect fluorescence antibody test, seroprevalence.

RESUMO

Neospora caninum é um parasita intracelular obrigatório que pode infectar canídeos domésticos e selvagens, ruminantes e eqüídeos. Esse parasita foi descrito em 1988 e, desde sua descoberta, tem emergido como uma das principais causas de aborto em bovinos, além de causar alterações neuromusculares e morte em cães. Para estimar a prevalência da neosporose bovina em 22 municípios que compõem a sub-região denominada Estrato 1 do estado de Mato Grosso do Sul, foram analisados soros obtidos no período de dezembro de 2003 a março de 2004 de fêmeas com idade igual ou superior a 24 meses. Durante a colheita das amostras foi preenchido um questionário com informações de interesse epidemiológico. A determinação da presença de anticorpos anti-*N. caninum* foi feita pela Reação de Imunofluorescência Indireta, sendo as prevalências encontradas nos animais e nos rebanhos amostrados de 14,9% (449/2488) e 69,8% (143/205), respectivamente. A variável que apresentou associação de soropositividade com *N. caninum* foi o aborto (OR 2,52; IC 1,25-5,06). Os resultados indicam que a infecção por *N. caninum* está presente nos rebanhos e que deveria ser considerada como uma potencial causa de aborto em Mato Grosso do Sul.

PALAVRAS-CHAVE: *Neospora caninum*, bovinos, imunofluorescência indireta, soroprevalência.

INTRODUCTION

Neospora caninum, a parasitic protozoan that forms cysts in animal tissues, was, as late as 1988, confused with *Toxoplasma gondii* because of their biological and structural similarities, although they are antigenically distinct (DUBEY et al., 1988).

Since its discovery, *N. caninum* has gained attention, as it is responsible for abortions and premature births in animals

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(DUBEY; LINDSAY, 1996). In cattle, abortions are the unique clinical sign of neosporosis and can occur in any period of gestation, though most frequently in the fifth and sixth months. Fetuses can die in the uterus, be absorbed, mummified, or autolyzed. Calves that are born alive may be diseased or, if clinically normal, have chronic infection (DUBEY, 2003). Moreover, clinical neosporosis has been described in canids, equines, domestic ruminants (cattle, sheep, goats), and wild ruminants (deer, rhinoceroses).

Dogs (MCALLISTER et al., 1998a) and coyotes (GONDIM et al., 2004) are regarded as definitive hosts, as they harbor *N. caninum* in its breeding phase, with a consequent shedding of oocysts in their feces.

Of the transmission routes of the disease, the vertical one is reported as the most important, though most cases of congenital infection result in apparently normal calves (DAVISON et al., 1999), whereas the horizontal route is of importance in herds with high seroprevalence (DIJKSTRA et al., 2002). A strong correlation between dogs seropositive to *N. caninum* and a high prevalence of bovines having antibodies against the parasite was reported by Wouda et al. (1999).

Studies on the prevalence of anti-*N. caninum* antibodies have revealed that neosporosis has a wide geographical distribution, including Australia, New Zealand, Europe, Korea, Japan, Thailand, and the Americas. Dogs and bovines are the chief species exposed to the parasite; both dairy and beef cattle can be affected (ANDERSON et al., 2000).

In Brazil, the occurrence of anti-*N. caninum* antibodies in cattle was first reported in the state of Mato Grosso do Sul (BRAUTIGAM et al., 1996). More recent investigations have reported rates of 7.7% in Mato Grosso do Sul (ANDREOTTI et al., 1999), 14.09% in Bahia (GONDIM et al., 1999a), 11.2% in Rio Grande do Sul (CORBELLINI et al., 2002), 14.3% in Paraná (GUIMARÃES et al., 2004), 12.61% in Minas Gerais (MELO et al., 2004), 8,8% in Rondônia (AGUIAR et al., 2006) and as much as 30.4% in Goiás (MELO et al., 2006). The parasite has been found in cattle fetuses both in São Paulo and in Rio Grande do Sul (GONDIM et al., 1999b; CORBERLLINI et al., 2002). Strains have been isolated from dogs in Bahia (GONDIM et al., 2001), cattle fetuses in Paraná (LOCATELLI-DITTRICH et al., 2003), and buffaloes from São Paulo (RODRIGUES et al., 2004).

The purpose of the present study was to use IFAT to estimate the prevalence of anti-*N. caninum* antibodies in cattle in the 22 municipalities located in a stretch of the state of Mato Grosso do Sul to identify risk factors likely to be associated with *N. caninum* infection.

MATERIAL AND METHODS

Study area

The so-called Estrato 1 subregion of Mato Grosso do Sul is comprised of 22 municipalities and is one of the four subregions of equivalent cattle herd numbers into which the state is divided by the Brazilian Program for the Control and Eradication of Bovine Brucellosis and Tuberculosis (PNCEBT).

The stretch investigated includes municipalities belonging to three of the four geographical mesoregions of Mato Grosso do Sul (Center-North, East and Southwest) and its 70 214.1 km^2 account for 19.7% of the state's area (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 1989, 2002). Beef and/or dairy cattle-raising, and in some cases crop cultures, are the activities developed in properties of varying sizes located in the subregion, harboring as many as 5.7 million head of cattle, of a total of 24.9 million in the state (AGÊNCIA ESTADUAL DE DEFESA SANITÁRIA ANIMAL E VEGETAL DE MATO GROSSO DO SUL, 2003). The stretch, furthermore, plays an important role as a corridor for cattle across the state, given its central geographical location.

Sampling methods

Herds were defined as the primary sampling units. Sample size was calculated as $N = Z^2 \times P(1 - P)/E^2$, as proposed by Noordhuizen et al. (1997), with a 95% confidence interval and a maximum error of 5%. For the expected prevalence, a 15% level was adopted, which corresponds to the mean value found in epidemiological studies conducted in other Brazilian localities (GONDIM et al. 1999a; MELO et al. 2004; GUIMARÃES et al., 2004; CORBELLINI et al., 2005). This resulted in a minimum total number of 196 properties, but 205 were included in the investigation instead.

The properties were randomly chosen from the list used by the Agency for Animal and Crop Health Protection of Mato Grosso do Sul (IAGRO-MS). The sampling interval was calculated by dividing the total number of properties by the number of properties to be sampled. Next, animals in each property were randomly selected. Inclusion criteria were: female animals at least 24 months old living under similar management conditions within any given property. From each property having up to 99 female animals that met the criteria for inclusion, 10 females were randomly selected, or all females aged 24 months and older if they were less than 10. When the number was greater than 99, 15 were randomly selected.

Selection was carried out by draw, using either simple or systematic random sampling, depending on the following calculation: In each property, the number of female animals aged 24 months and older was divided by the number of animals to be sampled; when the result of the division was less than two, simple random sampling was adopted; otherwise, systematic random sampling was used.

Collection of samples

Sera were collected as described by Monteiro et al. (2006), from December 2003 to March 2004. At collection time, a questionnaire was used to gather epidemiological information such as history of abortion, presence of dogs, origin of cattle, presence of marshy terrain as source of contamination, and type of exploitation.

Indirect fluorescence antibody test (IFAT)

Samples were subjected to IFAT to detect the presence of

antibodies. The antigen was produced by culturing tachyzoites of *N. caninum*, strain NC-1 (DUBEY et al., 1988) in Vero cells (LOCATELLI-DITTRICH, 2002; OLIVEIRA et al., 2004). Bovine anti-IgG commercial conjugate (Sigma) was used and the samples were tested at a dilution of 1:50 (PARÉ et al., 1998). Each slide included negative and positive control sera.

Databank

The results of serological tests and the information gathered from the questionnaires were stored in a databank developed for PNCEBT using the program Microsoft Access 97.

Statistical analysis

Calculations of the actual prevalence of infected animals and apparent prevalence of herds were conducted according to Martin et al. (1987, 1992).

To assess the risk factors associated with seropositivity to *N. caninum* in the herds sampled, univariate analysis was performed using interval estimates of odds ratio (OR). Data were processed with the program Win Episcope 2.0 (THRUSFIELD et al., 2001).

RESULTS AND DISCUSSION

This investigation of a representative sample from an area that accounts for 23% of the entire cattle herd of Mato Grosso do Sul reveals an actual prevalence of 14.9% (449/2488) for animals and an apparent prevalence of 69.8% (143/205) for the herds sampled. The parasite appears to have wide geographical distribution in all the 22 municipalities where cattle was sampled, with herd prevalences ranging from 28.6% to 100% and animal-level prevalences ranging from 4.8% to 37.5% (Table 1).

Table 1. Results of the indirect fluorescence antibody test (IFAT) for detection of anti-*Neospora caninum* antibodies in cattle in Mato Grosso do Sul, Brazil, by county. December 2003 to March 2004.

Municipalities	Herds			Animals		
	Sampled	Positive	%	Sampled	Positive	%
Angélica	6	3	50.0	49	8	16.3
Bandeirantes	7	3	42.9	95	6	6.3
Caarapó	8	6	75.0	107	11	10.3
Campo Grande	16	12	75.0	176	23	13.0
Deodápolis	4	9	44.4	6	99	6.0
Douradina	2	1	50.0	21	1	4.7
Dourados	17	16	94.1	197	65	33.0
Fátima do Sul	5	5	100.0	45	13	28.9
Glória de Dourados	9	8	88.9	113	31	27.4
Itaporã	6	6	100.0	74	19	25.7
Ivinhema	14	4	28.6	164	8	4.90
Jaraguari	9	7	77.8	95	19	20.0
Jateí	7	5	71.4	96	17	17.7
Maracaju	10	6	60.0	88	10	11.4
Nova Alvorada do Sul	8	7	87.5	160	13	8.1
Novo Horizonte do Sul	18	18	100.0	153	34	22.2
Ribas do Rio Pardo	18	18	100.0	261	58	37.5
Rio Brilhante	6	5	83.3	87	15	17.2
Rochedo	6	3	50.0	89	5	5.6
Sidrolândia	17	6	35.3	88	8	9.1
Terenos	10	6	60.0	169	16	9.5
Vicentina	5	3	61.9	62	23	37.1
Total	205	143	69.8*	2448	449	14.9**

Seroprevalence: *apparent; **actual.

Studies conducted in the Brazilian states of Paraná (GUIMARÃES et al., 2004), Bahia (GONDIM et al., 1999a) and Rondônia (AGUIAR et al., 2006) showed prevalences of 14.04%, 14.09%, and 8,8%, respectively. Similar results were found for other parts of South America, including Argentina (MOORE et al., 2002) and Uruguay (BAÑALES et al., 2006).

Ragozo et al. (2003), in a study with bovine samples from different Brazilian states, found a prevalence of 28% (31/110) for Mato Grosso do Sul. The difference between their results and those of the present study is probably due to differences in the cut off points adopted (1:25 in their study) and to their use of sera from a bank instead of sera collected from cattle ranches. The earliest studies that used IFAT for diagnosis of *N. caninum* (DUBEY; LINDSAY, 1996) adopted a cut off value of 1:640 or higher. Venturini et al. (1999) later concluded that this value should be lowered. Currently, several laboratories adopt cut off values of 1:200 or lower for IFAT, though no consensus has yet been reached over the issue.

The apparent prevalence of 69.8% in the present study indicates that most herds examined had at least one positive animal, showing that the disease can be maintained at endemic equilibrium in the region. Although herd exposure to the agent was relatively high, these figures might underestimate true prevalence, since only up to 15 animals were sampled on each farm. Similar results were found by Aguiar et al. (2006) in the state of Rondônia.

Table 2 summarizes the results of the univariate analysis of risk factors associated with herd-level seroprevalence.

An association was found between seropositivity to *N. caninum* and abortion, with seropositive herds being 2.52 times as likely to have abortions as seronegative ones (OR 2.52; CI 1.25-5.06). Positive cows with a higher risk of abortion also have a higher probability of transmitting congenital infection to their offspring (PARÉ et al., 1997), thus maintaining the parasite in the herd by vertical transmission.

In addition, the data reveal that abortion plays an important role in infection in the region, as dogs or other major hosts in the biological cycle can be infected by ingesting tissues from contaminated cattle, such as aborted fetuses or placentas, later shedding oocysts in the environment (DIJKSTRA et al., 2002; WOUDA, 2005). Even with a very high probability of vertical transmission, some type of horizontal transmission is required for the disease to be endemic in a herd (FRENCH et al., 1999). In herds with endemic infection, there is serological evidence that a low level of post-birth infection driven by unknown factors can still occur (ANDERSON et al., 2000), but cattle that seroconverts have an increased risk of abortion and will vertically infect their offspring (FRENCH et al., 1999).

No association between *N. caninum*–positive herd

Table 2. Risk factors for the presence of antibodies anti- *N. caninum* in cattle herds from Mato Grosso do Sul, Brazil. December 2003 to March 2004.

Variables	Description	Positive	Negative	OR	Lower CI* (%)	Upper CI* (%)
Presence of dogs	Yes	128	57	0.96	0.35	2.63
	No	14	6			
History of abortion	Yes	55	13	2.52	1.25	5.06
	No	84	50			
Cattle origin	Purchased	61	27	1	0.55	1.83
	Locally bred	81	36			
Presence of marshy terrain	Yes	104	47	0.93	0.47	1.84
	No	38	16			
Type of exploitation	Beef	57	24	1.25	0.65	2.38
	Dairy	59	31			
	Beef	57	24	0.73	0.29	1.84
	Mixed	26	8			
	Dairy	59	31	0.59	0.24	1.45
	Mixed	26	8			

*CI: 95%

serostatus and presence of dogs was found (OR 0.96; CI 0.35-2.63). This finding is similar to that obtained by Guimarães et al. (2004) and Aguiar et al. (2006) with the use of IFAT and by Bañales et al. (2006) using ELISA. Bartels et al. (1999), also using ELISA for serological diagnosis in a case-control study, demonstrated that the presence of dogs constitutes a risk factor. Otranto et al. (2003), showed that farms with two or more dogs had higher seropositivity for *N. caninum* than those with one dog or none.

It was not possible to draw any definitive conclusion on the involvement of dogs in the transmission of bovine infection in Mato Grosso do Sul, since no samples from dogs living on the farms investigated were collected for anti-*Neospora* antibody testing. In Campo Grande municipality, however, a prevalence of 26.53% for dogs was found by Oliveira et al. (2004) and a herd prevalence of 75% was found in the present study. Thus, the possibility of horizontal transmission occurring in beef cattle in this area cannot be ruled out.

As shown in Table 2, no association was found between type of exploitation and *N. caninum* serologic status (OR 1,25; CI 0.65-2.38), and when the apparent prevalence was determined for beef, dairy, and mixed herds, the values were 70.4% (57/81), 65.6% (59/90), and 76.5% (26/34), respectively. Studies conducted in the same region of the country (Brazil's Center-West region) also failed to show any differences between beef and dairy herds (MELO et al., 2006), whereas Moore et al. (2002) found in Argentina a higher prevalence for dairy herds. It is worth pointing out, however, that the average lifespan of dairy cattle is usually longer than that of beef cattle, increasing the chances of exposure to sources of infection, however in Western Amazon (Rondônia), Aguiar et al. (2006), found herd prevalence in beef herds significantly ($p < 0,05$) higher (100%) than in dairy (70%) and mixed (64%) herds.

In Mato Grosso do Sul, extensive production predominates and both beef and dairy herds tend to be raised under similar management conditions, a feature that may contribute to the comparable transmission rates found for both types of herd.

No association was found between cattle origin (purchased or locally bred) and *N. caninum* serologic status (OR 1; CI 0.55-1.83), possibly because both sources of replacement pose risks of infection: locally bred animals spend a longer time in the herd, with a consequently longer time of potential exposure to infection sources; in the case of purchased animals, additional infected ones may be added to the herd.

Although the complexity of this variable warrants more detailed studies, the practice of breeding calves instead of purchasing them obviously increases the proportion of vertical transmission. Despite the efficiency of vertical transmission, theoretical modeling has shown that infection with *N. caninum* cannot be sustained in cattle herds without horizontal transmission (FRENCH et al., 1999).

Even though a previous study conducted in the same region had revealed a higher positivity to *N. caninum* in cattle having access to marshy terrain (ANDREOTTI et al., 2004), in the present study no association was found with the presence of this type of terrain as a source of contamination (OR; 0.93 CI 0.47-1.84). Water contaminated with oocysts is reported as a potential risk factor (MCALLISTER et al., 1998b) and might contribute to the occurrence of horizontal transmission, though more thorough investigations are need to clarify this aspect.

The presence of anti-*N. caninum* antibodies in cattle from Mato Grosso do Sul has been demonstrated and the prevalence levels have been shown to be similar to those in other Brazilian states. *Neospora caninum* was present in all the 22 municipalities sampled, irrespective of type of exploitation.

Although the risk of *N. caninum* infection was not associated with cattle origin (purchase or local breeding) or presence of dogs on the farm, a balance apparently exists between horizontal and vertical transmission, with the difference that the latter route keeps transmission endemic, contributing significantly to the persistence of *N. caninum* in a herd by propagating infection to successive generations (ANDERSON et al., 2000).

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