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The occurrence of anti-*Neospora caninum* in bovine female animals and bovine fetuses in Nossa Senhora do Livramento County, Mato Grosso

Ocorrência de anticorpos anti-*Neospora caninum* em fêmeas e fetos da espécie bovina do município de Nossa Senhora do Livramento, Mato Grosso

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Abstract

Neosporosis is a parasitic disease caused by the obligate intracellular protozoan *Neospora caninum*, which is responsible for miscarriage cases in cattle countrywide. The vertical or transplacental route with prevalent miscarriages between the 3rd and 6th months of pregnancy is the main transmission form in cattle. The aim of the present study is to investigate the presence of anti-*N. caninum* antibodies in (*Bos indicus*) Nelore female animals and fetuses in Nossa Senhora do Livramento County - MT. Blood samples from 489 female bovines and from 128 bovine fetuses over three months of pregnancy were collected. The research about the anti *N. caninum* antibodies in bovine females and fetuses was conducted through the Immunofluorescence technique (IFA). We found 2.45% (12) seropositive and 3.91% (5) seropositive to *N. caninum* in the present study.

Key words: Immunofluorescence Indirect. Nelore. Neosporosis. Transplacental.

Resumo

A neosporose é uma doença parasitária ocasionada pelo protozoário intracelular obrigatório *Neospora caninum*, responsável por quadros de abortos em bovinos em várias regiões do país. A principal forma de transmissão entre os bovinos é a via vertical ou transplacentária com abortos prevalentes entre 3 a 6 meses de gestação. Este trabalho teve como objetivo pesquisar a presença de anticorpos anti-*N. caninum* em fêmeas e fetos bovinos da raça Nelore (*Bos indicus*), do município de Nossa Senhora do Livramento-MT. Durante o abate dos animais foram coletadas amostras de sangue de 489 fêmeas e de

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128 fetos acima de três meses de gestação. A pesquisa de anticorpos anti *N. caninum* nas fêmeas e fetos foi determinada pela Reação de Imunofluorescência Indireta (RIFI). A ocorrência foi de 2,45% para as fêmeas e de 3,91% para os fetos.

Palavras-chave: Imunofluorescência Indireta. Nelore. Neosporose. Transplacentário.

Neosporosis is an emergent disease, which has *Neospora caninum* as its etiological agent. This disease is responsible for miscarriages and neonatal death in bovines worldwide (DUBEY; SCHARES, 2011). Infected calves may be born alive and sick or clinically normal and with chronic infection (INNES et al., 2002).

The two known infection mechanisms of *N. caninum* are the horizontal transmission or the post-natal infection, when it occurs through the ingestion of contaminated water and food, and the vertical transmission or congenital infection, which occurs when the mother transmits the coccidia to the offspring via placenta (McALLISTER et al., 1998). The vertical transmission is the main dissemination form of *N. caninum* in bovine cattle, and it keeps the infection for many generations (INNES et al., 2002).

Studies comprising many species of susceptible animals have been conducted in order to better understand neosporosis. These studies take the agent, the host and the environment into consideration (GUIMARÃES JUNIOR; ROMANELLI, 2006). It is worth highlighting that the researches are conducted to set the serological prevalence of *N. caninum* and show considerable differences from country to country, inside the countries, from region to region, and between the beef and dairy cattle. Therefore, it is necessary to be careful at the time to assess these results due to the differences in the serological techniques, in the study design and in the size of the used sample (DUBEY et al., 2007).

Due to the livestock production potential of Mato Grosso State, which holds the biggest bovine herd in the country, 28 million animals on average (INDEA, 2015), and to the few information about the real epidemiological situation about this disease

in the state, the aim of the present study is to search for the presence of anti-*N. caninum* antibodies in (*Bos indicus*) bovine female animals and fetuses in Nossa Senhora do Livramento County. The animals were slaughtered in a meatpacking industry in Várzea Grande County - Mato Grosso State.

The samples were obtained in a meatpacking industry located in Várzea Grande County, in the metropolitan region of Cuiabá - Mato Grosso State, which is subjected to state sanitary inspection. Bovine female samples (*Bos indicus*) slaughtered in the aforementioned meatpacking industry and the over 3-month pregnancy fetuses were selected. All the samples came from rural properties in Nossa Senhora do Livramento County - MT, south latitude 15°46'30" and west longitude 56°20'44". This county has a herd of approximately 61,664 bovine female animals; 3,040 female animals are slaughtered annually on average (MATO GROSSO, 2011a), and 1,720 (50%) of them were slaughtered in 2010/2011 at SISE 16 (MATO GROSSO, 2011b), the year before the herein conducted sample collection procedure.

The size of the collected sample was set in the Epiinfo 6.4 software, through the formula $n = Z^2 [p(1-p) N] / d^2 (N-1) + Z^2 [p(1-p)]$; wherein *n* is the size of the sample; *N* is the size of the population; *Z* is value used for the confidence interval 99%; *p* is the prevalence; and *d* is the absolute error (THURSFIELD, 2007). The calculation was done by adopting the value of 50% for the expected prevalence and, since the prevalence of *N. caninum* infection in bovines in Mato Grosso State is not known, we accepted a 5% error in the prevalence to be set in the present study. The amount of serological samples collected per month was calculated according to the proportion of the average of female

animals monthly slaughtered from April to October 2010 and 2011, thus the total of 1533 and 1906 of animals slaughtered between the mentioned period between 2010 and 2011 was obtained (MATO GROSSO, 2011b). Thus, the minimum sample of 663 animals - in total - was obtained; these animals and properties met those found in the slaughter line.

From May to September 2012, blood samples (10 mL) from 489 female animals were collected; blood samples (5 mL) were also collected through cardiac puncture from 128 over 3-month pregnancy fetuses. These fetuses were obtained during the female's evisceration; they were identified by the lot numbers of the slaughtered lots and by the respective slaughter dates. However, female and fetuses' identification was not possible to get due to the handling procedures in the meatpacking unit. The age of the fetuses was estimated according to their length (cm), which was measured from the

Atlato-occipital joint to the first coccygeal vertebra (above 13 cm), according to Grunert and Birgel (1989).

The study was approved by the Ethics Committee on Animal Research of Cuiabá University, under registration n. 069 CEP/UNIC - protocol n. 2012/069.

The sera were aliquoted, identified and stored at -20°C for further analysis in the presence of antibodies through indirect immunofluorescence test, according to Conrad et al. (1993). Initially, the sorting of the bovine sera and of the fetuses was conducted through 1:100 and 1:25 dilution (DUBEY; LINDSAY, 1996), respectively, and through further titration of the positive samples.

The presence of anti-*N. caninum* antibodies in 12/489 (2.45%) of the female animals and 5/128 (3.91%) of the assessed fetuses was found in the present study (Table 1).

Table 1. Distribution of blood collection, number of collected female animals and fetuses and the number of *Neospora caninum* seropositives obtained during the slaughters performed from May to September 2012 in a meatpacking industry in Varzea Grande County - MT (SISE 16).

Collection date	Number of female animals		Number of fetuses	
	Collected	Positive (%)	Collected	Positive (%)
05/14th	42	0 (0)	2	0
05/16th	29	2 (6.8)	2	0
05/24th	40	1 (2.5)	11	0
06/06th	48	2 (4.1)	17	1 (5.8)
06/14th	14	1 (7.1)	4	0
06/15th	32	0	5	0
07/13th	97	0	29	0
07/17th	42	1 (2.3)	8	1 (12.5)
07/24th	44	2 (4.5)	14	2 (14.2)
07/27th	17	1 (5.8)	6	0
07/28th	20	1 (5)	7	0
09/16th	12	0	2	0
09/17th	52	1 (1.9)	21	1 (4.7)
Total	489	12 (2.4)	128	5 (3.9)

The antibody titrations found in the female animals ranged from 100 (2.24%) and 200 (0.2%); and on the fetuses they were 25 (1.56%), 200 (1.56%) and 400 (0.78%).

The presence of anti-*N. caninum* antibodies in the cows and fetuses in the current study was lower than that found by other authors, for example, in the Midwest region the prevalence of the disease in beef cows, in Goiania County, was 29.6% (MELO et al., 2006), and in the North of Mato Grosso State, 37.56% seropositive animals were detected (JUSTO et al., 2013). Dubey et al. (2007) reported that the different obtained prevalence result from the different serological techniques used to diagnose the disease, from the study design and from the size of the used sample.

It is worth highlighting that, even in percentages lower than those found in other studies, there was the presence of *N. caninum* positive fetuses in the current study. The fetuses were, consequently, infected by vertical transmission from the pregnant cows, since the antibodies detected against the parasite indicate that the fetuses have produced antibodies, because there is no maternal antibody transmission through the placenta in bovine animals (CARDOSO et al., 2008).

According to Innes et al. (2002), the pregnant animal may have miscarriages, generate a weak calf or a clinically healthy animal that may be persistently infected. These congenitally infected animals may transmit the parasite to other generations, and it helps keeping the parasite in the environment. Serological tests such as the Indirect Immunofluorescence Test become important instruments to detect antibodies in subclinical neosporosis animals, because there is a positive correlation between the presence of anti-*N. caninum* antibodies and non-conception (ANDREOTTI et al., 2010; JUSTO et al., 2013).

McInnes et al. (2006) reported that the presence or absence of *N. Caninum* antibody or DNA does not support or exclude the parasite's participation

in the bovine miscarriage etiopathogeny. The low anti-*N. Caninum* antibody detection in fetuses may be due to the parasite infection *in utero* and to the immune system of the developing fetus; thus, there is no immune response to be detected (WILLIAMS; TREES, 2006). Factors such as increased number of pregnancies and aging may help diminishing the congenital *N. Caninum* infection rates (ROMERO et al., 2002; DIJKSTRA et al., 2003).

Therefore, we may conclude that the current study showed the exposition of pregnant animals (*Bos indicus*) to *N. caninum* and the first indication of vertical transmission in beef cattle (3.91%) in Mato Grosso State.

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