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Anti-*Neospora caninum* antibody detection and vertical transmission rate in pregnant zebu beef cows (*Bos indicus*): *Neospora caninum* in pregnant beef cows (*Bos indicus*)

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ABSTRACT

The aim of the present study was to evaluate anti-*Neospora caninum* antibodies and the vertical transmission rate in naturally infected pregnant zebu beef cows (*Bos indicus*) reared on pasture. The present study began with 200 cows from four farms (50 cows from each farm), and these animals were submitted to timed artificial insemination (TAI). After ultrasonography, 76 pregnant cows were selected, 22, 15, 22, and 17, respectively, from farms 1, 2, 3, and 4. Blood samples were taken from cows thrice during the first, second, and third trimester of gestation, and a blood sample was collected from 31 calves before colostrum milking. From 76 cows 23 (30.3%) had anti-*N. caninum* antibodies detected by indirect ELISA (Idexx), and 53 (69.7%) did not. Sixty-four cows that initiated the experiment were negative to *N. caninum* and 11 became positive either during the second or third trimester of gestation, this mean an infection incidence of 17.2% (11/64). OD for ELISA was higher (OD = 2.08) during the second and third (OD = 2.10) trimesters of pregnancy when compared with the first (OD = 1.81), however, there were no statistical differences ($P = 0.45$). The vertical transmission was calculated to be 29.0% (9/31), and the risk of vertical transmission of *N. caninum* in seropositive dams was 26.25 times higher than seronegative animals ($OR = 26.25$, $2.38 < OR < 289$, $P = 0.007$). In conclusion, the rate of vertical transmission of *N. caninum* in pregnant zebu beef cows was 29%, and the risk was 26.25 higher in seropositive dams relative to than seronegative animals.

1. Introduction

Neospora caninum is a protozoan parasite with a worldwide distribution. Its life cycle was determined in 1998 [1], dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*), dingoes (*Canis lupus dingo*), and gray wolfs (*C. lupus*) are presently described as its definitive hosts [1–4]. However, under natural conditions, oocysts have only been observed in the feces of dogs and gray wolfs [4,5].

Cattle can contract neosporosis by vertical or horizontal transmission [6,7]. Vertical (transplacental) infections can be exogenous (infections by oocyst ingestion) or endogenous (reactivation of infection in a chronically infected cow) in origin. Vertical transmission is described as the main route, and abortions due to

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neosporosis are most prevalent at 3 to 6 months of pregnancy [8].

Brazil has approximately 205.3 million bovines [9], which constitute the largest commercial herd in the world [10]. Approximately 80% of Brazilian cattle are zebu animals (*Bos indicus*, Nellore and crossbreeding), and the great majority of the beef herd is raised at pasture with an annual production of approximately 9.3 million tons of meat [10].

Although the rates of vertical transmission of neosporosis have been estimated in dairy herds [7,11,12], little is known about neosporosis transmission in beef herds. Marques et al. [13] reported that 4.8% of fetuses from Brazilian zebu beef cows slaughtered in an abattoir were serologically positive for *N. caninum*. Additionally, the vertical transmission rate was reported as 24% in crossbreeding cattle (zebu/Holstein) based on the seroconversion of calves before ingestion of colostrum [14].

The aim of the present study was to evaluate the detection of anti-*Neospora caninum* antibodies and the vertical transmission (from mothers to precolostrum calves) in naturally infected zebu (Nellore breed) beef cows from Paraná state, Brazil.

2. Material and methods

2.1. Study area and sampling

This study was conducted at four farms located in the northern region of the state of Paraná in Southern Brazil. The selected farms utilized a herd vaccine program that included inoculations against abortion agents, such as leptospirosis, bovine viral diarrhea virus (BVDV) and infectious bovine rhinotracheitis virus (IBRV), however, where *N. caninum* vaccine were not used. The cows were kept in pastures with ad libitum access to water and mineral supplements. Two hundred zebu beef cows (Nellore breed, *Bos indicus)* were subjected to timed artificial insemination (TAI; 50 cows from each farm). The pregnant animals were selected after insemination, and gestation was diagnosed by ultrasonography. Seventy-six pregnant cows were detected (farm A, $n = 22$; farm B, $n = 15$, farm C, $n = 22$, and farm D, $n = 17$), and their blood was drawn during the first, second, and third trimesters of gestation. After delivery, blood samples from 31 calves were collected by puncture of the jugular vein immediately after calving and before they ingested colostrum. The sera were stored in 1.5 ml plastic tubes and kept at −20 °C until analysis. This study was approved by the Animal Experimentation Ethics Committee (CEEA, protocol number 54/12) of Universidade Estadual de Londrina.

2.2. Anti-Neospora caninum antibody detection

Antibodies against *N. caninum* were detected by HerdCheck *Neospora caninum* Antibody Test Kit (Idexx laboratories) following manufacture instructions. Sera from cows were diluted 1:100 (following manufacture suggestions) and calves 1:25[15]. A cow was considered to be seropositive if two or more of the test results were positive (OD ≥ 0.5). Results obtained during first, second, and third trimesters of gestation such as: +, –, – or –, +, – were considered false positives. A calf was considered seropositive when OD ≥ 0.5.

2.3. Statistical analysis

The experimental variables were analyzed by the chi-square test (χ^2) with the Yates correction and the Fisher exact test (F) using the Epi Info program (CDC, 6.04b version). The associations among the variables and the occurrence of seropositive animals were estimated from values obtained by the odds ratio (OR) with a confidence interval of 95%. ANOVA was performed to calculate differences between serum ODs. A P-value of ≤0.05 was considered significant.

3. Results

3.1. Seroprevalence of N. caninum

The serologic results for *N. caninum* are summarized in Tables 1 and 2. Of 76 pregnant cows, 23 (30.3%) were positive for *N. caninum*, and 53 (69.7%) tested negative. The prevalence of *N. caninum* in pregnant cows was statistically different between the farms ($\chi^2 = 9.03$, $p = 0.02$), showing a prevalence of 9.1% (2/22), 26.7% (4/15), 50.0% (11/22), and 35.3% (6/17) at farms A, B, C, and D, respectively. Sixty four cows began the experiment negative for *N. caninum* and 11 become positive in the second or third trimester of gestation, which

Table 1
Outcome of anti-*Neospora caninum* antibody responses in sera from cows monitored during pregnancy.

	Trimester of gestation			
	First	Second	Third	Total (%)
Serologic dam condition	–	–	–	53 (69.7)
	–	–	+	3 (3.9)
	–	+	+	8 (10.5)
	+	+	+	7 (9.2)
	+	+	–	2 (2.6)
	+	–	+	3 (3.9)
Total				76 (100)
ELISA[1]	1.81[a]	2.08[a]	2.10[a]	
Prevalence				30.3% (23/76)
Incidence				17.2% (11/64)

[1] OD average from commercial ELISA (Idexx) –, negative +, positive.
[a] $P = 0.45$.

Table 2
Relationship between cow and calf sera against *Neospora caninum* antibody.

Dams[1]	Calves[2]		
	+ (%)	–(%)	Total (%)
+	5 (16.1)	2 (3.2)	6(19.3)
–	4 (12.9)	20 (67.8)	25 (80.7)
Total	9 (29.0)	22 (71.0)	31 (100)
$P = 0.007$[3] (OR = 26.25, 2.38 < OR < 289)			

[1] A cow was considered to be seropositive if two or more of the test results were positive (OD ≥ 0.5). Results such as: +, –, – or –, +, – were considered false positives.
[2] A calf was considered seropositive when OD ≥ 0.5.
[3] Fisher Exact.