Assessment of transplacental transmission of *Neospora caninum* in dairy cattle in the Agreste region of Pernambuco

Avaliação da transmissão transplacentária do *Neospora caninum* em bovinos de leite na região Agreste de Pernambuco

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Abstract

The transplacental transmission is the primary route of *Neospora caninum* infection in bovine herds around the world. This study aimed to determine the frequency of transplacental transmission of the parasite in dairy cattle of Agreste region of Pernambuco through serological tests (IFAT and ELISA). Three hundred sixteen serum samples from cows and heifers and their offspring were analyzed. The transplacental transmission rate was 72.22% (13/18) for cows and 69.23% (9/13) for heifers by IFAT. ELISA test showed transplacental transmission rate of 43.58% (17/39) for cows and 50% (9/18) for heifers. The transplacental transmission rates were similar, in both groups in test, but a higher seropositivity was found in cows by IFAT. Data were statistically analyzed using the chi-square and Fisher’s exact test. A significant relationship of dependence between seropositivity of mothers and their offspring was found. The more frequent IFAT antibody titers and ELISA levels for *N. caninum* were, respectively, 200 and between four (cows) and five (heifers and offspring). In the Spearman correlation, no association was found between the magnitude of antibody titers for *N. caninum* between mothers and their offspring. The kappa test showed an index of 0.35, indicating a mild correlation between the serological tests used. The study suggests that cows and heifers are the main transmitters of *N. caninum* in the studied region and that vertical transmission is the major form of transmission in dairy herds of the Agreste region of Pernambuco.

Keywords: Neosporosis, dairy cows, IFAT, ELISA, Northeastern Brazil.

Resumo

A transmissão transplacentária é a principal via de infecção do *Neospora caninum* nos rebanhos bovinos em todo o mundo. O presente estudo teve como objetivo determinar a frequência da transmissão transplacentária do parasita em bovinos leiteiros do Agreste Pernambucano, por meio de testes sorológicos (RIFI e ELISA). Foram analisadas 316 amostras de soro de fêmeas bovinas (vacas e novilhas) e de suas crias. A taxa de transmissão transplacentária pela RIFI foi de 72,22% (13/18) para vacas e 69,23% (9/13) para as novilhas. O ELISA teste mostrou taxa de transmissão transplacentária de 43,58% (17/39) para as vacas e 50% (9/18) para as novilhas. As taxas de transmissão transplacentária foram semelhantes para os dois testes em geral, porém uma maior soropositividade foi encontrada nas vacas pela RIFI. Os dados foram estatisticamente analisados pelo teste de qui-quadrado e teste exato de Fischer. Foi encontrada uma relação significativa de dependência entre a soropositividade das mães e de suas crias. Os títulos de anticorpos anti- *N. caninum* foi de 200 na...
Neosporosis is a disease that shows a global importance, with prevalence ranging from 0.07% to 97.2% (DUBEY & SCHARES, 2011). Transplacental transmission is the main form of dissemination of neosporosis through cattle herds, and is considered to be one of the main causes of abortion in livestock. The parasite may cause chronic infection, remaining within the herd across the generations and giving rise to reproduction losses (DUBEY et al., 2007). When infection occurs during the final third of gestation, fetal deaths and abortions hardly ever occur and offspring appear to be healthy when in fact they are persistently infected (DUBEY & SCHARES, 2011). The horizontal infection occurs due to the presence of the main definitive hosts, represented by dogs, coyotes (Canis latrans) and Australian dingos (Canis lupus dingo) (McALLISTER et al., 1998; GONDIM et al., 2004; KING et al., 2010), which eliminate Neospora caninum oocysts in their faeces and can contaminate food items and water, keeping the infection between generations through of transplacental transmission (DUBEY et al., 2007).

Transplacental transmission of N. caninum can be estimated from the number of seropositive offspring born from seropositive mothers, soon after their birth and before ingestion of colostrum (PARÉ et al., 1996). Among the techniques used to show whether neosporosis is involved as a disease with in the reproductive sphere, the Indirect Fluorescent Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assay (ELISA) are the major serological tests used in the routine (PARÉ et al., 1996; JENKINS et al., 2002).

Serological studies characterizing the frequency of transplacental transmission among offspring, before ingestion of colostrum, have been reported in Brazil (VIANNA et al., 2008; MARQUES et al., 2011; HEIN et al., 2012; SANTOS et al., 2012; MACEDO et al., 2013), but none have been conducted in the state of Pernambuco up to now. In this state, the dairy cattle has been established historically in the Agreste region, counting with approximately 169.581 million dairy cattle (IBGE, 2010). The present study aimed to evaluate the frequency of transplacental transmission of N. caninum between dairy cattle (cows and heifers) and their respective offspring before ingestion of colostrum, in the Agreste region of Pernambuco, by serological techniques.

One hundred fifty eight female cattle (113 cows and 45 heifers), without previous reports of reproductive problems, that were assisted during delivery at the Cattle Clinic, Garanhuns campus, Federal Rural University of Pernambuco (UFPR), and their respective offspring (n = 158), were evaluated, totaling 316 animals. Samples were collected at delivery, during the period of 2003-2013. Animal offspring (n = 158), were evaluated, totalizing 316 animals. Samples were subjected to sequential dilutions at base 2, to determine the antibody titers (DUBEY et al., 1988; MINEO et al., 2009). The positive and negative controls serum samples were tested on each slide and were compared with negative and positive controls. The positive control serum samples were obtained from the serum bank of the Immunoparasitology Laboratory, UNESP Jaboticabal. Test serum samples and positive control serum samples were tested on each slide and were compared with negative and positive controls. The positive control serum samples were obtained from the serum bank of the Immunoparasitology Laboratory, UNESP Jaboticabal. Test serum samples and positive control serum samples were diluted at 1:200. After dilution (1:200) in phosphate-buffered saline solution (PBS) (pH 7.2; 1.3 M NaCl, 27 M KCl, 56 mM NaHPO₄, 10 mM KHPO₄ and 9.2 mM NaH₂PO₄), 10 μL of diluted serum samples were deposited in each slide well. The slides were then incubated at 37 °C for 30 minutes in humid chamber. After incubation, slides were washed three times with PBS for five minutes, consecutively, and then dried at room temperature. Following this, 10 μL of conjugate (anti-IgG-bovine-SIGMA®, St. Louis, Missouri, United States) diluted in polypropylene microtubes and then kept frozen in ultrafreezer (−80 °C), until the time of use.

For IFAT, tachyzoites of the isolate Nc-1 of N. caninum cultivated in Vero cells were used as antigens, which were deposited on previously marked wells (MINEO et al., 2009). Ten serum samples were tested on each slide and were compared with negative and positive controls. The positive control serum samples were obtained from the serum bank of the Immunoparasitology Laboratory, UNESP Jaboticabal. Test serum samples and positive control serum samples were diluted at 1:200. After dilution (1:200) in phosphate-buffered saline solution (PBS) (pH 7.2; 1.3 M NaCl, 27 M KCl, 56 mM NaHPO₄, 10 mM KHPO₄ and 9.2 mM NaH₂PO₄), 10 μL of diluted serum samples were deposited in each slide well. The slides were then incubated at 37 °C for 30 minutes in humid chamber. After incubation, slides were washed three times with PBS for five minutes, consecutively, and then dried at room temperature. Following this, 10 μL of conjugate (anti-IgG-bovine-SIGMA®, St. Louis, Missouri, United States) diluted in PBS at 1:300 was added to each slide well. Then, slides were incubated in humid chamber again, at 37 °C for 30 minutes, with subsequent washing, as described previously.

After the slides had been dried at room temperature, buffered glycerin was added (glycerin and 0.5 M carbonate-bicarbonate buffer at pH 9.6), and then covered with a cover slips, for observation at a magnification of 400X, under a microscope equipped with fluorescent light (Olympus BX-FLA). Test positivity was determined through observation of total peripheral fluorescence of the tachyzoites, using a cutoff point of 1:200. Positive samples were subjected to sequential dilutions at base 2, to determine the antibody titers (DUBEY et al., 1988; MINEO et al., 2009).

The indirect ELISA was performed as previously described by Machado et al. (1997), with some modifications. There was...
Table 1. Serological results for *Neospora caninum* by IFAT and ELISA, according to the animal category (cows, heifers and their offspring), in Garanhuns, 2003-2013.

<table>
<thead>
<tr>
<th>Offspring</th>
<th>IFAT</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow</td>
<td>Heifer</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>TOTAL</td>
<td>18</td>
<td>95</td>
</tr>
</tbody>
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p-value = 0.0001 for IFAT, 0.0106 for ELISA.
Transplacental transmission of *Neospora caninum*

HEIN et al., 2012), Santa Catarina (MACEDO et al., 2013), São Paulo (SARTOR et al., 2003), Tocantins (MARTINS et al., 2011), Mato Grosso do Sul, Rio de Janeiro (MUNHOZ et al., 2006), Rondônia (AGUIAR et al., 2006; MARQUES et al., 2011; ANDREOTTI et al., 2004), Alagoas (SOUZA et al., 2012) and Bahia (GONDIM et al., 2004), where the values ranged from 6% to 58%. In the state of Pernambuco, Silva et al. (2008) found a seropositive frequency of 31.7% in herds belonging to large milk producers located in Gravesend, by IFAT. Moreover, Amaral et al. (2012), evaluating beef cattle from slaughterhouses of the micro-region of Brejo/PE, found a seropositivity rate of 17.3%, which was also consistent with our results.

In this study, some municipalities of the Agreste region of Pernambuco (Lagoa do Ouro, Canhotinho, Caetés, Lajedo and

Figure 1. Map of distribution of seropositive (n = 53) and seronegative (n = 263) animals (cows, heifers and their offspring) for *Neospora caninum* by IFAT assisted in the Garanhuns cattle Clinic 2003-2013, in the Agreste region of Pernambuco, Brazil. Municipalities with higher seropositivity (Caetes, Lajedo, Canhotinho, Palmerina and Lagoa do Ouro).

Figure 2. Distribution of animals according to IFAT titers of antibodies to *N. caninum* and animal categories (cow, heifer and offspring), in Garanhuns, 2003-2013.

Figure 3. Distribution of animals according to ELISA levels of antibodies to *N. caninum* and animal categories (cow, heifer and offspring), in Garanhuns, 2003-2013. ELISA level ≥ 4 = Positive; according to the formula previously described by Machado et al. (1997).
Palmerina), which concentrated a large number of milk producers (SEBRAE, 2013; DUBEY & SCHARIES, 2011), showed higher rates of seropositivity than those found among animals in other municipalities.

By serological tests (IFAT or ELISA) revealed that there was significant association (p ≤ 0.05) of seropositivity between mother and offspring, except by ELISA for heifers and offspring. This supports hypothesis that transplacental route is a major source of transmission and maintenance of neosporosis in dairy cattle to successive generations (ANDERSON et al., 1997). The transplacental transmission rate was similar between the two groups studied, using both serologic techniques, but higher seropositivity was found by IFAT for cows. Higher vertical transmission rate in cows may be related to chronic infections in dairy herds, where animals remain much active time in the production system, spreading the agent over the generations (INNES et al., 2005; DUBEY & SCHARIES, 2011). Innes et al. (2000) reported that older animals showed better immune adaptation to the parasite than younger animals, but may have the reactivation of infection in immunosuppressive situations.

It is also important to highlight that the occurrence of birth of seropositive calves from seronegative mothers (cows [3/10] and heifers [3/6]), totaling six seropositive offspring from sixteen negative females by IFAT, could be due to the infection of mother have happened some time ago and the antibody level is maintained at a level below the detection threshold of serological tests (CONRAD et al., 1993; FRÖSSLING et al., 2005; LÓPEZ-GATIUS et al., 2005; SAGER et al., 2001). This condition was also observed by Frössling et al. (2005) and López-Gatius et al. (2004) in cattle from Sweden.

The presence of antibody titer in calves before colostrum feeding proves transplacental transmission, since maternal antibodies do not cross the placenta in cattle, and can assume that the presence of antibodies in calves immediately after birth, before the intake of colostrum, are generated in uterus resulting N. caninum infection. The frequency of transplacental transmission can be estimated by the number of seropositive calves soon after birth before colostrum intake, born from seropositive mothers (BARTELS et al., 2007).

In this study, there was no correlation between the magnitude of anti-N. caninum antibodies titers between mothers and their offspring. On the other hand, Dubey et al. (2007) reported that the magnitude of antibody titers of infected calves showed concordance with the magnitude of those from their mothers. Hictala & Thurmond (1999) emphasizes the need of special attention of the researchers when interpreting the serological results for N. caninum in cattle aged between 13 and 24 months of age, because of the possibility of false negative results, due to the decline of antibodies in the old congenitally infected animals. Serological studies have suggested that titles ≥ 200 (IFAT) are specific to infection by N. caninum (DUBEY et al., 1996), while the highest titer have been associated with occurrence of abortions (TREES et al., 1993). According to Cox et al. (1998), high titer of anti-N. caninum antibodies quickly decline after the abortion, but low positive titers may persist for a long time.

According to the kappa index, IFAT and ELISA crude antigen presented concordance mild (0.35). IFAT was the first serological test used to demonstrate the presence of antibodies for N. caninum (DUBEY & SCHARIES, 2011). Cross-reactions with other coccidia are almost non-existent, so it is used as a benchmark for comparison with other serological tests. However, comparing indirect ELISA based on NcSRS2 recombinant antigen for detecting anti-N. caninum antibodies in sheep, Andreotti et al. (2009) found that these two tests showed similar concordance with an excellent kappa (0.98). Indirect ELISA based on the recombinant protein NcSRS2 was shown to be a highly sensitive and specific tool for serological diagnosis of N. caninum, since it reduces the occurrence of non-specific cross reactions (ANDREOTTI et al., 2009).

Conclusion

The high rates of transplacental transmission observed in this study, either by IFAT or ELISA, may indicate that this type of transmission plays an important role in the maintenance and transmission of N. caninum in dairy cattle herds in the Agreste region of Pernambuco.

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