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Antibody kinetics in goats and conceptuses naturally infected with *Neospora caninum*

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ABSTRACT

Neospora caninum is a protozoan which can cause abortions in caprines. However, information regarding the humoral immune response and the occurrence of reproductive disorders is scarce. This is the first study in which the kinetics of antibodies is studied in pregnant goats naturally infected by *N. caninum*, as well as their respective conceptuses. The subclasses of IgG (IgG1 and IgG2) were also evaluated in pregnant goats. Reproductive problems related to neosporosis (abortion and stillbirth) occurred in 15.38% of the goats. There was a statistically significant association between the increased titres of maternal IgG in the second half of the gestational period with the occurrence of endogenous transplacental transmission. The rate of congenital transmission was 77%. During the gestational period of the seropositive goats, there was mainly a predominance of the subclass IgG2, although mixed patterns of IgG2–IgG1 and the IgG1 pattern were also observed. These results indicate that *N. caninum* is responsible for the occurrence of important alterations in the humoral immune response of naturally infected goats, and is also a potential causative agent for reproductive disorders in goats. The high proportion of infected conceptuses reinforces the suggestion that congenital infection is one of the main routes of parasite transmission in goats.

1. Introduction

Neospora caninum is an obligate intracellular protozoan and is considered one of the main agents responsible for abortions in bovines in various regions of the world (Dubey et al., 2007). However, the epidemiological, clinical and economic importance of infection by *N. caninum* in small ruminants has not been properly established (Dubey and Schares, 2011).

Trees and Williams (2005) used the term endogenous transplacental infection (TPI) to refer to the reactivation of infection during pregnancy by an agent which has established a chronic infection in the animal before pregnancy, and vertical transmission was used to refer to infection through successive generations. In bovines, TPI is one of the main routes of neosporosis infection, and studies have demonstrated high rates of congenitally infected calves, which can be born healthy and transmit the parasite to the next generation (Paré et al., 1996; Schares et al., 1998).

In goats and others ruminants, there is no prenatal transfer of immunoglobulin across placenta. Consequently, parasite-specific antibodies that are detectable in precolostral sera have most likely been prenatally synthesised by the foetus, indicating an active immune response against the invading parasite (Staubli et al., 2006). After

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birth, the goats acquire passive immunity by ingestion of immunoglobulin-rich colostrums from its dam. According to Pearson and Brandon (1976), the half-life of passively acquired immunoglobulins in small ruminants is 25 days for IgG, 6 days for IgM and 2 days for IgA.

In naturally infected cows, an increase in the antibody titres has been observed in the second half of the gestational period, which may be strongly related to the recrudescence of infection with consequent endogenous TPI (Paré et al., 1997; Stenlund et al., 1999; Guy et al., 2001; Nogareda et al., 2007). This increase in specific antibodies in naturally infected cows was mainly related to greater values for the subclass IgG2 (Guy et al., 2001; Andrianarivo et al., 2005). Predominant patterns of the subclass IgG1, as well as mixed patterns of IgG1–IgG2, have also been observed in cows that were experimentally and naturally infected (Andrianarivo et al., 2001; Moore et al., 2005). In experimentally infected goats, increases in the IgG titres occurred during gestation, with evidence of the occurrence of TPI (Lindsay et al., 1995). However, no studies have reported the humoral response during the gestation of goats naturally infected by *N. caninum*, mainly in relation to the subclasses of IgG, which can indicate the type of polarisation of the immune response. In order to establish potential alterations in the humoral immune response and their relation to the occurrence of endogenous TPI and reproductive disorders, the objective of this study was to evaluate the kinetics of IgG anti-*N. caninum* in goats and conceptuses naturally infected by *N. caninum*, and report some findings about the subclasses of IgG1 and IgG2 in pregnant goats.

2. Materials and methods

2.1. Goats

In this study, 15 multiparous Saanen, Pardo-Alpina and mixed breed goats were studied. Thirteen animals were naturally infected by *N. caninum*, identified by the presence of specific antibodies using the indirect fluorescent antibody test (IFAT). All goats selected for this study were seronegative by IFAT to *Toxoplasma gondii*. Two other goats were also seronegative by IFAT to *N. caninum* and *T. gondii*, and were used as negative controls. The goats were kept in pens to avoid potential exposure to sporulated *N. caninum* oocysts from the environment or other sources of infection. This study was approved by the Ethics Committee of Animals Use (CEUA) from Universidade Federal de Lavras (UFLA) under the protocol number 013/2011.

2.2. Diagnosis of pregnancy and collection of blood samples

Thirty days after mating, the gestation was diagnosed by transrectal ultrasound, which was used on a monthly basis to verify the foetal viability. Blood samples were collected from each goat on the day of mating (day 0), on days 30, 60, 90 and 120 of pregnancy, on the day of parturition or abortion and three months after parturition or abortion. For goats kids that were born healthy, the samples were collected on the day of birth, before the ingestion of colostrum (day 0), on day 2 (48 h after birth), and 30, 60, 90, 120, 150 and 180 days after birth. The blood, which was obtained from the jugular vein in Vacutainer® tubes without anticoagulant, was centrifuged at 1090 × *g* for 10 min. The serum obtained was frozen and maintained at −20 °C until the serological tests were carried out.

2.3. Indirect fluorescent antibody test (IFAT) in goats and kids

The IFAT was used to detect IgG anti-*N. caninum* antibodies, according to the technique described by Paré et al. (1995). An initial dilution of 1:50 was used as the cut-off point (Lindsay et al., 1995) and two-fold dilutions were carried out based on this. Samples for which there was complete peripheral fluorescence of the tachyzoites were considered positive. Positive and negative controls were added to each slide. Tachyzoites of isolate NC-1 were used on the slides as antigens. The caprine fluorescein-conjugated anti-IgG antibody (Sigma–Aldrich®) was diluted 1:200 in PBS. Even in goats that were negative in the first sampling, the IFAT was used to detect IgG anti-*Toxoplasma gondii* antibodies in pregnant goats, using a similar method to that described for *N. caninum*, with an initial dilution of 1:64 as the cut-off value (Figliuolo et al., 2004).

2.4. Detection of the specific IgG subclasses against N. caninum (IgG1 and IgG2) in pregnant seropositive goats

The subclasses anti-*N. caninum* IgG1 and IgG2 were evaluated during the pregnancy of 13 seropositive goats. The levels of antibodies were measured applying the enzyme-linked immunosorbent assay (ELISA), using a commercial kit (Chekit *N. caninum* Antibody ELISA, Idexx®) according to the manufacturer's instructions, with small modifications. The HRP-conjugated sheep anti-bovine IgG1 and HRP-conjugated sheep anti-bovine IgG2 (Bethyl®) antibodies were used as detection antibodies at dilutions of 1:100 and 1:50, in PBS, respectively (Marinaro et al., 2009). These antibodies may cross-react with the subclasses of goat IgG (Marinaro et al., 2009). Positive and negative controls, previously tested by IFAT, were used. The plates were read at A450 nm. In order to support the results, the Relative Index (RI) was calculated for the IgG1 and IgG2 values. IgG1 and IgG2 values were obtained for each blood collection from the pregnant goats, along with the IgG2/IgG1 ratio of the individual RI values.

2.5. Diagnosis of abortion and stillbirths

For the four aborted and two stillborn foetuses originating from two seropositive goats, lesions compatible with neosporosis were evaluated. The presence of *N. caninum* was established by immunohistochemistry (IHC), and PCR as described by Varaschin et al. (2012). The DNA was extracted from 40 mg of tissue that was collected and stored at −20 °C until analysis; this was from the central nervous system and cardiac muscle of all foetuses and stillbirths. DNA extraction was performed with a commercial kit (Wizard SV Genomic DNA Purification System, Promega,