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Detection of *Neospora caninum* DNA in semen of experimental infected rams with no evidence of horizontal transmission in ewes

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ABSTRACT

Recent reports from New Zealand indicate *Neospora caninum* has a possible role in causing abortions in sheep. Transmission of *N. caninum* via semen has been documented in cattle. This study aimed to investigate if horizontal transmission through semen was also possible in sheep. Initially, 6-month old crossbred ram lambs ($n = 32$), seronegative to *N. caninum*, were divided into 4 equal groups. Group 1 remained uninoculated whilst the remainder were inoculated with *N. caninum* tachyzoites intravenously as follows: Group 2 – 50 tachyzoites; Group 3 – 10^3 tachyzoites; Group 4 – 10^7 tachyzoites. Semen samples were collected weekly for 8 weeks for the detection of *N. caninum* DNA and quantified using quantitative PCR (qPCR). Plasma collected 1 month post-inoculation was subjected to ELISA (IDEXX Chekit) and Western blot. At 2 weeks post-infection, three rams from Group 1 (uninoculated) and three rams from Group 4 (10^7 tachyzoites/ml) were mated with two groups of 16 ewes over two oestrus cycles. Ewe sera collected 1 and 2 months post-mating were tested for seroconversion by ELISA and Western blot. All experimentally infected rams seroconverted by 1 month with ELISA S/P% values ranging from 11% to 36.5% in Group 2, 12–39.5% in Group 3 and 40–81% in Group 4. However, none of the ewes mated with the experimentally infected rams seroconverted. For the Western blot, responses towards immunodominant antigens (IDAs) were observed in ram sera directed against proteins at 10, 17, 21, 25–29, 30, 31, 33 and 37 kDa. Rams in Group 2, 3 and 4 were noted to have at least 3 IDAs present. None of the ewes showed any of the 8 prominent IDAs except for the one at 21 kDa which was seen in 30 out of 32 ewes in both groups. *N. caninum* DNA was detected intermittently in the ram's semen up to 5 weeks post-inoculation with the concentrations ranging from that equivalent to 1–889 tachyzoites per ml of semen. Low concentrations of *N. caninum* DNA were also detected in the brain tissue of two rams (Groups 1 and 4). These results suggest that although *N. caninum* DNA can be found in the semen of experimentally infected rams, the transmission of *N. caninum* via natural mating is an unlikely event.

1. Introduction

Neospora caninum is an obligate intracellular parasite which is recognized as the leading cause of bovine abortion (Thornton et al., 1991). However, *N. caninum* infections have also been reported in horses, goats, deer, and sheep

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(Dubey, 2003). Natural infection of *N. caninum* in sheep was first identified in 1990 in a congenitally infected lamb with signs of ataxia and weakness after birth (Dubey et al., 1990). Subsequently, naturally occurring ovine neosporosis has been reported worldwide, including Japan, South America, Australia, Switzerland, Italy, Spain and New Zealand (Koyama et al., 2001; Kobayashi et al., 2001; Hassig et al., 2003; Moore, 2005; Masala et al., 2007; Moreno et al., 2012; Reichel et al., 2008; Howe et al., 2008; Bishop et al., 2010). Repeat abortions in subsequent years have also been reported (Jolley et al., 1999). In addition, studies have shown that experimentally infected sheep will seroconvert and abort in a dose dependent manner (Buxton et al., 1998, 2001; Weston et al., 2009). These reports suggest that *N. caninum* infections can and do occur and cause disease, although it has generally not been regarded as a significant cause of abortion in sheep (Dubey et al., 1990; Dubey and Lindsay, 1990; Otter et al., 1997; Buxton et al., 1998; Helmick et al., 2002).

Seroprevalence studies using ELISA-based assays in sheep flocks from around the world show the prevalence ranges from 2% to 14% worldwide (Figliuolo et al., 2004; Gaffuri et al., 2006; Panadero et al., 2010; Langoni et al., 2011). Seroprevalence has been reported at 2.2% in New South Wales, Australia, and slightly lower at 0.63% and 1.4% in New Zealand (Reichel et al., 2008) as compared to the seroprevalence in dairy and beef cattle in New Zealand which is about 30–35% and 2.8%, respectively (Reichel, 2000; Tennent-Brown et al., 2000). However, the role of *N. caninum* as a significant pathogen and abortifacient in sheep is still unclear although there have been sporadic reports of *N. caninum* being involved in ovine abortions (Otter et al., 1997; Helmick et al., 2002; Hassig et al., 2003; Moreno et al., 2012). At present, knowledge about the epidemiology of neosporosis in sheep is limited and particularly the mode of transmission.

Transmission of *N. caninum* via semen was thought to be possible when bulls were found to be seropositive towards *N. caninum* and *N. caninum* DNA was detected in the semen of naturally (Moore et al., 2003; Ortega-Mora et al., 2003; Caetano-da-Silva et al., 2004; Ferre et al., 2005) and experimentally infected bulls (Serrano-Martinez et al., 2007a; Ferre et al., 2008). Dose dependent seroconversion and parasitaemia were also seen in heifers inseminated with semen spiked with *N. caninum* tachyzoites, though no seroconversion or detection of *N. caninum* DNA in the embryo or calves was observed (Serrano et al., 2006; Serrano-Martinez et al., 2007b). The possibility that semen was a potential mode of transmission for *N. caninum* was further supported when Masuda et al. (2007) showed that CB-17 SCID and BALB/c male mice inoculated with 2×10^5 *N. caninum* tachyzoites were able to transmit the infection through mating to immunodeficient female SCID mice and their neonates.

Due to the increasing evidence for the possible role of *N. caninum* in poor reproductive performance in New Zealand (Howe et al., 2012) and lack of transmission studies in sheep under natural farming conditions, this study was designed to examine the potential for transmission of *N. caninum* through semen in sheep. The specific objectives were to determine if *N. caninum* DNA could be detected in the semen of rams experimentally inoculated with *N. caninum* with doses ranging from 50 to 10^7 tachyzoites (controlled challenge study) and to determine if *N. caninum* could be horizontally transmitted in sheep through semen via natural mating (controlled exposure study). The findings from this research will help to better understand the epidemiology of *N. caninum* among sheep flocks in New Zealand and worldwide.

2. Materials and methods

2.1. Experimental design

This trial consisted of two parts. The first part was a controlled challenge study to determine if *N. caninum* DNA would be found in the semen of rams experimentally infected with *N. caninum* tachyzoites. The second part was a controlled exposure study to determine if *N. caninum* could be transmitted to ewes via natural mating with rams experimentally infected with *N. caninum*.

2.2. Animals

For the first part of the trial, 32 young rams aged approximately 6 months were selected from a farm with no known history of reproductive problems. The rams were a cross between Borderdale, Romney and Suffolk breeds and were free from *N. caninum* as indicated by negative serology using ELISA when sampled prior to removing them from their home farm and again prior to the experiment commencing. The rams were randomly divided into 4 groups with 8 rams per group. Group 1 was left uninoculated (Control); Group 2 rams were inoculated with 50 *N. caninum* tachyzoites intravenously (i/v); Group 3 rams were inoculated with 10^3 *N. caninum* tachyzoites i/v and Group 4 rams were inoculated with 10^7 *N. caninum* tachyzoites i/v. Inoculated rams grazed in one mob in a paddock separated from the uninoculated control rams in an adjacent paddock.

Semen was collected by electroejaculation using a battery powered intra-rectal ejaculator probe (Ruakura probe, Shoof Int., NZ). Repeated 5 s stimulations separated by a similar length break were applied and semen was collected in a sterile container. Semen collection was done once pre-inoculation and then weekly up to 8 weeks post-inoculation. Blood samples were also collected 1 month post-inoculation to determine serum antibody levels and to look for the presence of *N. caninum* DNA circulating in whole blood.

For the second part of the trial 32 adult ewes aged approximately 4 years were purchased from the same farm. These ewes had successfully reared a lamb the previous year. All ewes had been vaccinated for *Toxoplasma gondii* and *Campylobacter foetus foetus* and were determined by a blood sample taken prior to leaving their farm of origin to be seronegative by ELISA for *N. caninum*. In addition, prior to mating, blood samples were collected 3 times via venipuncture to confirm that the ewes were serologically negative by ELISA to *N. caninum*.

The 32 ewes were randomly divided into 2 groups of 16. One group was mated with 3 rams from the control group (Group 1) while the other group was mated with 3