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The role of *Neospora caninum* in three cases of unexplained ewe abortions in the southern North Island of New Zealand

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Abstract

Unexplained abortions observed in three southern North Island sheep farms were investigated to determine if there was an association with *Neospora caninum* infection. Ewe hoggets and ewes on three farms that exhibited a persistent abortion problem, despite vaccination against *Toxoplasma* and *Campylobacter* infections, were chosen for this study. Blood was obtained from aborting, non-aborting and selected ewes that had dead foetuses at scanning. Some ewes were euthanized and examination of their uterine contents, gross examination and histopathology were performed. Serology was conducted for *N. caninum*, *Toxoplasma gondii* and *Leptospira interrogans* serovar Pomona and *Leptospira borgpetersenii* serovar Hardjo antibodies. Whole blood and selected tissues were used in polymerase chain reactions (PCR) to detect DNA from *N. caninum* and *T. gondii*. Gross examination and histopathology was not diagnostic and no bacterial pathogens were isolated. *N. caninum* indirect fluorescent antibody test (IFAT) serology identified 19 of 67 samples as positive, with titres ranging from 1:100 to 1:800. Serological results for *Toxoplasma* and *Leptospira* were generally negative or had low (1:100 dilution) titres, indicative of vaccination or previous exposure. *N. caninum* DNA was also detected by PCR in 23 of 64 samples, including in aborted foetal material. *T. gondii* DNA was detected by PCR in only 2 of the 64 blood samples. The results of these current investigations are suggestive of an association between *N. caninum* infection and abortion in sheep within New Zealand.

Clinical relevance: *N. caninum* may be an important cause of sheep abortions.

Keywords: *Neospora caninum*; PCR; IFAT; Sheep; Abortion; *Toxoplasma gondii*

1. Introduction

Neospora caninum is an obligate intracellular parasite similar in structure and development to *Toxoplasma gondii*, to which it is also genetically related (Dubey, 2003). It is the most frequently diagnosed cause of abortion of cattle in New Zealand and many countries worldwide. It also causes infections in dogs, horses, goats, deer, and sheep (Dubey, 2003). Little is known about the involvement of

Abbreviations: PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; IFAT, immunofluorescent antibody test; IgG-FITC, immunoglobulin G conjugated to fluorescein isothiocyanate isomer I; dNTP, 2′-deoxynucleoside 5′-triphosphates; min, minutes; s, seconds; UV, ultraviolet light.

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N. caninum in foetal mortality of sheep in New Zealand.

N. caninum was first described as a natural infection in sheep in a congenitally infected lamb in England (Dubey et al., 1990). Subsequently, naturally occurring ovine neosporosis has been reported in Japan, South America and Switzerland (Kobayashi et al., 2001; Koyama et al., 2001; Hassig et al., 2003; Moore, 2005). Although *N. caninum* was shown to cause mortality in newborn lambs and congenital infection in naturally exposed sheep, it is not regarded as a significant cause of abortion in sheep (Dubey et al., 1990; Dubey and Lindsay, 1990; Buxton et al., 1998; Hurtado et al., 2001; Helmick et al., 2002). However, experimental infection of pregnant ewes with tachyzoites has been shown to induce abortion or congenital infection (McAllister et al., 1996; Buxton et al., 1997, 1998, 2001). Repeat abortions in subsequent years have also been reported (Jolley et al., 1999). Sheep can also be infected by ingesting oocysts, suggesting the possibility of horizontal infection (O'Handley et al., 2002).

There has been little research on ovine neosporosis in New Zealand and there is no prevalence data in the general sheep population. Antony and Williamson (2003) recommended that *N. caninum* infection of sheep required further investigation after noting the highest seroprevalence of infection in dogs from sheep and beef farms. The presence of serum antibodies against *N. caninum* in aborting ewes was demonstrated in 2003 and 2004 (West et al., 2006). Historically, it has been easy to confuse abortion due to *Toxoplasma* with that caused by *Neospora*. However, the widespread use of Toxovax® (AgVax Research Limited, Inc., New Zealand) vaccine, that dramatically reduces the prevalence of abortion due to toxoplasmosis, means that alternative causes of abortions in New Zealand sheep can now be investigated.

This report describes preliminary studies to investigate the role of *Neospora* infection in *Toxoplasma*-vaccinated sheep with unexplained abortions on three southern North Island sheep farms.

2. Materials and methods

2.1. Flock descriptions

Farm A is a research farm that consisted of several groups of mixed-aged ewes. One of these groups included 125 spring-lambing ewes which were part of a larger study investigating year-round lambing (Morris et al., 2004). These ewes had been vaccinated in February 2005 with Toxovax® and Campylovexin® (Schering-Plough Coopers Animal Health, New Zealand). Ewe mating occurred during March and April. At the end of May, 18 of the 115 pregnant ewes appeared to be carrying dead foetuses at ultrasound scanning. A further 16 ewes were found to have aborted in early August and another group of 10 ewes had aborted by September. Lambing occurred from late August until the end of October 2005.

Farms B and C consisted of 7000 and 2000 one-year-old maiden ewes (hoggets), respectively, that were mated during May 2005. They were scanned by ultrasound in August, when a large number of dead intra-uterine foetuses and recent abortions were noted. The hoggets had been vaccinated with Toxovax® in March 2005 and with a Campylovexin® booster at the end of May 2005. The hoggets were noted to be behind their target weights before breeding but were fed well through mating.

Serum and heparinized blood samples were collected from selected affected ewes when being scanned as absorbing or identified as aborting from Farm A ($n=38$), Farm B ($n=6$), and Farm C ($n=3$) for serological and polymerase chain reaction (PCR) analysis. Some of these selected affected ewes were euthanized at either Massey University (Farm A; $n=4$) or on the respective farms (Farm B; $n=6$, or Farm C; $n=3$). The entire uteri and contents were collected from each of the euthanized animals and submitted for gross and histopathology (Massey University, Palmerston North). Samples of each uteri, placenta and foetal brain collected were also used for PCR analysis. Tissue samples and stomach contents were tested for the presence of contaminating bacteria by standard enteropathogenic microbiological culture. On Farm A, an additional 20 heparinized and whole blood samples were taken in October from ewes which had lambed normally and had produced healthy, live lambs (full-term ewes). Unfortunately, foetal tissues were not available from this group at the time of sampling. Serum and heparinized blood was stored at −20 and 4 °C respectively until tested.

2.2. Serology

Serum samples (Farm A $n=38$ affected and 20 normal, Farm B $n=6$, Farm C $n=3$) were tested for *N. caninum* antibodies using an indirect fluorescent antibody test (IFAT), developed at Massey University, as described by West et al. (2006). A test was considered positive if the titre was ≥1:100. Positive samples at 1:100 were serial two-fold diluted to 1:64,000 to determine the fluorescence end-point. A positive control serum sample from a *Neospora*-infected ewe (1:6400) and a negative control serum sample from an uninfected ewe were included in every test.

Serum samples (Farm A $n=37$ affected and 20 normal, Farm B $n=6$, Farm C $n=3$) were tested for *T. gondii* antibodies using a commercial latex agglutination test kit (LAT) (Eiken Chemical Co., Tokyo, Japan) as per the manufacturer's instructions with included controls. Serum samples were also tested for the presence of serum antibodies against *Leptospira interrogans* serovar Pomona (Farm A $n=37$ affected and 20 normal, Farm B $n=6$, Farm C $n=3$) and *Leptospira borgpetersenii* serovar Hardjo (Farm A $n=21$ affected and 20 normal, Farm B $n=6$, Farm C $n=3$) at serial two-fold dilutions, starting from 1:50, using a microscopic agglutination test (MAT). The titre