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Efficacy of a *Neospora caninum* killed tachyzoite vaccine in preventing abortion and vertical transmission in dairy cattle

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ABSTRACT

A clinical trial was undertaken to assess the efficacy of Bovilis® Neoguard, a killed *Neospora caninum* tachyzoite vaccine on 5 commercial dairy farms in New Zealand with a history of *Neospora*-associated abortion. Cattle were enrolled in the trial at 30–60 days of gestation and randomly allocated to treatment or control groups. Treatment consisted of 5 mL doses of Bovilis Neoguard administered subcutaneously at enrolment then 4 weeks later. Isotonic saline was administered to the control group. Of 2246 cattle enrolled in the trial, 10.7% of cows and 12.6% of heifers were seropositive to *N. caninum*. Sampling of a randomly selected proportion of enrolled animals 6 weeks after the second treatment showed that 188/232 (81.0%) vaccinated with Bovilis® Neoguard had seroconverted, while 11/130 (8.5%) cows and 10/36 (27.8%) heifers in the control group had seroconverted. Forty-eight vaccinated and 63 control animals aborted. On one farm 12.5% of control animals and 6.1% of vaccinated animals aborted (vaccine efficacy 0.61; $p = 0.03$). On another farm with a high level of abortion 8.4% of control animals and 8.7% of vaccinates aborted. On the remaining 3 farms fewer abortions occurred than expected. A modified Poisson regression approach was used to calculate relative risks for abortion and vertical transmission. Overall vaccine efficacy was 0.25 ($p = 0.12$). Heifer replacement calves from the animals enrolled in the trial were sampled for antibodies to *N. caninum* at 6–9 months of age. Fourteen of 17 calves from vaccinated, seropositive cows were seropositive as were 13/23 calves from seropositive cows in the control group. The interaction between dam serostatus and treatment group was significant ($p = 0.05$) with vaccination increasing the risk of vertical transmission. It was concluded that vaccination after conception prevented 61% abortions in one of five herds and that vaccination may have increased the risk of early embryonic death.

1. Introduction

Neospora caninum is an important cause of bovine abortion in many countries (Dubey et al., 2007). Losses occur in dairy (Anderson et al., 1995) and beef cattle (Waldner et al., 1999) but losses are more likely to be noticed, investigated and reported on dairy farms due to daily observation of cows at milking. Epidemic and endemic abortion patterns have been associated with *N. caninum* infection (Wouda et al., 1999). Epidemic abortion, with up to 33% of animals aborting, has been reported (Thornton et al., 1994) and is thought to occur when a naive herd is exposed to *N. caninum* oocysts excreted by a definitive host (Jenkins et al., 2000) or in a chronically infected herd with recrudescence of latent infection (Wouda et al., 1999). Endemic abortion may occur in herds following epidemic abortion (Moen et al., 1998; Pfeiffer et al., 2002). Heifer replacements infected by vertical transmission maintain a proportion of seropositive cows in a herd despite infected and aborting cows having an increased risk of being culled (Thurmond and Hietala, 1996). Economic losses attributable to *N. caninum* include an increase in calving interval, a decrease in

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milk production, a decrease in stock value and increased culling (Trees et al., 1999; Bartels et al., 2006).

The immune response to *N. caninum* infection includes specific cell-mediated and antibody responses, and the Th1-type response important in controlling intracellular parasitic infections (Innes et al., 2002). An antibody response is commonly detected within 14 days of experimental challenge and may help prevent parasite invasion of other host cells (Innes et al., 2002). The bradyzoite stage of the parasite is thought to evade the host's immune response and it has been postulated that immunomodulation during pregnancy alters the balance of Th1 and Th2-type immune responses, allowing recrudescence of the parasite and subsequent parasitaemia (Entrican, 2002). Parasitaemia in a pregnant cow leading to transplacental transmission and/or abortion may occur in cattle experiencing a primary infection (exogenous transplacental transmission) or where a chronic infection has recrudesced (endogenous transplacental transmission; Trees and Williams, 2005). Abortion may be due to tissue destruction in the fetus, placental insufficiency due to inflammation, the maternal immune response or a combination of these (Dubey et al., 2006).

Recommendations for the control of bovine neosporosis include undertaking serological testing and culling of infected cattle (Hall et al., 2005), quarantine and testing of replacement and purchased cattle (Haddad et al., 2005), preventing transmission from dogs and other potential definitive hosts (Reichel and Ellis, 2002) and minimising exposure to stressors such as mycotoxins (Bartels et al., 1999) and bovine viral diarrhoea virus (Quinn et al., 2004) that may promote recrudescence of infection. Transfer of embryos from infected cows to uninfected recipients can prevent endogenous transplacental transmission (Baillargeon et al., 2001). Treatment of infected calves and mice with toltrazuril (or its derivative ponazuril) has shown some efficacy in preventing vertical transmission in small-scale trials (Gottstein et al., 2005; Haerdi et al., 2006) but is not currently recommended as a treatment.

A killed tachyzoite vaccine (Bovilis Neoguard; Intervet International B.V., Boxmeer, The Netherlands) was registered for use in cattle in New Zealand in 2001 as an aid in the prevention of abortion due to *N. caninum*. The product had been assessed in experimental challenges for safety (Choromanski and Block, 2000; Barling et al., 2003) and had been trialled under controlled and field conditions. A dairy herd in Minnesota reported 25% abortions annually for 2 years (with some confirmed as being associated with *N. caninum* infection) prior to introducing Neoguard vaccination, after which the abortion incidence dropped to less than 4% (Choromanski and Shawnee, 2002). A challenge trial of seronegative heifers showed a significant reduction in abortion among the vaccinated animals (Choromanski and Shawnee, 2002). It was also suggested that the vaccine may protect against vertical transmission since 6 heifer calves from the Minnesota herd were seronegative at age 6 months despite their dams being seropositive prior to vaccination (Choromanski and Shawnee, 2002). A field trial in Costa Rica in 25 dairy herds involving 876 cows reported 11.2% abortions in vaccinated animals and 20.8% abortions in the control group, a vaccine efficacy of 0.46 [95% confidence interval (CI): 0.26, 0.61; (Romero et al., 2004)].

The objectives of this clinical trial were to assess the efficacy of Bovilis Neoguard in reducing abortion on commercial dairy farms in New Zealand with a history of *Neospora*-associated abortion and its effect on the vertical transmission of *N. caninum*.

2. Material and methods

2.1. Animals and serological testing

The owners of five commercial dairy farms from three regions in New Zealand (three from Taranaki, one from Canterbury and one from Southland) were recruited to participate in this clinical trial due to the documented history of high abortion rates (>8%) on their farm for at least two previous years that had been diagnosed as due to *N. caninum* infection. Cows were predominantly Friesian or Friesian/Jersey crossbreds and calved annually from late July to early October. Feed consisted predominantly of grazed pasture, supplemented with grass or maize silage at times during lactation, or with hay during the winter (dry period). Multiparous cows and home-bred, replacement heifers that would calve for the first time at approximately 24 months old were included in the trial. All cattle had been vaccinated against leptospirosis [serovars *Hardjobovis* and *Pomona* (Leptavoid 2, Schering Plough Animal Health, Upper Hutt, NZ) or serovars *Hardjobovis, Pomona* and *Copenhageni* (Lepto-3-way, Virbac, Auckland, NZ)] with annual boosters administered in mid-gestation (March–May). A bovine viral diarrhoea virus vaccination (BVDV) programme (Bovilis® BVD, Intervet International B.V., Boxmeer, The Netherlands) had been instigated on Farm A.

Cows and primiparous heifers were enrolled once pregnancy was confirmed by transrectal ultrasonography conducted 8 weeks after the planned start of mating for each herd. Allocation to the treatment or control group was by systematic random allocation using a coin toss for the first, then every alternate animal. A second pregnancy test was conducted 4 weeks later to confirm pregnancy in the enrolled animals and to allow enrolment of further animals. Animals were 30–60 days pregnant at the time of enrolment. Antibodies to *N. caninum* were measured by indirect fluorescent antibody test (IFAT; VMRD Inc., Pullman, Washington, USA) in serum collected from enrolled animals. Initial dilution was 1:100 then positive samples were serially diluted two-fold to determine a fluorescence end-point. An IFAT titre of $\geq$1:200 was considered positive. Sera were also tested for antibodies to BVDV by enzyme-linked immunosorbent assay (ELISA; Institut Pourquier, Montpellier, France). Pregnant cattle were enrolled in the trial in December 2001 and January 2002.

2.2. Treatment

Vaccinated animals received 5 mL of Bovilis® Neoguard (batch 244016; Intervet International B.V., Boxmeer, The Netherlands) administered twice, at an interval of 4 weeks, by subcutaneous injection in either the anterior neck or