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Associations between *Neospora caninum* specific antibodies in serum and milk in two dairy herds in Scotland

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Abstract

The study evaluated the use of the Mastazyme® ELISA for quantification of *Neospora caninum* (*N. caninum*) specific IgG in bovine milk and examined the relationship between serum and milk antibodies in two dairy herds. The serum and milk antibodies both had bimodal distributions in each herd. This was mainly due to between cow variation: in both herds, approximately two thirds of cows were either clearly and consistently seropositive or seronegative for *N. caninum* with one third consistently near the threshold. Milk and serum *N. caninum* IgG were strongly related. This relationship was modelled using a linear mixed model including a polynomial term for serum, the effect of herd, and between and within cow variance components. The latter gave a significantly better fit to the data than a model that allowed for a different relationship for the positive and negative (according to the serum test) groups of observations. The sensitivity and specificity (based on serum percentage positivity (pp)) of the milk antibody was determined for different milk pp thresholds.

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In spite of the differences between the relationship of milk to serum seen for the two herds, for those estimates with sufficient precision, sensitivity and specificity greater than 0.73 for both herds were obtained using single thresholds of 14 and 15.5 for milk pp in both herds based on, as our gold standard, serum antibody pp thresholds of 22.5 and 25, respectively. If milk antibody is to be used for detecting persistently infected cows, the higher threshold of 15.5 may be suitable while for epidemiological screening 14 would be preferable. Further validation in a greater number of herds is required, but our results suggest that this test may prove to be a useful adjunct to serum *N. caninum* IgG assays in the monitoring of *N. caninum* infection as part of herd health programmes and epidemiological studies.

Keywords: Cattle; Abortion; *Neospora caninum*; Diagnostic screening; Milk; ELISA

1. Introduction

Neospora caninum (*N. caninum*) is an apicomplexan protozoan parasite which is an important cause of abortion in dairy and beef herds worldwide (Dubey, 1999; Anderson et al., 2000) resulting in considerable economic loss associated with fetal death and reduced milk and meat production (Thurmond and Hietala, 1996; Barling et al., 2001). In Scotland, antibody to *N. caninum* was detected in 15.9% of aborted and stillborn calves (Buxton et al., 1997) while in England and Wales, 12.5% of bovine abortions were considered to be caused by the parasite (Davison et al., 1999). *N. caninum* also causes neurological disease in dogs, and dogs can act as a definitive host (Basso et al., 2001).

Vertical (transplacental) transmission from dam to fetus is considered to be the major means of transmission and results in endemic neosporosis in affected herds. Although less common than endemic infection, point-source outbreaks do occur (horizontal transmission) (McAllister et al., 2000; Crawshaw and Brocklehurst, 2003), and dogs have been implicated as the source of infection in some outbreaks (Wouda et al., 1999). In cattle, the detection of serum antibody (IgG) to *N. caninum* above a selected threshold is indicative of the presence of infection in that individual. The immunofluorescent antibody test (IFAT) was originally used to demonstrate bovine serum *N. caninum* specific IgG (Conrad et al., 1993). This technique has now been largely replaced by enzyme-linked immunoassays (ELISAs) incorporating whole tachyzoite antigen (Paré et al., 1995a; Williams et al., 1997; Williams et al., 1999), recombinant *N. caninum* protein fragments (Louie et al., 1997) or water-soluble fractions of tachyzoites (Osawa et al., 1998). Commercially available ELISAs have been developed from some of the original methods described (Paré et al., 1995a; Williams et al., 1999; Baszler et al., 2001) and have the advantage of ease of analysis of large sample numbers. Avidity ELISAs have also been valuable in epidemiological studies to distinguish between recent and persistent infection (Björkman et al., 1999; Schares et al., 2002).

In recent years, screening programmes for a variety of bovine infectious diseases have adopted bulk milk antibody assays, as a convenient means of assessing the herd status for diseases such as bovine virus diarrhoea (BVD), bovine herpesvirus-1 and *Leptospira hardjo* infection (e.g. Scottish Agricultural College Premium Cattle Health Scheme).