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Performance characteristics and optimisation of cut-off values of two enzyme-linked immunosorbent assays for the detection of antibodies to *Neospora caninum* in the serum of cattle

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Abstract

Aim: To determine the performance characteristics of two enzyme-linked immunosorbent assays (ELISAs) manufactured by Institut Pourquier (IP) for the detection of antibodies against *Neospora caninum* in bovine sera.
Methods: Sera from 526 cattle were assayed in two ELISAs (IP) for the detection of anti-*N. caninum* antibodies. Results from a further ELISA (IDEXX) were used to provide the ''gold standard'' *N. caninum* infection status of the cattle and the ELISA results assessed by two-graph receiver operating characteristic (TG-ROC) analysis.
Results: TG-ROC analysis suggested changes to one of the IP ELISA protocols, arriving at a cut-off threshold that was different to the one recommended by the manufacturer. With that change, both of the ELISAs performed with high sensitivity and specificity (in excess of 98%) for bovine sera.
Conclusions: The analysis of the two IP ELISAs when used on individual bovine sera demonstrated high sensitivity and specificity. TG-ROC analyses optimised the cut-off point suggested by the manufacturer for one of these commercial diagnostic assays and found agreement with the manufacturer's cut-off regarding the other assay. This will help with the accurate identification of infected animals and thereby contributing to the control of neosporosis.
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Keywords: *Neospora caninum*; Protozoa; Cattle; Serology; ELISA; ROC analysis

1. Introduction

Neospora caninum is known to infect both dairy and beef cattle and has been described as a cause of bovine abortion in many countries around the world, including New Zealand and Australia (Reichel, 2000).

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The enzyme-linked immunosorbent assay (ELISA) has assumed an important, central role in the development of knowledge about the distribution, prevalence and importance of neosporosis. Several ELISA tests have now been described in the literature (Paré et al., 1995; Björkman et al., 1997; Williams et al., 1997; Schares et al., 2000) and some are available commercially.

A recent study (Hall et al., 2005) showed that a commercially available ELISA was able to diagnose *N. caninum* infection with a high degree of sensitivity (Se) and specificity (Sp) and that, by using a test-and-cull strategy, it was possible to effectively eliminate *N. caninum* infection from a dairy herd. In the present study, the performance of this inhibition (or blocking) ELISA on serum samples was further analysed as was an indirect *N. caninum* ELISA. The performance characteristics of these two assays, including Se, Sp and cut-off thresholds are further described.

If test-and cull approaches are to be used on a larger scale, serological assays that lend themselves to quickly and efficiently testing large numbers of samples are required (i.e. for testing whole-herd bleeds). ELISA technology appears to fit this premise well, more so if these tests can be demonstrated to be reliable and shown to produce accurate and reproducible results. The correct diagnosis of cattle infected with *N. caninum* is also important when undertaking epidemiological studies and diagnosing abortion.

ELISAs are widely used in this process and so it is important that they are validated and their performance characteristics and limitations are known. A recent study comparing 11 ELISAs to detect *N. caninum* antibodies found that the IDEXX ELISA had excellent Se and Sp (100 and 99.7%, respectively) when the results of the majority of tests were used as the gold standard (von Blumröder et al., 2004).

When TG-ROC analyses were performed using the computer program '*Computational methods for diagnostic tests*' (CMDT, Free University Berlin, Germany), this also produced cut-offs similar to that of the manufacturer with high Se and Sp (99.6 and 98.1%, respectively). The IDEXX ELISA was shown to have good agreement with the indirect fluorescent antibody test (IFAT). It has also been subject to comparisons with an 'in-house' ELISA and TG-ROC analysis in Australasia (Reichel and Pfeiffer, 2002), which suggested relatively high specificity (95%) at the manufacturer's suggested cut-off of 0.5 (SP%). Thus the IDEXX ELISA is an excellent choice as a gold standard test when comparing serological tests.

2. Materials and methods

2.1. Herd sampling

The serum samples came from two dairy herds located in New South Wales, Australia. There were 266 samples from Herd 1 (Hall et al., 2005) and 260 samples from Herd 2. The latter herd had experienced a *N. caninum* abortion storm 5 years earlier (April–July 2000) during which approximately 70 foetuses were lost. In September 2001 the whole herd was blood sampled and the prevalence of *N. caninum* was 24% (53/224) (P. Windsor, personal communication).

2.2. Sera

Serum samples ($n = 526$) were available from individual animals with naturally acquired *N. caninum* infections and from non-infected cattle.

The serum samples were assayed for anti-*N. caninum* antibodies using three commercially available ELISAs. Of the two ELISA manufactured by Institut Pourquier (Montpellier, France), one was a blocking ELISA (cat. no. P00510/01) in its configuration, the other one an indirect ELISA (cat. no. P00511/01). The third ELISA (5N05.00) was manufactured by IDEXX Laboratories (Westbrook, Maine, USA) and was used to determine the 'gold standard' status of the sera.

2.3. IDEXX ELISA

The *N. caninum* IDEXX ELISA was performed on all sera (in duplicate) according to the manufacturer's specifications and the *N. caninum* infection status of each serum determined. Sera where the S/P was ≥ 0.5 was regarded as positive and those where the S/P was <0.5 were regarded as negative for *N. caninum* antibodies.

2.4. Indirect ELISA (Institut Pourquier)

The *N. caninum* indirect ELISA was performed on all sera according to the manufacturer's instructions. The samples were tested in single wells unless there

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