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Neospora caninum IgG avidity tests: An interlaboratory comparison

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Abstract

Avidity tests can be used to discriminate between cattle that are acutely and chronically infected with the intracellular parasite *Neospora caninum*. The aim of this study was to compare the IgG avidity ELISA tests being used in four European laboratories. A coded panel of 200 bovine sera from well documented naturally and experimentally *N. caninum* infected animals were analysed at the participating laboratories by their respective assay systems and laboratory protocols. Comparing the numeric test results, the concordance correlation coefficients were between 0.479 and 0.776. The laboratories categorize the avidity results into the classes ''low'' and ''high'' which are considered indicative of recent and chronic infection, respectively. Three laboratories also use an ''intermediate'' class. When the categorized data were analysed by Kappa statistics there was moderate to substantial agreements between the laboratories. There was an overall better agreement for dichotomized results than when an intermediate class was also used. Taken together, this first ring test for *N. caninum* IgG avidity assays showed a moderate agreement between the assays used by the different laboratories to estimate the IgG avidity. Our experience suggests that avidity tests are sometimes less robust than conventional ELISAs. Therefore, it is essential that they are carefully standardised and their performance continuously evaluated.

Keywords: IgG avidity; *Neospora caninum*; Interlaboratory; Cattle; Antibody; ELISA

1. Introduction

The intracellular parasite *Neospora caninum* is an important cause of bovine abortion world-wide (Dubey, 2003). Abortion outbreaks in cattle herds

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have been connected with recent infection in the pregnant heifers and cows, whereas endemic abortion patterns are thought to be consistent with presence of chronically infected dams (Thurmond et al., 1997; McAllister et al., 2000; Schares et al., 2002; Dubey, 2003).

Demonstration of specific antibodies in serum from an animal is indicative of *N. caninum* infection (Björkman and Uggla, 1999; Dubey, 2003). However, because the antibody levels fluctuate during pregnancy in persistently infected animals (Stenlund et al., 1999), antibody levels or titres cannot be used to discriminate between acutely and chronically infected animals. For this purpose avidity ELISA tests, based on the fact that the first antibodies synthesized after an antigenic challenge have lower affinity for the antigen than those produced later, can be used. The first *N. caninum* IgG avidity test utilised membrane proteins incorporated into iscoms as antigen (Björkman et al., 1999). It has later been accompanied by tests based on tachyzoite extracts (Maley et al., 2001; Sager et al., 2003; Aguado-Martinez et al., 2005) and the *N. caninum* membrane protein NcSRS2 (p38) (Schares et al., 2002) and an IgG avidity Western blot assay (Aguado-Martinez et al., 2005). Avidity testing has proved a powerful tool for epidemiological investigations on bovine *Neospora* infection and related abortion problems (Dijkstra et al., 2002; Schares et al., 2002; Sager et al., 2003; Björkman et al., 2005; Frössling et al., 2005).

Although *N. caninum* avidity testing has been used for some years, no evaluation of the agreement between the different avidity assays has been done so far. This study was part of a larger European research project and aimed to compare the various *N. caninum* IgG avidity tests being used in our laboratories.

2. Materials and methods

2.1. Study design

A coded panel of 200 bovine sera from well documented naturally and experimentally *N. caninum* infected cattle were tested in four laboratories (FLI Wusterhausen, UCM Madrid, IPB Berne and SLU/SVA Uppsala) for IgG avidity by their respective assay systems. The sera were coded by SLU/SVA Uppsala and aliquots were sent to the other participating laboratories.

2.2. Sera

(a) One hundred and fifty two serum samples came from 11 naturally *N. caninum* infected dairy herds in Germany, The Netherlands and Sweden. Five of the herds had recently before sampling experienced epidemic abortions whereas five had had endemic abortion problems. In the remaining herd several of the animals had seroconverted during 1 year but no detrimental effects on reproduction were observed (Dijkstra et al., 2002). Initially, all sera collected in these herds were sent to SLU/SVA Uppsala where they were analysed by IgG avidity iscom ELISA (Björkman et al., 1999, 2003). Sera to be included in the study were selected to cover the whole range of possible avidity values. For two of the herds with epidemic abortion (Herd 3 and Herd 4 in Schares et al., 2002) more detailed information was available. Fourteen of the sera were collected from dams that recently aborted and 22 from dams without abortion. The animals had been sampled 17–31 days after the epidemic abortion started on these farms.

(b) Forty-eight serum samples came from six experimentally *N. caninum* infected pregnant cows. Three of the cows had been given 600 *N. caninum* oocysts (Nc-Liv) orally at 70 days gestation (Trees et al., 2002) whereas three cows had been subcutaneously injected with 5×10^8 tachyzoites of the Nc-1 isolate 140 days into gestation (Innes et al., 2001). Samples collected from all cows before inoculation and 2, 4, 6, 8, 12, 16 and 20 weeks after inoculation were included in the avidity comparison.

2.3. Avidity tests

The samples were analysed at the participating laboratories using their respective assay systems and laboratory protocols (Table 1). The laboratories categorized the avidity results into the classes ‘‘low’’ and ‘‘high’’ which are considered indicative of recent and chronic infection, respectively. Three of the laboratories also used an ‘‘intermediate’’ class.