Development of Rapid Immunochromatographic Test with Recombinant NcSAG1 for Detection of Antibodies to *Neospora caninum* in Cattle

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Received 26 December 2004/Returned for modification 9 February 2005/Accepted 19 April 2005

An immunochromatographic test (ICT) with recombinant surface antigen 1 of *Neospora caninum* (NcSAG1) was developed for the rapid detection of antibodies to *N. caninum* in cattle. The ICT was used to clearly discriminate between immunofluorescent-antibody test (IFAT)-positive bovine sera and IFAT-negative bovine sera. Serum samples collected from cattle in Yanbian, China, were examined by the ICT. Of the 96 serum samples, 23 (24.0%) were positive by the ICT, and 19 (19.8%) samples were positive by a previously developed enzyme-linked immunosorbent assay (ELISA). Eighteen of 19 ELISA-positive samples were positive according to the ICT. A good agreement was found between the results of the ICT and the ELISA. The results presented here suggest that the ICT with recombinant truncated NcSAG1 fused to glutathione S-transferase is a useful and reliable method for the detection of antibodies to *N. caninum* in cattle.

*Neospora caninum* is an apicomplexan protozoan parasite that causes fetal abortion and neonatal mortality in cattle and neuromuscular paralysis in dogs. It occasionally causes clinical infections in horses, goats, sheep, and deer (5). Dogs are the only known definitive host for *N. caninum* (11). Since *N. caninum* was first recorded in 1984 (1), neosporosis has emerged as an economically important disease with considerable impact on the livestock industry worldwide.

As a major cause of abortion in cattle, *N. caninum* has provoked a great deal of attention with respect to its prevalence and diagnosis. *N. caninum* infection in cattle has been reported in many countries (3). Quantitative studies in the United States, New Zealand, The Netherlands, and Germany indicated that 12 to 42% of aborted fetuses from dairy cattle were infected with *N. caninum* (4). In cattle, transplacental transmission is the major route of transmission of *N. caninum*. Seropositive heifers remained clinically normal but gave birth to congenitally infected calves (4). Therefore, it is important to identify the potential cause of abortion by using a rapid and reliable serodiagnostic method.

Many serological diagnostic methods have been developed to diagnose *N. caninum* infection. The enzyme-linked immunosorbent assay (ELISA) with purified recombinant antigen was thought to be an effective way to diagnose *N. caninum* infection (8). Compared with the native antigens, recombinant antigens have an additional benefit: they are easily produced in large quantities and can be readily standardized for diagnostic assays. The diagnostic potential of surface antigen 1 of *N. caninum* (NcSAG1) expressed in *Escherichia coli* was previously evaluated, and the result indicated that the recombinant NcSAG1 could be a reliable reagent for use as an antigen in an ELISA for the serodiagnosis of *N. caninum* infection in cattle (2). However, in general, the ELISA is time-consuming and laborious and requires special materials and equipment, which make it unsuitable for clinical or field applications. In contrast to the ELISA, the immunochromatographic test (ICT) is a simple, rapid method, which makes it suitable for clinical or field applications. Here, we report the development and evaluation of the ICT with recombinant NcSAG1 for detection of specific antibodies to *N. caninum* in cattle.

The expression and purification of recombinant truncated NcSAG1, without signal peptide and with C-terminal hydrophobic regions fused to glutathione S-transferase (GST-NcSAG1t), were performed as described previously (2). The production and preparation of mouse immunoglobulin G (IgG) against GST-NcSAG1t, as well as the preparation of ICT strips, were carried out according to a method described previously (7), with some modifications. Briefly, purified GST-NcSAG1t (200 μg/ml), GST (200 μg/ml), and anti-GST-NcSAG1t IgG (1.5 mg/ml) were jetted linearly on a nitrocellulose membrane, as test, GST, and control lines, respectively. For the preparation of the conjugated antigen, 1 ml of purified GST-NcSAG1t (100 μg/ml) was combined with 10 ml of gold colloid (British BioCell International, United Kingdom), and the conjugated antigen was then sprayed on the glass fiber. The nitrocellulose membrane with GST-NcSAG1t, GST, and IgG, as well as the conjugated pad, sample pad, and absorbent pad, was assembled on an adhesive card and cut into 3-mm-wide strips (Fig. 1, lane 1).

To evaluate the potential use of the ICT with GST-NcSAG1t for the detection of specific antibodies to *N. caninum*, serum samples collected from four mice and four dogs pre- and postexperimental infection with *N. caninum* were
tested. All serum samples from postinfected mice (2 months postinfection) and dogs (2 months postinfection) were positive (Fig. 2), whereas all serum samples from preinfected mice and dogs were negative (Fig. 2). On the other hand, serum samples from four mice experimentally infected (2 months postinfection) with a closely related parasite, *Toxoplasma gondii* Beverley strain, were negative according to the ICT (Fig. 2). These results suggest that the ICT with GST-NcSAG1t not only could detect the specific antibodies to *N. caninum* but also could discriminate between neosporosis and toxoplasmosis, which has been thought to be important because some animals, such as dogs, cattle, sheep, and horses, can be naturally infected with both *N. caninum* and *T. gondii* (5, 10, 11, 12, 13).

The ICT with GST-NcSAG1t was evaluated by using 20 known seropositive bovine serum samples and 20 seronegative bovine serum samples previously diagnosed by immunofluorescent-antibody test (IFAT) (14). All IFAT-positive bovine sera were positive and all IFAT-negative bovine sera were negative by the ICT. A total of 96 field serum samples collected from cattle in Yanbian, China, were then investigated by use of the ICT, and the results were compared with those of the previously developed ELISA with GST-NcSAG1t (2). As shown in Fig. 3 and Table 1, of 96 serum samples, 23 (24.0%) were positive according to the ICT, and 19 (19.8%) were positive according to the ELISA. The relative sensitivity and specificity of the ICT were 94.7% and 93.5%, respectively, when the ICT was considered positive when both control and test lines turned purplish red at a dilution of 1:10.

**TABLE 1. Comparison of ICT with ELISA for detection of antibodies to *N. caninum* in cattle**

|          | ELISA<sup>a</sup> results | ICT<sup>b</sup> test results |
|----------|---------------------------|-------------------------------|
|          | No. (%) positive | No. (%) negative | Total (%) |
| No. (%) positive | 18 (18.8)     | 1 (1.0)                 | 19 (19.8) |
| No. (%) negative | 5 (5.2)        | 72 (75.0)               | 77 (80.2) |
| Total (%)       | 23 (24.0)      | 73 (76.0)               | 96 (100)  |

<sup>a</sup> Antibodies to *N. caninum* were detected by ELISA with GST-NcSAG1t (2). The ELISA was considered positive when an optical density at 415 nm of ≥0.1 was observed at dilutions of 1:100 and above.

<sup>b</sup> Antibodies to *N. caninum* were detected by ICT with GST-NcSAG1t. The ICT was considered positive when both control and test lines turned purplish red at a dilution of 1:10.
corresponding ELISA was used as a reference. All ELISA-positive samples except one (18/19) were ICT positive. In addition, five ELISA-negative samples were ICT positive. The optical densities at 415 nm of five ELISA-negative samples that were positive by the ICT were near the cutoff point of 0.1 (ranging from 0.07 to 0.1). Western blot analysis with whole-tachyzoite lysate as an antigen was used to further verify the ICT with recombinant NcSAG1t would be reliable. The ICT was negative by the ICT was also negative in the Western blot analysis (data not shown). The degree of agreement between the ICT and ELISA was estimated by calculating the kappa value (16). The kappa value (0.99) indicates a very good agreement between the two tests. All of these results suggested that the ICT with recombinant NcSAG1t showed positive reactions in Western blot analysis, and the one ELISA-positive sample that was negative by the ICT could be attributed to its ability to detect all classes of immunoglobulins.

Recent studies have shown the prevalence of *N. caninum* infection in cattle and dogs in Asia. In Taiwan, in a dairy farm where 18 cases of abortion had been observed, up to 76.3% of cattle contained IgG and/or IgM antibodies to *N. caninum* (6). Kim et al. confirmed that 12.1% of cattle abortions in Korea were attributed to *N. caninum* (9). In Japan, dogs reared on dairy farms where cases of abortion had been observed or where the cattle were seropositive for *N. caninum* infection had a higher infection rate (31.3%) than dogs in urban areas (7.1%) (15). In this study, 24% of serum samples collected in this study, had a higher infection rate (31.3%) than dogs in urban areas.

Program (A-1), Ministry of Education, Culture, Sports, Science, and Technology, Japan, and by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

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