Evaluation of the Prevalence of Neosporosis Using the ImmunoComb Commercial Kit

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ABSTRACT

Neosporosis is a parasitic disease and a major cause of abortions in cattle, leading to considerable economic consequences. Since, and apart from abortions, cows are generally asymptomatic, and there is no effective treatment or vaccine. The main approach to address neosporosis is implementation of control programs. Control strategies are usually based on serological screening for evaluation of herd seroprevalence, followed by selective culling, selling of breeding animals or other management decisions. Serological testing for neosporosis is usually performed in the laboratory, using various methods, including IFAT and ELISA. This study was aimed to evaluate the use of the ImmunoComb bovine *Neospora* antibody test kit (Biogalc, Kibbutz Galed, Israel), for the detection of neosporosis. The kit was designed as a portable test which allows field use by veterinarians or farm personnel. A total of 288 serum samples were collected from heifers during days 110-120 of their first pregnancy. Each sample was serologically tested for neosporosis both by the ImmunoComb kit and by the gold standard IFAT. The agreement between both tests was evaluated using 1:200 and 1:800 IFAT cutoff titers. The agreement between tests was moderate (Cohen's kappa= 0.45, 72.56% agreement, P<0.001) for the 1:200 cutoff titer, and substantial (Cohen's kappa= 0.69, 84.72% agreement, P<0.001) for the 1:800 cutoff titer. The cutoff titer of 1:800 was selected since it was preciously shown to be the clinically relevant titer, associated with increased risk of abortion. For this cutoff titer, the ImmunoComb test had positive (PPV) and negative (NPV) predictive values of 84.7%. Therefore, it was considered that the ImmunoComb kit may be useful for farm owners wishing to conduct in-house screening for cows with a high probability of abortion.

Keywords: *Neospora*; Neosporosis; Cattle; Serology; IFAT; ImmunoComb; Israel.

INTRODUCTION

Neosporosis, caused by the cyst-forming parasite *Neospora caninum,* is a major cause of abortions in cattle (1, 2). Although infected cows do not show clinical signs of disease, during pregnancy dormant parasites in tissue cysts may re-emerge and infect the fetus. Fetal infection during pregnancy may result in fetal death and abortion or may result in the birth of congenitally infected calves (1, 3, 4). This route of vertical transmission is very efficient in cattle, and considered the main source of infection. Horizontal transmission by oocysts secreted by canids, the definitive hosts, is also possible (2, 5, 6).

Chronically infected cows are at an increased chance of abortions and repeated abortions. It had been shown that the risk of abortion increases in correlation with anti-*Neospora* antibody titer of the dam. Low antibody titers of 1:200 do not associate with abortions, while titers of 1:800 and higher are clinically significant regarding the chance of abortion (7). Antibody titers of infected cows tend to fluctuate over time, principally in cows with borderline titers such as 1:200, and may be influenced by the animal's immune status (3, 8).

Circulation of neosporosis in endemic cattle herds may lead to considerable economic consequences, due to abortions and repeated abortions (9). Moreover, vertical transmission, may extend the problem in the herd, due to the considerable rate of healthy calves borne congenitally infected. Since no effective treatment or vaccine is currently available for neosporosis, many infected farms aim to implement control programs to reduce or eliminate infection in the herd. These programs are often based on serological screening to detect infected cows and either remove them from the herd or use selective breeding to reduce the rate of congenital infection in replacement heifers.

Serological testing for neosporosis may be performed by several different methods. Immunofluorescence antibody test (IFAT) is a well validated method, which allows assessment of the antibody titer. However, this method is relatively labor-intensive and requires skilled personnel to interpret the results. In recent years, several commercial enzyme-linked immunosorbent assay (ELISA) kits became available, which still require laboratory equipment and trained personnel to perform. The results of these kits are calculated from a optical density (OD) reading, but are interpreted as dichotomous (yes/no) results. The ImmunoComb bovine *Neospora* antibody test kit (Biogal©, Kibbutz Galed, Israel) is unique, as it is adapted for use in field conditions, and does not require laboratory equipment or trained personnel. This test also produces dichotomous results. Although the results are presented in a semi-quantitative scale of 0 to 6, with only a result of 3 or higher being considered seropositive. The possibility of using such a kit on the farm, either by the attending veterinarian or farm personnel, may make such testing more accessible and affordable for farms wishing to implement *Neospora* control programs.

The aim of this study was to evaluate the use of the ImmunoComb kit for the detection of *Neospora* seropositivity in dairy cows.

MATERIAL AND METHODS

Sample collection

The study was conducted with the farm owner's consent and was approved by the Animal Experiments Welfare Committee of the Kimron Veterinary Institute (020_b18181_6).

The study population comprised of 288 heifers from four farms. All farms had a history of neosporosis. Blood samples were collected from the heifers on day 110-120 of their first pregnancy, between November 2020 and June 2021.

Blood was collected from the tail vein of each heifer into a serum sterile collection tube without anticoagulant. Serum was separated following centrifugation at 4000 *g* for 4 min, and kept frozen (at -20˚C) until testing.

Serological testing

The presence of anti-*Neospora* spp. antibodies was determined by immunofluorescence antibody test (IFAT), as previously described (10). In addition, each serum sample was also tested using the commercial ImmunoComb bovine *Neospora* antibody test kit (Biogal©, Kibbutz Galed, Israel), according to the manufacturer's instructions.

Statistical analysis

The agreement between the serological detection using IFAT and ImmunoComb was evaluated using spearman's correlation (ρ) and Cohen's kappa. Positive and negative predictive values were calculated for ImmunoComb in reference to IFAT as the gold standard.

Statistical significance was set at $p<0.05$. The analysis was performed using SPSS 29.0® (IBM Corp, Armonk, NY, USA).

RESULTS

Anti-*Neospora* antibody titers, measured by IFAT, ranged between 0 and 1:12,800 (Median=400, Interquartile range=800). Seventy-seven samples tested negative (26.7%), 67 samples had antibody titer of 1:200 (23.3%), 79 had antibody titer of 1:800 (27.4%), 58 had antibody tier of 1:3200 (20.1%) and seven had antibody titer of 1:12,800 (2.4%).

Using ImmunoComb, 144 (50%) samples tested positive for the presence of anti-*Neospora* antibodies, while 144 (50%) tested negative. ImmunoComb scores ranged between 0 and 5.5, but since this assay is not designed as a quantitative or semi-quantitative measure, only the dichotomous results are relevant, with a cutoff score of 3, as set by the manufacturer.

The correlation between the IFAT and ImmunoComb semi-quantitative results was moderate and significant ($\rho=0.727$, $P<0.001$).

The agreement between both tests was evaluated using two IFAT diagnostic cutoff values: 1:200 and 1:800 (Table 1). The 1:200 cutoff value was set to detect all positive indi-

viduals. The cutoff value of 1:800 have been demonstrated as the clinically relevant titer for increased risk of abortion. When seropositivity was defined by the IFAT titer of 1:200 the agreement between diagnostic methods was moderate (Cohen's kappa= 0.45, 72.56% agreement, P<0.001). When seropositivity was defined by an IFAT titer of 800 (clinically relevant) than the agreement was highly signficant (Cohen's kappa= 0.69, 84.72% agreement, P<0.001).

Referring to the IFAT results as the gold standard reflecting the true prevalence, the ImmunoComb had a positive predictive value (PPV) of 95.8% and 84.7% and a negative predictive value (NPV) of 49.3% and 84.7% for the detection of cows with antibody titers of 1:200 and 800 or higher respectively (Table 1).

Table 1. *Neospora* serological diagnosis using IFAT and ImmunoComb tests on 288 heifers. The diagnostic cutoff was set as 1:800 in IFAT and 3 usng the Immunocomb.

		ImmunoComb		Total
		Negative	Positive	
IFAT	**0**	71 (92%)	6	77
	1:200	51 (76%)	16	67
	≥1:800	22	122 (85%)	144
	Total	144	144	288

DISCUSSION

The ImmunoComb bovine *Neospora* antibody test kit is designed as a portable test that does not require laboratory equipment or scientific training and can be used in the field by veterinarians or breeders. Although in this study the test was performed in the laboratory, and by trained laboratory personnel, the use of the kit was quite simple, and could easily be applied in the field. Short training of designated farm workers is probably warranted, as the test does involve several consecutive steps that require attention. The interpretation of the results was relatively straight-forward, with the use of the reference color scale provided by the manufacturer. The fact that the final results are dichotomous makes it clearer for farm managers implementing control strategies.

IFAT was selected as the reference test for comparison, as it is semi-quantitative and allows for finer resolution with selection of different cutoff values for evaluating the sensitivity of the commercial kit. The results demonstrated good efficacy of the ImmunoComb kit for the detection of seropositive cows with titers of 1:800 or higher, but had lower sensitivity for the detection of titers of 1:200.

The titer of 1:200 had been considered a borderline titer as the impact in the risk of abortion is relatively low and in endemic herds this titer represents an unstable, varying and non-conclusive sero-status (11).Although the PPV for this titer was higher (95.8%), the NPV was lower (49.3%) thus, the test is less sensitive (65.4%) using this cutoff titer. It is important to consider that cows with titers of 1:200 are still infected with *Neospora* parasites, and the dynamics of infection may change in the future. Such cows may still give birth to congenitally infected calves. Therefore, if the aim of the control program is to eliminate neosporosis from the farm, a more sensitive detection method may be warranted.

Titers of 1:800 and higher have been shown to be associated with increased chance of abortions. In addition, *Neospora* seropositive aborting cows normally develop high antibodies titer following abortion. The immunocomb kit tested in this study detected such cows with a sensitivity and specificity of 85%, with similar positive and negative predictive values. Thus, it appears that the use of this test for selective management of seropositive cows is likely to help in detection of cows of high risk of abortion. Nevertheless, a diagnostic accredited laboratory is still recommended for more precise and reliable results.

CONCLUSIONS

In this study, the Immunocomb bovine *Neospora* antibody test kit was evaluated as an alternative to laboratory testing for screening of cows for *Neospora* infection. The test had 85% sensitivity and specificity, in cows with titer associated with abortion and was found to be useful for farm owners wishing to conduct in-house screening for cows with high chance of abortion or for diagnosing the cause of abortion in aborting dams.

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