NOTE *Parasitology*

Seroprevalence of antibody to NcSAG1 antigen of *Neospora caninum* in cattle from Western Java, Indonesia

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ABSTRACT. *Neospora caninum* can cause fetal abortion and neonatal mortality in cattle, and is a cause of economic concern worldwide. This study aimed to determine the prevalence of *Neospora caninum*-specific antibodies in cattle from Western Java, Indonesia. Serum samples from 991 cattle from 21 locations were tested for antibodies to *N. caninum* by using an enzyme-linked immunosorbent assay (ELISA) on the basis of recombinant NcSAG1. The overall seroprevalence was 16.6%, ranging from 0 to 87.5% in the sampled locations. The results of this study indicate latent infection rates of sampled animals were different in each location. Further studies are necessary to elucidate the relationship between *N. caninum* infection and abortion in cattle, and to identify risk factors for infection in high-prevalence environments.
KEY WORDS: cattle, ELISA, Indonesia, NcSAG1, *Neospora caninum*

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Neospora caninum is an intracellular Apicomplexan protozoan parasite, closely related to *Toxoplasma gondii* [6]. Neosporosis, the disease caused by *N. caninum,* is mainly observed in dogs and cattle. Dogs and cattle act as the definitive host and intermediate host, respectively [5]. Canine neosporosis causes neuromuscular paralysis [8], whereas bovine neosporosis causes fetal abortion and neonatal mortality [5]. Abortion, stillbirth and neurological disease associated with *N. caninum* are a cause of major economic problems in the livestock industry worldwide [6].

Neospora caninum infection has been detected serologically using an indirect fluorescent antibody test (IFAT) [2], immunoblotting [1] and several enzyme-linked immunosorbent assays (ELISA) [7, 10]. *Neospora caninum*-specific antibodies are a useful marker to identify animals at risk of abortion, however, information on seroprevalence of this pathogen is limited in Indonesia. Damriyasa [4] reported the seroprevalence was 5.1–8.0% in a sample of 438 Bali cattle in Bali by using an ELISA on the p38 surface antigen (NcSRS2) of *N. caninum* tachyzoites. Sardjana [9] reported 24% of a sample of 25 dairy cattle from the Batu-Malang region in Eastern Java were positive for *N. caninum* using an ELISA and direct agglutination test. NcSAG1 has been identified as a useful antigen to detect both acute and chronic infections of *N. caninum* [7, 10]. To date, there are no data on the seroprevalence of *N. caninum* in cattle from Western Java. Therefore, the aim of this study is to determine the seroprevalence of *N. caninum* in cattle from Western Java, Indonesia, using an ELISA based on the tachyzoite surface antigen, NcSAG1.

Blood samples were obtained from 991 cattle at 21 locations randomly selected in Western Java, Jakarta and Banten provinces, Indonesia. The sampling was performed in strict accordance with the recommendations in the Guidance for the care and use of animals for scientific purpose of the ethics consideration in Balai Veteriner Subang, Indonesia. The farms were located in 14 districts or cities; Tangerang, Jakarta, Bogor, Sukabumi, Karawang, Purwarka, Bandung Barat, Cimahi, Garut, Tasikmalaya, Cirebon, Kuningan, Ciamis and Banjar. (Fig. 1). Sera were separated by centrifugation and stored at −20°C until use.

Identification of *N. caninum*-specific antibodies was performed using an ELISA described previously [7] with slight modifications. The purified recombinant protein of NcSAG1 (rNcSAG1) fused with glutathione S-transferase was prepared as the antigen. Serum samples (1:200) and the horseradish peroxidase-conjugated anti-bovine total immunoglobulin (1:10,000, Bethyl Laboratories, Montgomery, TX, U.S.A.) were used. The absorbance at 415 nm (OD_{415nm}) was determined as the difference in the mean OD_{415nm} between the rNcSAG1 and blank wells. The cut-off point was determined as the mean OD_{415nm} value for standard *Neospora*-negative sera kept in our laboratory (n=5) plus 5 standard deviations. 95% confidence intervals for the seroprevalence were calculated using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, U.S.A.).

This is the first epidemiological study to detect *N. canunum* infection of cattle in Western Java, Indonesia, by using an ELISA on the basis of rNcSAG1 as a detection antigen.

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Fig. 1. Geographical distribution of the collection sites used in this study. White circles represent the location of sampled farms. The name of the district or city is displayed on the map.

Table 1. Seroprevalence for *N. caninum* antibodies to NcSAG1 in cattle from Western Java, Indonesia

District or city	Location ID	No. tested	No. of positive	Seroprevalence (%)	95% CI (%)
Tangerang	#1	24	3	12.5	2.7–32.4
	#2	47	0	0.0	0.0–6.2
	#3	51	1	2.0	0.0–10.4
Jakarta	#4	40	16	40.0	24.9–56.7
Bogor	#5	40	12	30.0	16.6–46.5
Sukabumi	#6	40	3	7.5	1.6–20.4
Karawang	#7	40	2	5.0	0.6–16.9
Purwakarta	#8	36	0	0.0	0.0–8.0
Bandung Barat	#9	40	35	87.5	73.2–95.8
	#10	40	19	47.5	31.5–63.8
	#11	153	17	11.1	6.6–17.2
	#12	80	12	15.0	8.0–24.7
Cimahi	#13	32	0	0.0	0.0–8.9
Garut	#14	40	9	22.5	10.8–38.5
Tasikmalaya	#15	40	11	27.5	14.6–43.9
Cirebon	#16	40	0	0.0	0.0–7.2
	#17	40	8	20.0	9.1–35.6
Kuningan	#18	8	1	12.5	0.3–52.7
Ciamis	#19	40	5	12.5	4.2–26.8
	#20	80	8	10.0	4.4–18.8
Banjar	#21	40	3	7.5	1.6–20.4
Total		991	165	16.6	14.4–19.1

CI: Confidence interval.

This in-house system can clearly differentiate sera infected with *N. caninum* from those infected with *T. gondii*, which has similar antigenicity. Additionally, sensitivity and specificity of the test are comparable with the IFAT [3]. Previous research has demonstrated anti-NcSAG1 antibody levels of experimentally-infected cows persisted long-term (over 12 months) [10], and therefore, this antigen could be used as both acute and chronic markers for *N. caninum* infection

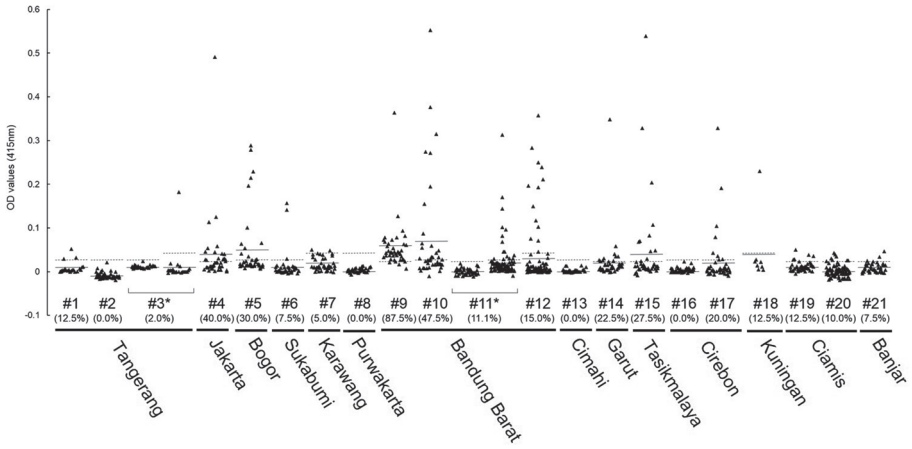

Fig. 2. Results of an ELISA to detect antibodies to rNcSAG1 in the surveyed areas. The seroprevalence for each farm is shown in brackets below the ID number. Dotted and solid lines indicate the cut-off and average values, respectively. The results of locations #3 and #11 were divided into the different reaction sets (*).

[7]. Antibodies to NcSAG1 of *N. caninum* were detected in 165 (16.6%) of 991 cattle. The highest seroprevalence was identified at location #9 (Bandung Barat) with a prevalence of 87.5%, followed by #10 (Bandung Barat), with a prevalence of 47.5%. The seroprevalence of the remaining locations ranged from 0 to 40.0% (Table 1). The higher OD_{415nm} values were observed at locations with high seroprevalence (Fig. 2). The occurrence of seropositive animals indicates that *N. caninum* is present in the areas with high seroprevalence. To date, transplacental transmission from a naturally infected dam to her fetus appears to be the only confirmed intraspecific, natural route of transmission for this parasite [6], and high seroprevalence in the present study may reflect latent infection in the sampled herds. Many cattle in locations with high seroprevalence have inactive tissue cysts containing bradyzoites, which may become active during pregnancy when the host is immunocompromised, increasing the risk of *N. caninum* transmission across the placental barrier. Further investigation is needed in locations with high seroprevalence to elucidate the role of *N. caninum* in abortion and neonatal mortality in cattle. In addition, it is necessary to determine whether domestic dogs or wild dogs shed *N. caninum* oocysts in the high seroprevalence locations. Many small farms in Western Java are managed by individual or groups of farmers, and therefore, the hygiene level varied among farms. The high seroprevalence and/or the high OD_{415nm} values may reflect contamination from feces of domestic dogs or wild dogs in these locations.

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