

Neosporosis In Cattle: Preliminary Study In Batu-Malang Region, Indonesia.

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ABSTRACT

Neospora caninum has been associated with abortion in cattle. Therefore, the present study using ELISA and Direct Agglutination tests on 25 dairy cattle in Batu-Malang region was performed to determine the infection rate. It was found that 6 (24%) of the dairy cattle were positive to *Neospora caninum*. The affected animals in the pregnancy condition were imported from Australia and New Zealand, believed to have brought in the disease.

Keywords: *Neospora caninum*, abortion, cattle.

Introduction

Neospora caninum is a protozoan parasite that is closely related to *Toxoplasma gondii*. Dogs are the definitive host but it was discovered in 1988 that it can infect cattle when administered orally. Horizontal transmission to cattle from a canine definitive host is unknown. Over the past decade, tachyzoites or tissue cyst of *Neospora* have been detected in naturally infected dogs, cattle, sheep, goats, deer and horse (Dubey, 2000 . Georgia *et al*, 2006), but infection in animal was often mistakenly diagnosis as Toxoplasmosis.

Neosporosis in cattle is characterized by abortion (Journell *et al*, 1999). Cows of any age may abort from 3 months gestation to term with most abortions occurring at 5-6 month gestation (Dubey and Schares, 2006). In France, Klein *et al*. (1997) have reported that 14%-26% of the abortions in cows are positive of *Neospora caninum* while Danatt *et al*. (1995) have been reported 29% infection. This is a preliminary study in Indonesia involving 25 dairy cows in Batu-Malang region, from which serum samples were obtained on the day or several days after abortion.

Material And Methods

Samples

Serum samples were collected from 25 dairy cows of KUD Batu - Malang region. The animals were between 2 to 4 years old with 400-500 kg of body weight. The sera collection involved 10 cows that aborted in the year 2000 and 15 cows that aborted in the year 2001. The serum samples were processed at the Laboratory of Reproduction, Pathology and Biotechnology of Ecole Nationale Veterinaire de Nantes in France.

Sample Processing

Sera samples were analyzed for antibodies to *Neospora caninum* using ELISA technique. *Neospora* antigen was coated

onto the 96 well plates at the concentration of dilute test samples one hundred-fold (1:100). Upon incubation for 30 minutes at room temperature with the test sera, antibody-antigen complex was formed. After washing four times with approximately 300 ul of phosphate buffered wash solution, remove the unbounded materials from the wells, an anti-bovine peroxidase conjugate was added, which bound to the bovine antibody in the wells. In the final step of the assay, the unbounded conjugate was washed away, and the enzyme (hydrogen peroxidase), substrate (hydrogen peroxides) and chromogen (3,3', 5,5' tetra methyl benzidine), were added into the wells. Subsequent color intensity was proportionally related to the amount of antibody present in the test sample. The absorbance was measured at 650 nm and results were calculated to interpretation samples were considered positive: $(O.D. \text{ samples} - O.D. \text{ control negative}) / (O.D. \text{ control positive} - O.D. \text{ control negative})$ should be greater or equal to 0,5 (Bjorkman *et al*, 1994; Dubey and Lindsay, 1996).

The other technique used to detect of *Neospora caninum* was the Direct Agglutination test, described by Romand *et al*. (1998). Sera were diluted 1:40 with phosphate buffer saline (pH 7.2) containing 2 mercaptoethanol and screened at 1:40 and 1:80 dilutions. Serum positive at 1:80 were subsequently submitted to serial 2 fold dilution (1:100 to 1:800). The interpretation of samples were considered positive is present of formation agglutination.

Results

The 10 sera collected in 2000 revealed 1 positive *N. Caninum* (Table 1). The 15 sera of 2001 revealed 6 positive *Neospora caninum* with 4 dubious results (Table 2).

The results by the direct Agglutination test is not to compare to the ELISA test but to confirm the accuracy of the ELISA test results.

Table 1: The first examination of 10 serum cattle aborted collected in the year 2000.

No.	Cow (Number)	Cow (Number)	Chlamydiosis	Q fever	Rhinotracheitis bovine infection	Neospora caninum
1	2302	0	0	0	0	0
2	3626	0	0	0	0	0
3	1603	0	0	+++	0	0
4	1511	0	0	0	0	0
5	5138	0	0	0	0	0
6	5142	0	0	0	++	0
7	1614	0	0	0	0	0
8	3182	0	0	0	0	0
9	3065	0	0	+++	0	0
10	3468	0	0	0	0	0

- : Negative ; + : Positive ; ++ : Strong Positive ; +++ : Very strong Positive.

The result of the study was done for the first examination of 10 serum cattle aborted collected in the year 2000, researchers used several test to detect the disease causing abortion in cattle such as Brucellosis, Chlamydiosis, Q fever, Rhino tracheitis bovine infection and Neospora caninum but

the objective of the study to confirm the potential role of Neospora caninum in abortion in cattle. The result of these test showed that one positive of Neospora caninum and Chlamydiosis, 6 positive of Q fever and Rhino tracheitis bovine infection (Table 1).

Table 2: The second examination of 15 serum cattle aborted collected in the year 2001.

No.	Cow (The owners cow)	ELISA Test	Direct Agglutination Test	Interpretation of Neospora caninum
1	Mitun	75	450	0
2	Sutomo	213	150	+/-
3	Sutomo	1200	150	+++
4	Mistioni	165	75	0
5	Deno	638	>600	++
6	Akuwan	365	150	0
7	Yarmanto	324	75	0
8	Rasid	64	75	0
9	Supriyanto	94	<50	0
10	Nawi	223	<50	+/-
11	Rahmad	254	150	+/-
12	Jono	414	300	0
13	Paidi	131	150	0
14	Asmanu	1011	150	+++
15	Rusdiono	269	<50	+/-

- : Negative ; + : Positive ; ++ : Strong Positive ; +++ : Very strong Positive.

In table 2, the second examination of 15 serum cattle aborted collected in the year 2001 as a follow up to the first examination in the year 2000 to determine incidence of Neosporosis in Indonesia by ELISA test and Direct Agglutination test. The result of the first test by ELISA confirm with the second test by Direct Agglutination test that revealed 6 positive Neospora caninum with 4 dubious.

Discussion

The aim of this study was to detect *Neospora caninum* infection in dairy cattle in Batu-Malang region of Indonesia. The diagnostic tool used was ELISA and the Direct Agglutination test. Several assays are available for detecting

antibodies to *Neospora caninum* in cattle, all of these assays are based on *tachyzoite* antigens.(Dubey and Schares, 2006). This test should provide easily available and inexpensive tools for serologic testing for antibodies to *Neospora caninum* as described by Romand *et al.* (1998). Serological test have the advantage that they can be applied antemortem and may provide information on the stage of infection. This enables us to detect the disease and assess the potential role it plays in causing abortion in cattle. (Dubey and Schares, 2006).

Neospora caninum was recognised in the early 1990 as a major cause of bovine abortion worldwide (Pare *et al.*, 1996). It have eventually established the transplacental transmission of *Neospora caninum* as a major route of infection in cattle. The

antibody response was observed in late gestation with abortion during the second trimester of pregnancy. In cattle herds with endemic abortion due to Neosporosis there is often a positive association between the sero status of mothers and daughters, i.e. there is evidence that the major route of transmission in these herds is vertical. (Dubey and Schares, 2006).

Hemphill and Gottstein (2000) reported that the infection may be propagated vertically through successive generation. However, trans-placental or vertical transmission seemed to be very efficient for transmitting *Neospora caninum* in cattle. Conrad (1993) and Wouda (1997) have reported that *Neospora caninum* seropositive cows have an increased risk of abortion. The incidence of this disease in dairy cattle in Indonesia was perhaps the result of importing cows in probably pregnant condition from Australia or New Zealand.

Hall *et al.* (2006) have reported that prevalence of *Neospora caninum* in Australia (NSW) dairy cattle was higher. Previously, the only one prevalence data available has been from individual dairy herds and the prevalence range from 10 % - 31 % in other Australian states estimates of *Neospora caninum* prevalence are 4 % in Western Australian beef and dairy cattle to 15 % in central Queensland beef cattle. The prevalence in other countries has range from 2 % to 45 % among non aborting herds.

Conclusion

This study was done to confirm that *Neospora caninum* that infected dairy cattle in Batu-Malang were imported from Australia and New Zealand in their pregnancy condition.

Recommendation

This study recommendation for the next step of this study should be examination for all the dairy cattle how to treat the cows was infected and to prevent for the cows that will be imported from Australia and New Zealand by health examination.

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