SEROPREVALENCE OF *NEOSPORA CANINUM* ANTIBODIES IN CATTLE IN KERALA – A PRELIMINARY INVESTIGATION

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Abstract

A total of 309 blood samples were collected from cattle of various organized livestock farms of Kerala to investigate the presence of antibodies to Neospora caninum. The animals tested were all adult animals.The samples were subjected to N.caninum antibody test,cELISA using the kit from VMRD. The test procedure as instructed in the manufacturer's manual was followed.A test sample that produced .>/ 30 % inhibition was taken as positive and that which produced< 30% inhibition was taken as negative. Accordingly, it was found that 86 samples out of 309 had antibodies to N.caninum (27.83 %). The results indicate the likelihood of this abortifacient being prevalent in cattle population in our state.

Keywords: *Neospora caninum*,cattle, cELISA

Neosporosis, caused by *Neospora caninum* once considered as emerging, is now well established in most parts of the world. It is known to cause abortions and still birth in cattle, sheep and goats.Loss of pregnancy will lead to significant loss of potential income to the farmer. Anderson *et al.* (2000) recorded high seroprevalence of this organism in cows that had aborted. Hence it seems vital to look into the problem of neosporosis in cattle and a study was thus undertaken to screen the presence of *N.caninum* antibodies in cattle sera so as to examine the magnitude of the infection among cattle in Kerala.

Materials and Methods

A total of 309 blood samples were collected from cattle of various Government/ Kerala Veterinary and Animal Sciences University livestock farms of Kerala (Table) to investigate the presence of antibodies to *Neospora caninum*.

All the animals were adult animals. History of abortion if any was recorded.The samples were subjected to *Neospora caninum* antibody test; cELISA using the kit from VMRD (Meenakshi *et al.*,2007). The test procedure as instructed in the manufacturer's manual was followed.

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Kit Contents

A. Antigen coated wells	5 plates
B. Positive control	3.6 ml
C. Negative control	3.6 ml
D. 100X Antibody peroxidase conjugate	500 µl
E. Conjugate diluting buffer	60 ml
F. 10x wash solution concentrate	2*120 ml
G. Substrate solution	60 ml
H. Stop Solution	60 ml

Working Solutions

1. 1X Antibody peroxidase conjugate was prepared by diluting one part of 10X wash solution concentrate with 9parts of distilled water

Test Proper

1. 50µl of the undiluted test serum samples were transferred to the antigen coated wells.
2. Equal amount of positive and negative control sera were also transferred in duplicate in each single plate.
3. The plate was tapped on the sides to ensure proper coating and mixing of samples.
4. Incubated the plate uncovered for one hour at room temperature.
5. Washed the plate three times by striking the plate on a paper towel and rinsing with 1X wash solution. Washing procedure was repeated three times.
6. Added 50µl of diluted antigen peroxidase conjugate and incubated for 20 min at room temperature, with the plate uncovered.
7. Washing procedure was repeated three times.
8. Added 50µl of substrate solution to each well. The sides were tapped to ensure proper mixing and coating. The plate was incubated at room temperature for 20 min uncovered.
9. The stop solution (50µl) was then added to each well and the contents gently mixed by tapping the sides of the plate several times.
10. The plate was then read in an ELISA reader at 620 nm.

Test Validation

The mean of negative controls must produce an optical density (OD) ≥0.30 and ≤2.5 while that of positive controls must produce an OD≥30% inhibition.

The per cent inhibition (% I) was calculated as follows:

%I= 100 –(sample O>D * 100) divided by(Mean negative control O.D))

Interpretation

A test sample that produced >/ 30% inhibition was taken as positive and that which produced < 30 % inhibition was taken as negative.

Results and Discussion

86 out of 309 cattle sera examined had antibodies to *N. caninum* (27.83 %) as furnished in the table.

Table: Prevalence of *N.caninum* in cattle by cELISA

Sl.No	Farm	Number examined	Number positive	Percent positive
1	University Livestock Farm,Mannuthy	65	29	44.60
2	University Livestock Farm,Pookode	29	6	20.69
3.	Cattle Breeding Farm,Thumburmuzhi	8	1	12.5
4	Government Livestock Farm, Kudappanakkunnu	28	6	21.43
5	Jersey Farm,Chettachal	29	5	17.24
6	Jersey Farm,Vithura	24	6	25.00
7	Buffalo Breeding Farm ,Kollam	32	16	50.00
8	Cattle presented at clinics	74	17	18.08
	Total	**309**	**86**	**27.83**

The present observation indicates a high rate of prevalence of antibodies against *N.caninum*. Meenakshi *et al.* (2007) detected *N.caninum* antibodies in 8.19 percent of cattle and 50% of water buffaloes by ELISA in Punjab, suggesting the possibility of post natal transmission and revealing a significant association between prevalence and abortion. Sengupta *et al.* (2012) also spotted a prevalence of 12.61 and 9.97 percent in cattle and water buffaloes respectively from Maharashtra and they found a higher prevalence in unorganized herds than in organized farms. They also pointed out a vital association between seroprevalence and abortion.

In the present work, the sera were collected from cattle population at random. Only one buffalo from the Buffalo breeding farm, Kollam had a history of abortion and it was found positive for antibodies. Nevertheless, the presence of antibodies in other cattle highlights the significance of this infectious agent in causing abortion and loss of productivity in cattle population. The present study is an eye opener to the extent of *N.caninum* infection in cattle in Kerala and forms the pioneer work projecting the possible risk factors that could be involved in the transmission of this infection.

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