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REGULAR ARTICLES

Seroepidemiology revealed neosporosis as an under-realised entity in dairy cattle reared in South India

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Abstract

From the dairy herds ($n=16$) reared in few localities of South India with the history of reproductive inefficiency and incidental abortion, 176 sera samples from Jersey ($n=108$) and Holstein Friesian ($n=68$) crossbred cows were collected to detect prevalence of bovine neosporosis antibodies induced by *Neospora caninum* (*N. caninum*) through competitive enzyme linked immunosorbent assay (cELISA). The overall true prevalence was found as 23.5% whereas 7.7, 19.1, 25.7 and 40.5% was observed in cows of less than 1 year, 1 to 3 years, 3 to 6 years and above 6 years of age, respectively, denoting that increase in prevalence of *N. caninum* antibody correlated directly with the age. Among the cattle with and without abortion, 41.1 and 20.6% of true prevalence was found, respectively. The breed-wise true prevalence was 24.3 and 22.2% in Jersey and Holstein Friesians crossbred cows, respectively, indicating that crossbred cows of both breeds are equally susceptible to neosporosis. The prevalence of *N. caninum* antibody might be attributed to coexistence of dogs resulting in contamination of feed with dog faeces. The presence of dogs with the cattle herd predisposed the herd 3.59 times more to acquire neosporosis than the herd without dogs. The annual estimated economic loss in an aborted herd of having 11 animals was 0.23 million INR due to loss of both calf and milk yield.

Keywords *Neospora caninum* · Prevalence · Crossbred cows · Competitive ELISA · Economic loss

Introduction

India has 305 million female cattle which is the highest in the world (Statista Research Department, 2022). Among various disease aetiologies, *Neospora caninum* which is the cyst-forming coccidian parasite causes abortion and neonatal mortality in cattle with high economic loss to the dairy and beef industry and also causes clinical diseases in dogs. Abortion may be sporadic, endemic and epidemic storm like pattern which is the most shattering as more than 10% cows are affected over a short period of time (Dubey et al. 2007; Reichel, et al. 2013). It is also associated with decrease in milk production (Thurmond and Hietala 1997). The affected cattle may act as lifelong carrier (Dubey and Schares 2006) and number of animals may attain seronegative status occasionally due to fluctuation in antibody responses (Conrad et al., 1993). Vertical transmission (transplacental) appear to be highly efficient ranges from 75 to 100% (Anderson et al. 1997; Hall et al. 2005) and horizontal (postnatal) infection is comparative as low as 10% (Davison et al. 1999; Hietala and Thurmond 1999; Hall et al. 2005). The worldwide estimated economic loss due to *N. caninum* abortion was reported around US$1298.3 million.

In India, cattle is mainly reared for milk and manure as around 80 million households are depending on dairy farming to fulfil daily needs. Among them, the majority are small scale to marginal farmers without possessing grazing lands. Hence, the neosporosis certainly would impact on dairy farmers' economy and social status, as it can lead to huge economic losses by virtue of abortion and loss of milk production.

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The serological diagnosis is playing a major role to develop control strategies for neosporosis in adult cattle and precolostral calves (Dubey et al. 2007). The serological techniques primarily used to diagnose neosporosis comprise a variety of ELISA (in-house and commercial kits), indirect fluorescent antibody tests (IFAT) and *N. caninum* agglutination test (Ortega-mora et al. 2007). Seroprevalence of neosporosis in cattle was reported in other parts of India than the current study area (Meenakshi, et al. 2007; Sengupta, et al. 2012; Himachala, 2014). Hence, the aim of this study was to determine the seroepidemiology of neosporosis in the selected cattle herds of Tamil Nadu, South India for the first of its kind and its associated risk factors with economic impact in dairy cattle farming.

Materials and methods

One hundred and seventy six blood samples were collected from Jersey ($n=108$) and Holstein Friesian ($n=68$) cross-bred cows of the selective dairy herds ($n=16$) with a history of reproductive problem and incidental abortion cases at Dharmapuri, Erode, Karur, Namakkal and Tiruchirappalli districts of Tamil Nadu in Southern India. Among the cows ($n=176$) considered for the study, only 25 animals had the history of abortion. The sera samples were collected and stored at − 20 °C until tested.

Out of 176 sera samples, 36, 47, 55 and 38 were collected from below 1 year, 1 to 3 years, 3 to 6 years and above 6 years of age group, respectively. Nine out of 36 samples were collected from below 1 year old male animals. Among the samples, 153 and 23 samples originated from the herd with dogs and herd without presence of dog, respectively. During sample collection, data pertaining to the study was gathered using questionnaire to correlate the results with the risk factors and economic impact of the infection.

Competitive Enzyme Linked Immuno-sorbent Assay kit from Veterinary Medical Research and Development (VMRD), Pullman, Washington, USA was utilized to test the prevalence of *N. caninum* antibody. The reagents were prepared as per the manufacturer's guidelines, and test was performed as per the protocol of Baszler et al. (2001). The results were read at 620-nm wavelength in a microplate absorbance spectrophotometer (iMARK™—Biorad). Samples producing ≥ 30% inhibition were considered as positive, and samples producing < 30% inhibition were taken as negative using the formula: percentage of inhibition (% I) = 100 [1 − (sample O.D. ÷ mean negative control O.D.)].

Epidemiological data

The data were collected through semi-structured questionnaires from the farm owners of the selected herds in order to obtain information about the risk factors such as herd size, location, cattle age, breed, presence of dogs in the farm, and abortion history.

Statistical analysis

The animal apparent prevalence (*AAP*) was calculated using number of positive cases divided by total number of animals tested and the estimated animal true prevalence (*ATP*) was calculated using the Roagan-Gladen estimation (Greiner and Gardner 2000)

$$ATP = \frac{AAP + Sp - 1}{Se + Sp - 1}$$

where *Sp* is the test specificity, and *Se* is the test sensitivity and the *ATP* at 95% confidence interval (*CI*) for animal true prevalence using normal approximation was calculated as $ATP \pm 1.96 \times \sqrt{var(ATP)}$. The variance was expressed as

$$var(ATP) = \frac{AAP \times (1 - AAP)}{n \times Y^2}$$

Y $Se + Sp$-1

n no. of samples

The chi-square test was used to analyze the significance.

Economic loss

The economic loss for the selective herds was calculated as per the methodology mentioned by Chi et al. (2002).

Results

The mean optical density of negative control sera was 0.447 with standard deviation of 0.0884 against the Veterinary Medical Research and Development (VMRD) recommended value range between ≥ 0.30 and < 2.50, and the mean of the positive control sera's optical density was 0.172 with standard deviation of 0.014 against the recommendation of ≥ 30% inhibition value which was 62.52% in the current study. The sensitivity and specificity of cELISA test were 96% and 99%, respectively as per the manufacturer's (VMRD) reference.

The apparent prevalence of *N. caninum* antibodies among 176 sera samples tested was 23.3%, and true prevalence was found 23.5% (95% *CI*, 22.8–24.1%) in cattle. The result revealed that the seropositivity in various locations was statistically significant ($p < 0.05$) (Table 1).

Table 1 Overall prevalence of *N. caninum* antibody in dairy animals by cELISA

Herds	No. of samples tested	No. of positive	Apparent prevalence (%)	True prevalence (%)	True prevalence at 95% *CI* (%)
Dharmapuri	35	4	11.4	11.0	9.9–12.1
Erode	37	4	10.8	10.3	9.3–11.4
Karur	34	25	73.3	76.3	74.8–77.9
Namakkal	40	3	7.5	6.8	6.0–7.7
Tiruchirappalli	30	5	16.7	16.5	15.1–17.9
Total	176	41	23.3	23.5	22.8–24.1

Table 2 Prevalence of *N. caninum* antibody in dairy animals with a history of reproductive problem and incidental abortion

Herds	No. of samples tested	No. of positive	Apparent prevalence (%)	True prevalence (%)	True prevalence at 95% *CI* (%)	Odd ratio at 95% *CI*
Aborted	25	10	40.0	41.1	39.0–43.1	2.58 (1.06– 6.30)
Non aborted	151	31	20.5	20.6	19.9–21.2	

Table 3 Age-wise prevalence of *N. caninum* antibody in dairy animals

Herds	No. of samples tested	No. of positive	Apparent prevalence %	True prevalence %	True prevalence at 95% *CI* (%)
< 1 year	36	3	8.3	7.7	6.8–8.7
1–3 years	47	9	19.1	19.1	17.9–20.3
3–6 years	55	14	25.5	25.7	24.5–27.0
> 6 years	38	15	39.5	40.5	38.9–42.1

Table 4 Breed-wise prevalence of *N. caninum* antibody in dairy animals

Herds	No. of samples tested	No. of positive	Apparent prevalence %	True prevalence %	True prevalence at 95% *CI* (%)
Jersey crossbred	108	26	24.1	24.3	23.4–25.1
Holstein–Friesian crossbred	68	15	22.1	22.2	21.1–23.2

Table 5 Prevalence of *N. caninum* antibody associated with the presence and absence of dogs in the dairy herd

Herds	No. of samples tested	No. of positive	Apparent prevalence %	True prevalence %	True prevalence at 95% *CI* (%)	Odd ratio at 95% *CI*
Presence of dogs	153	39	25.5	25.8	25.1–26.5	3.59 (0.81–16.02)
Absence of dogs	23	2	8.7	8.1	6.9–9.3	

Based on the history of abortion, it was found 41.1% of true prevalence in aborted cases and 20.6% in non-aborted cases and were found to be significant ($p < 0.05$) (Table 2). The odd ratio revealed that the aborted animals were 2.58 times at more risk than non-aborted animals.

The findings of age-wise prevalence of neosporosis revealed more number of cases in the age group of above 6 years old, and statistically significant differences were observed between different age groups ($p < 0.05$) (Table 3).

Difference in positivity noted between Jersey ($n = 108$) and Holstein Friesian ($\mathbf{n} = 68$) crossbred cows was not statistically significant in the study ($p > 0.05$) (Table 4).

In the presence of dogs, true prevalence was 25.8%, and the animals were 3.59 times at more risk than the herd without dogs (Table 5). The calculated annual economic loss for the animals having herd size of eleven animals was 0.23 million INR.

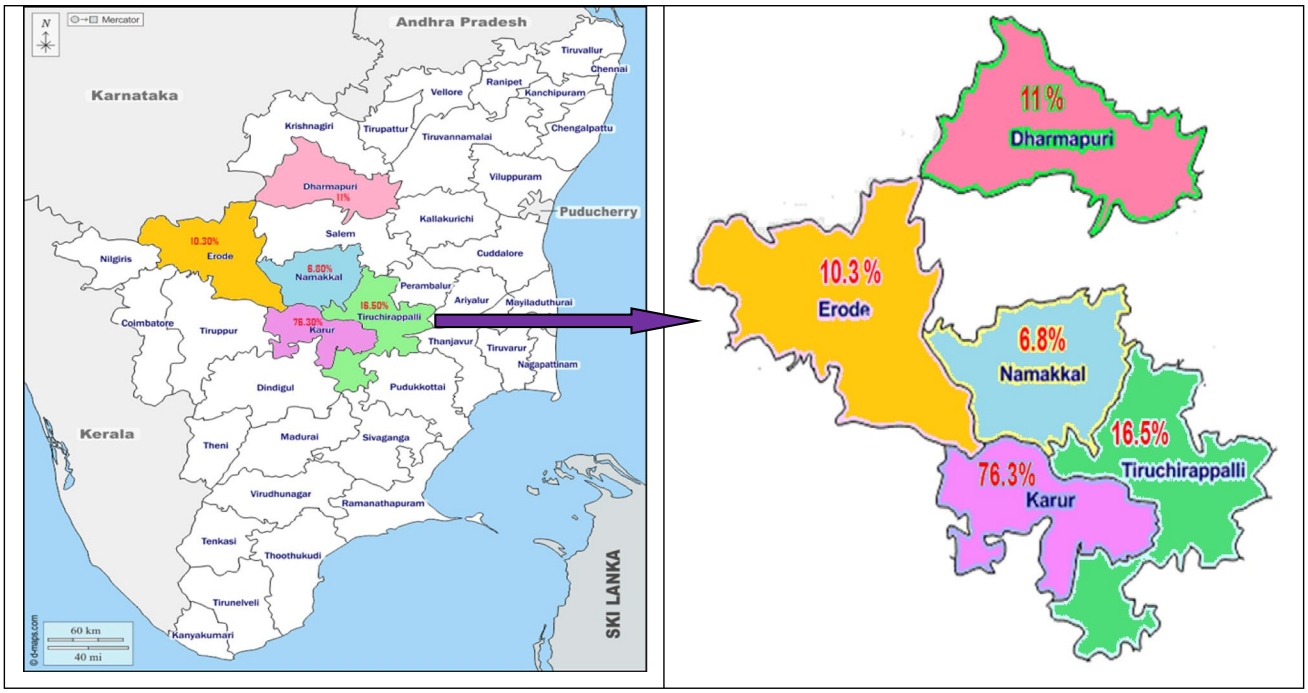

Fig. 1 Spatial distribution of *N. caninum* antibody in the study area

The spatial distribution of true prevalence among the five districts of Tamil Nadu in Southern India was found highest in Karur district (76.3%), followed by Tiruchirappalli, Dharmapuri, Erode and the lowest in Namakkal district (6.8%) (Fig. 1).

Discussion

The present study investigated the seroprevalence of *N. caninum* in 176 cattle in five districts of Tamil Nadu in South India using a commercial c-ELISA kit to establish associated risk factors in dairy cattle farming. The overall antibodies to *N. caninum* was 23.5% ($n=41$) in cattle of selected herds of Tamil Nadu, South India. The level of prevalence was comparable with earlier findings of global reports and even more than what was reported in Sweden (0.5%), Japan (5.74%), Brazil (7.6%), Algeria (19.6%), Northern Egypt (20.43%) (Bartels et al. 2006; Koiwai et al. 2006; Sousa et al. 2012; Ghalmi et al. 2012; Ibrahim et al. 2012) and in India (8.2% in Punjab state and 12.61% in Karnataka state) (Meenakshi et al. 2007; Sengupta et al. 2012), whereas the prevalence was slightly lower than the reports of Imre et al. (2012) in Western Romania (27.7%) and Himachala (2014) in Kerala state of India (28%). Thus, the findings of the current study denote that the *N. caninum* infection is not uncommon among cattle reared in parts of Tamil Nadu in southern India.

In the present study, the highest prevalence (76.3%) was observed in all three herds of Karur district and lowest (6.8%) was found in the four herds of Namakkal district of Tamil Nadu, India. The difference in positivity between districts might be influenced by varying degrees of co-existence of cattle and dogs. By extrapolating similar scenario in other area, it could be assumed that a huge economic loss could be caused by *N. caninum* in milch animals.

Higher prevalence of *N. caninum* antibodies among aborted cows (41.1%) than in non-aborted cows (20.6%) noted in the present study was incongruous to the findings of other reports (Vural et al. 2006; Yadav et al. 2016). There was a strong correlation between the prevalence and time lapsed from last abortion. The prevalence in cows which aborted within 2 years was higher (68.6%) than in cows with no history of abortions (26.2%) which could be compared with reports of Llano et al. (2018), as higher prevalence was noticed among recently aborted cows.

Based on current findings, it could be said that the risk of being seropositive for neosporosis increased with age or gestation number in dairy cattle. This was in line with reports of Bartels et al. (2006) from Spain and Wei et al. (2022) from China. The 'increased positivity' for neosporosis in older animals might be ascribed to the high probability of getting exposed to *N. caninum* in aged cattle through horizontal route of transmission from the positive cows or herd. In contrast, 13 to 24 months old dairy cattle was associated with lower prevalence than in cattle 7 to 12 months old and

cattle older than 24 months in England (Davison et al.1999), and an increased age was associated with decreasing prevalence of neosporosis in Sweden (Bartels et al. 2006). The difference in age-related prevalence of neosporosis between various farming conditions might be attributable to variation in the number of co-existing dogs with or without proper deworming.

Among male (data not presented) and female animals in the study area, the prevalence was only observed in the females; moreover, the male calves were not reared beyond 6 months of age. Furthermore, the prevalence of neosporosis between Jersey and Holstein-Friesians crossbred cows differed non-significantly in prevalence indicating that there was no difference in the susceptibility to neosporosis among these two breeds. Furthermore, few reports found that there was less susceptibility in native breeds than in described breeds (Bartels et al. 2005, 2006).

The reported prevalence rate of neosporosis in the crossbred cows of the study area might be due to vertical transmission by which the infection might be maintained in the herds with persistently infected progeny and/or continuous exposure to contaminated feed and water from the defecation of affected dogs (Anderson et al. 1997; Dijkstra et al. 2002; Dubey et al. 2007), and the presence of farm dogs, its population and frequency of dogs defecating in a feed manger and water increase the risk of abortion in herd level due to *N. caninum*. The introduction of a persistently infected heifer to a seronegative herd favours the spread of infection. The life cycle of *N. caninum* in the cows of the study area might be expected to continue on the account of vertical transmission leading to persistent congenital infection and presence of dogs (Fioretti et al. 2003; Guy et al. 2001).

The results indicated that the presence of dogs is a major risk factor for the horizontal transmission of *N. caninum.* This finding was endorsed by the reports from USA (Barling et al. 2001). The presence of dogs increased 3.59 times more risk for neosporosis in the dairy cattle of present study locations. This finding was comparable with Yadav et al.'s (2016) report which stated the risk of acquiring neosporosis was enhanced to 4.16 times in the presence of dogs. It was found that deficiency in hygienic measures to prevent dogs from feeding on fetal membranes or other infective materials by farmers (Schares et al. 2004; Darijani et al. 2021).

Prevention of *N. caninum* exposure involves the removal of the seropositive cattle and/or rear the cattle in areas free from other seropositive cattle and dogs. The economic loss calculated for abortion induced by *N. caninum* and associated milk production loss was 0.23 million INR in a herd having 11 animals which was in line with the report of Chi et al. (2002) who assessed that the annual loss would be CND$ 2304 (0.14 million INR) per 50 dairy cattle herd.

It is concluded that the seroprevalence of neosporosis which was found to be higher than what was reported from other parts of India and was confirmed in one fourth of the cows from selective herds of Tamil Nadu, India. The presence of *N. caninum* carriers and dogs in the farm was the most important risk factors for the occurrence of the neosporosis. The findings of the study pave the way to develop strategies for prevention and control of neosporosis which can be a catastrophe to the robust and growing Indian dairy industry.

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Author contribution M. Saravanajayam contributed in the study design, blood sample collection and laboratory and data analysis and article preparation. K. M. Palanivel contributed in the scientific review, supervision of the study and article correction. S. Saravanan contributed in the procurement of reference materials and analytical chemicals. A. Balasubramaniam contributed in the scientific critical review and manuscript writing. All the authors read and approved the final manuscript.

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Data Availability The data are available upon request.

Declarations

Ethics approval Not required.

Statement of animal rights Blood collection was performed by a veterinarian with respect and preservation of animal health and welfare.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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