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Serological Studies on Neosporosis and Risk Factors Assessment among Bovines of Haryana, India

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ABSTRACT

Background: Bovine neosporosis causes reproductive disorders and abortion among bovines resulting in production and economic loss in meat and dairy industries. The current study was done to know about the status of neosporosis among bovines of Haryana State.

Methods: A total of 165 serum samples from bovines of Haryana, having history of reproductive disorders or abortion were screened for brucellosis as well as neosporosis by ELISA.

Result: All the samples were found as serologically negative for brucellosis, but seropositivity of 33.94% was observed for neosporosis by the monoscreen antibody ELISA. A significantly higher seropositivity was found among buffaloes (53.85%) than cows (21%), having an Odds ratio of 4.38. All the seropositive animals were associated with abortion with a prevalence rate of 36.36%, while none of male animals were diagnosed as seropositive. Bovines of age group 3-5 years were more seropositive. The bovines which had an abortion at third trimester of pregnancy were more seropositive (43.05%) than first (18.75%) and second trimester (38%) aborted bovines. The multiparous animals exhibited higher seropositivity (41.07%) in comparison to primiparous (23.81%) bovines. The present study confirms the presence of *N. caninum* antibodies in bovine population of Haryana State.

Key words: Abortion, Bovine, Haryana, Neosporosis, Risk Factor, Seropositivity.

INTRODUCTION

Bovine neosporosis is an emerging global concern, which results in production and economic loss in meat and dairy industries. Bovine neosporosis, a major cause of spontaneous abortion in cattle is caused by *Neospora caninum*. Global economic losses due to neosporosis to the beef and dairy industries are estimated up to one billion US dollars annually (Miroud *et al*., 2019; Semango *et al*., 2019). *N. caninum* is an obligate intracellular apicomplexan protozoan parasite having canines as natural definitive hosts. The sexual reproduction occurs within definitive host and they excrete environmentally resistant oocysts in faeces. Bovines are intermediate hosts in which asexual development occurs (Dubey *et al*., 2007; King *et al*., 2011).

Neosporosis in bovines has been reported from several countries (Yadav *et al*., 2016; Llano *et al*., 2018). *N. caninum* causes reproductive disorders like abortion, stillbirths, mummification, embryo resorption and increases calving intervals in bovines (Noori *et al*., 2019). It also causes reduced milk yield in dairy animals, decreased growth rate and feed deficiency. Both the vertical and horizontal routes of transmission are possible, while the dominant route of infection in bovines is transplacental method. Vertical transmission occurs when an infected dam of the intermediate host transfers tachyzoites through the blood stream to its fetus (Dubey *et al*., 2007). Horizontal transmission is possible by the ingestion of oocysts through contaminated feed and water (Talafha and Al-Majali, 2013; Ansari-Lari, 2020). The presence of definitive hosts like dogs that have access to the aborted foetuses and placentas in the dairy farm and subsequent shedding of faecal oocysts in the environment may aggravate the transmission by the horizontal route (Llano *et al*., 2018; Miroud *et al*., 2019; Darijani *et al*., 2021).

The clinical sign of neosporosis is abortion in pregnant bovines and infected calves may show neurological signs (Marugan-Hernandez, 2017). The neosporosis abortions are of epidemic, endemic or sporadic in nature. Abortion due to *N. caninum* may occur at any time of gestation (Sengupta *et al*., 2013), but highest rate of abortions observed during second trimester of pregnancy (Hall *et al*., 2005; Llano *et al*., 2018). Asymptomatic healthy calves which are infected congenitally through transplacental transmission may contribute to prevalence of neosporosis if they are maintained as breeding stock (Marugan-Hernandez, 2017).

The serological tests are usually used to diagnose neosporosis in epidemiological surveys. The enzyme linked immunosorbent assay (ELISA) is the common test which

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detect antibody against *N. caninum* from bovine sera. The presence of antibody in an aborted animal or foetal fluid can't be conclusively establish that the cause of abortion is neosporosis, as many other abortifacient agents are also known (Miroud *et al*., 2019). But the evidence of *N. caninum* specific antibodies in dam sera gives an indication of existence of the infection or contact with these protozoa in the animals (Sengupta *et al*., 2013). It has been reported that the seropositive cows are 3-7 times more prone to abortion when compared to seronegative cows (Serrano-Martinez *et al*., 2019). Due to the abortifacient role and subsequent economic significance of *N. caninum*, the current study was designed to know about the status of bovine neosporosis in Haryana State and its association with abortion in dairy bovines.

MATERIALS AND METHODS

Samples

A total of 165 serum samples from bovines (100 cattle and 65 buffaloes) received in the College Central Laboratory, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana during the period June 2020 to May 2021, were used for the current study. The details of the animals like species, age, breed, parity, abortion history etc were collected from dairy farmers/entrepreneurs. All these animals were associated with history of reproductive disorders or abortion. These animals were having no history of vaccination against bovine neosporosis. All the sera used for this study were tested for brucellosis by Rose Bengal Plate Test as well as ELISA.

ELISA

The serum samples were screened for the presence of *Neospora caninum* antibodies using a commercially available ELISA kit (Bio-X Diagnostics, BELGIUM) following manufacturer's guidelines. The microtitre plates sensitized with *Neospora caninum* SRS2 recombinant protein were used in the study.

Statistical analysis

Seroprevalence was determined by the ratio of positive animals to the total number of animals examined. Confidence interval (CI) was constructed at 95% level of confidence. Retrospective analysis of the epidemiological data was performed in order to obtain the statistical relationship between presence of antibody against *N. caninum* and the risk factors like species, sex, age, abortion history, parity and pregnancy status (in trimester) in which abortion occurred. The logistic regression model was applied along with Odds ratio (OR). The statistical significance was determined at $p \leq 0.05$ by chi-square test. The data collected were analyzed using Statistical package for Social Sciences (SPSS) software for windows (Version 15, USA).

RESULTS AND DISCUSSION

All the sera used for this study were diagnosed as serologically negative for brucellosis by Rose Bengal Plate Test as well as ELISA. So, the current study was further designed to know about the status of other abortifacient agents in these animals, other than *Brucella* organisms. *Neospora caninum* is considered one of the most frequent infectious organisms causing abortion in bovines worldwide. In our study, out of 165 serum samples, 33.94% samples were seropositive for neosporosis by this monoscreen antibody ELISA. Among these, 21 samples were from cattle and 35 samples were from buffaloes with a seropositivity of 53.85% and 21%, respectively (Table 1). The presence of *Neospora caninum* antibodies in the bovines indicates the existence of infection in these animals. Seroprevalence of *N. caninum* in dairy cattle has been reported in different regions of world. The reported seroprevalence of *N. caninum* exposure in cattle ranges between 7.6 and 41% in the Americas, 10.7 and 19.6% in Africa, 4.1 and 43% in Asia, 0.5 and 27.7% in Europe and 10.2% in Oceania (Semango *et al*., 2019). Seroprevalence of neosporosis in buffalo were reported upto 88.3% worldwide, while that from India was 9.97%-50% (de Barrows *et al*., 2020). Mahajan *et al*. (2019) reported seropositivity of 10.5% in cattle and 21.6% in

Table 1: Seropositivity and risk factor assessment of bovine neosporosis.

Risk factors	Categories	No. of animals examined	Sero prevalence (%)	Odd's ratio	95% Confidence Interval	P value
Species	Buffalo	65	53.85			
	Cow	100	21	4.38	2.21-8.7	<0.0001
Age	<3 year	21	14.29			
	3-5 year	86	37.21	3.556	0.970-13.022	0.0555
	>5 year	68	36.21	2.681	0.712-10.097	0.145
Stage of pregnancy in trimester	I	32	18.75			
	II					
	III	50	38.0	2.656	0.924-7.632	0.0697
		72	43.05	3.276	1.202-8.931	0.0204
Parity	Primiparous	42	23.81			
	Multiparous	112	41.07	2.25	0.983-5.15	0.0550

buffaloes from Punjab, while Abdeltif *et al.* (2022) reported seroprevalence of 36.2% in cows from Northeast Algeria. In our study, a significantly higher seropositivity was found among buffaloes than cows, having an Odds ratio of 4.38. Mahajan *et al*. (2019) also found higher seropositivity and abortion rate among buffaloes when compared to cows from Punjab.

It was found that 32 (37.21%) animals from the age group 3-5 years were seropositive for neosporosis. A seropositivity of 36.21% and 14.295 were observed from age groups of >5 years and <3 years, respectively. The results obtained were statistically analyzed and found that the seropositivity of *N. caninum* antibodies in different age groups were statistically non-significant. This result is in accordance with the findings of Yadav *et al*. (2016). Llano *et al*. (2018) and Serrano-Martinez *et al*. (2019) also reported that 3-5 age groups of animals were at more risk to the infection when compared to other age groups. However there have been reports that seroprevalence of *N. caninum* decreased significantly with the increase in age of cows (Noori *et al*., 2019), while some researchers couldn't find any relationship of the disease and the role of age (Darijani *et al*., 2021).

It was found that, all the seropositive animals in our study were associated with abortion or reproductive problems like infertility with seropositivity of 36.36%. None of the male animals were positive for neosporosis in this study. The animals which had abortion at third trimester of pregnancy were more seropositive (43.05%) for neosporosis, when compared to first (18.75%) and second trimester (38%). This may be due to the immune system of pregnant animals which is more exposed to *N. caninum* during middle and last trimester of pregnancy rather than first trimester (Abdeltif *et al*., 2022). Several other studies also reported association of abortion history with seroprevalence of *N. caninum* (Sengupta *et al*., 2013; Yadav *et al*., 2016; Noori *et al*., 2019; Llano *et al*. (2018; Miroud *et al*., 2019; Changoluisa *et al*., 2019; Mahajan *et al*. (2019). The multiparous animals exhibited higher seropositivity (41.07%) for neosporosis in comparison to primiparous (23.81%) bovines. Llano *et al*. (2018), Miroud *et al*. (2019) and Serrano-Martinez *et al*. (2019) also found a significantly higher proportion of *N. caninum* antibodies in multiparous dairy cattle.

The rural areas of the State where the bovine herds are reared have easy and constant access to the definitive host like dogs. The presence of definitive host in the premises of bovine farms may aggravate the transmission of the parasite by horizontal route. The lacunae in hygienic managemental farm practices can aggravate the infection level in bovines because of the ingestion of oocysts through contaminated food, fodder and water. Since this infection has significant impact on economy of the livestock industry, the awareness among farmers is required to prevent the neosporosis and biosecure the bovine population of the State. The presence of *Neospora caninum* antibodies in these bovines indicates the exposure of the animals for this organism. This indicates neosporosis may have been associated with abortion in these animals, giving a second thought on other abortifacient agents, which should be studied in future elaborately, involving the entire livestock population of the State.

CONCLUSION

The present study confirms the presence of *N. caninum* antibodies in bovine population of Haryana State. The transmission of infection is probably from the environment by horizontal route, due to the close association of definitive hosts like dogs in the premises of livestock farms in the State. The hygienic disposal of aborted materials in order to prevent the access of definitive hosts to the animal shed should be practised. The control measures for the prevention of *N. caninum* infection in bovines to be followed. The segregation or culling of seropositive cows and vaccination are also advised.

Conflict of interest

All authors declare that they have no conflict of interest.

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