The Haryana Veterinarian (March, 2023) **62(SI),** 44-47 ISSN 0033-4359 (P) **Research Article**

SEROPREVALENCE STUDIES AND ASSOCIATED RISK FACTORS FOR DETECTION OF NEOSPOROSIS IN BOVINE ABORTIONS

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ABSTRACT

Bovine neosporosis caused by obligate intracellular protozoan parasite *Neospora caninum* is mainly associated with abortions and consequently causing enormous economic losses to livestock farmers worldwide.The sera samples from animals were collected randomly from various organized and unorganized farms located in three agro-climatic zones of Punjab. Seroprevalence of bovine neosporosis was found to be 11.48% by indirect ELISA. The seropositivity found in animals with previous abortion history (30.36%) was significantly higher (Chi square=28.574; p=0.000) than animals without previous abortion (3.13%) and animals with previous abortion history were 9.7 times (RR=9.714, CI=3.294-32.350) more at risk to be seropositive than animals without previous abortion history. This present study revealed that there is no significant association of neosporosis with age and species of the animal and presence or absence of dogs in the farms. Thus, present study provides the information about the effect of various risk factors on seropositivity of neosporosis in cattle.

Keywords: Abortions, Buffalo, Cattle, Neosporosis, *Neospora caninum*, Seroprevalence

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Neospora caninum is an intracellular protozoan parasite which affects the reproductive performance of bovine and causing abortion. First report of neosporosis-like condition in dogs suffering from myositis and encephalitis was found in Norway (Bjerkas *et al.*, 1984). Toxoplasma gondiihas a structural similarity with *N. caninum* which leads to the misdiagnosis of bovine neosporosis until 1988. After being isolated from canine cases with symptoms of encephalitis and myositis, the aetiological agent was named as *Neospora caninum* (Dubey *et al.*, 1988). Dogs act as both definitive host and intermediate host for this parasite where the sexual multiplication happens that shows neuromuscular problems which leads to death while cattle were the principal intermediate hosts, with abortion being the notable clinical sign (Dubey *et al.*, 2003). In bovines, the parasite causes repeated abortions, neonatal deaths, stillbirths, embryo reabsorption and early fetal loss (Dubey and Schares, 2011). Brain and heart damage is considered as a primary cause of abortion in three to eight months old bovine foetuses. Abortions in most cases are found between 5 to 6 months of gestation. Clinically normal but congenitally infected calves which were born to seropositive dam accounts for as high as 95% of live births. It could cause abortion storms with incidences as high as 10%, but most commonly found as an endemic cause of bovine abortion (Dubey, 2003). Transmission of this protozoan in cattle includes transplacental infection through tachyzoites (vertical transmission) and infection by ingestion of sporozoites containing oocysts which are shed by a definitive host (horizontal transmission). Once infected, a cow remains infected for rest of its life and infection can be passed down through generations. Endogenous transplacental transmission following reactivation of this protozoan infection in persistently infected cow during pregnancy, ranging from 50 to 95%, thereby playing a major role in maintenance and continuance of its pathology in cattle population (Santolaria *et al.*, 2011). In last few years, lot of work has been done for diagnosis of bovine neosporosis, both in vivo and in aborted foetuses. Bovine neosporosis in live animals is usually diagnosed by an enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT). ELISA is a valuable test for finding an anti *N. caninum* antibodies in serum or milk (Meenakshi *et al.*, 2007; Yao *et al.*, 2009; Alvarez-Garcia *et al.*, 2013). In this study, the seroprevalence of neosporosis in bovine was discussed using commercially available indirect ELISAkit.

MATERIALS AND METHODS

The present study was conducted from October 2020 to September 2021 in Animal Disease Research Centre and Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Seroprevalence studies: Five ml blood from each animal drawn aseptically without anticoagulant. Serum separation from blood samples was done by centrifugation of blood at 3000 rpm for 10 minutes. After separation sera was collected in a plastic vial and stored at -20 degree

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celsius until evaluated. Out of 184 sera collected from various dairy cows, there was 135 cattle and 49 buffalo sera in all (Table 1). A commercial indirect ELISA kit was employed to analyse these samplesas per the manufacturer's guidelines (BioX diagnostics, Belgium). The optical density (OD) of control and test samples was measured at 450 nm in an ELISA plate reader. IRPC (Positive Index Calculation) of the sample was calculated using the following formula

$$\text{IRPC}(\%) = \frac{\text{Abs (Sample) - Abs (Control -)}}{\text{Abs (Control +) - Abs (Control -)}} \times 100$$

Samples IRPC ≥20 was disclosed as positive. Samples IRPC < 17 was disclosed as negative. Samples with values between both IRPC (17 and 20) was considered doubtful. In present study, risk factors of bovine neosporosis such as age, species, abortion history, dogs in the farms and various Agro-climatic zones were also studied.

RESULTS AND DISCUSSION

In this study, 184 serum samples have been collected randomly from various agroclimatic zones of Punjab and examined for presence of antibodies to Neosporacaninum using commercially available indirect ELISA kit. In 184 sera samples studied (Cattle = 135 and Buffalo = 49), seroprevalence of 11.41% (21/184) was detected for neosporosis. The seropositivity for neosporosis found in current study was similar to the work of Meenakshi *et al.* (2007), Lista-Alves *et al.* (2006) and Gottstein *et al.* (1999) who reported the seropositivity for neosporosis as 11.11%, 11.33% and 11.3%, respectively using indirect ELISA. However, higher seropositivity of 24.8% was recorded by Gharekhani and Yakhchali (2019) and 18.4% by Nematollahi *et al.* (2013) and 24.1% by Spilovska *et al.* (2015) by using commercial ELISA kit. Abortions caused by neosporosis can happen at any season of the year (Anderson *et al.*, 1991). Abortions are better to be expected in seropositive cows than seronegative animals (Davison *et al.*, 1999; Thurmond *et al.*, 1997), According to Pare *et al.* (1996), up to 95 percent of live calves from serology positive dams would have a congenital infection but clinically normal.

In 184 sera studied, seroprevalence of bovine neosporosis in cattle and buffalo using ELISA was detected as 9.63% and 16.32%, respectively (Table 2). Results of present study revealed that the buffalos showed non significantly higher (chi square =1.595; p =0.207, RR=0.590, 95% CI= 0.244-1.493) seropositivity (16.32%) than cattle (9.63%). Meenakshi *et al.* (2007) also reported significantly higher seropositivity in buffalos (p <0.01)

Table 1

List of districts selected for sampling

Zone	Districts	Number of samples collected		
		Total	Cattle	Buffalo
Sub mountain	Gurdaspur Hoshiarpur Ropar	34	25	09
Central plain	Amritsar Fategarh Sahib Ferozpur Jalandhar Kopurthala Ludhiana Moga NawanShehar Patiala Sangrur	104	75	29
Arid-irrigated	Bathinda Faridkot Mansa Muktsar	46	35	11

Table 2

Seroprevalence of bovine neosporosis based on species using indirect ELISA

Species	Number of animals examined	Number of animals found positive	Positivity in percentage
Cattle	135	13	9.63 %
Buffalo	49	8	16.32 %
Total	184	21	11.41 %

Table 3

Zone-wise seroprevalence of bovine neosporosis using indirect ELISA

Zone number	Name of the agroclimatic zone	Number of animals tested	Number of animals positive	Positivity in percentage
Zone 1	Sub mountain	34	04	11.76%
Zone 2	Central plain	104	11	10.58%
Zone 3	Arid irrigated	46	06	13.04%
Total		184	21	11.41 %

Table 4

Age wise seroprevalence of bovine neosporosis using indirect ELISA

Age group	Number of animals tested	Number of animals positive	Positivity in percentage
Heifer	41	2	4.88 %
Lactating animals	143	19	13.28 %
Total	184	21	11.41 %

Table 5

Relationship of bovine neosporosis with history of abortion using indirect ELISA

Animals with previous abortion history	Number of animals tested	Number of animals positive	Positivity in percentage
Yes	56	17	30.36 %
No	128	4	3.13 %
Total	184	21	11.41 %

Table 6

Seropositivity of bovine neosporosis in relation to presence of dogs in the farm using indirect ELISA

Presence of dogs in the farm	Number of animals tested	Number of animals positive	Positivity in percentage
Yes	90	13	14.44%
No	94	08	8.51%
Total	184	21	11.41%

than cattle in Punjab, India. Punjab has been categorized into three different agro-climatic zones, namely Sub Mountain zone, Central zone and Arid-irrigated zone. Number of samples from Sub Mountain zone is 34 followed by 104 in central and 46 in arid-irrigated zone. Out of 184 sera samples examined, the seropositivity of neosporosis was found to be 11.76%, 10.58% and 13.04% by ELISA in sub-mountain (zone 1), central (zone 2) and arid-irrigated (zone 3), respectively (Table 3). Slightly higher seropositivity was detected in arid irrigated zone as compared to other two zones.

Selected animals were divided into two different age groups i.e. heifers (13 to 24 months) and lactating animals (> 24 months). Age is a major factor, which helps in studying the distribution pattern of disease and also act as a salient risk factor on bovine neosporosis (Dubey *et al.*, 2007). The percent seropositivity in heifers was found as 4.88 % (2/41) and in lactating animals was about 13.28% (19/143) using ELISA (Table 4). The seropositivity was non-significantly higher (chi square = 2.228; p = 0.135, RR=0.367, 95% CI=0.060-1.502) in lactating animals (13.28%) than heifers (4.88%). Results obtained from current study was found similar to the work of Keefe and VanLeeuwen (2000). They found a non-significant association of Neospora seropositivity with age of the animals.

Out of 184 animals tested, 56 were detected to have a previous history of abortion and 128 animals were recorded with no previous abortions. Seroprevalence of 30.36% was recorded in animals with previous history of abortion as compared to seroprevalence of 3.13% in animals with no history of abortion (Table 5). The seropositivity was significantly higher (Chi square = 28.574; p=0.000) in animals which have the previous abortion (30.36%) than animals without previous abortion (3.13%). The animals with previous abortion history were 9.7 times (RR=9.714, CI=3.294-32.350) more at risk to be seropositive than animals without previous abortion history. Results obtained from this study was similar to the work of Davison *et al.* (1999) who reported significantly higher seroprevalence in aborted cattle (p<0.0001) than non-aborted cattle.

Dogs were found to be a definitive host for neosporosis which keep on shedding the oocysts of *Neospora caninum* thus increases the prevalence of infection in both cattle and buffaloes by close contact. In present study 90 out of 184 animals had close association with dogs, whereas 94 animals were recorded as no contact with dogs. Seropositivity of 14.44% was detected in animals where dogs were kept along the cattle and buffalo as contrast to lower seropositivity of 8.51% in animals which were not in contact with dogs (Table 6). Seropositivity in animals which was having close contact to dogs was found to be non-significantly higher (Chi square =1.601; p=0.206, RR=1.697, CI=0.690-4.328).

Large scale studies are needed to find out the impact of various risk factors on seropositivity of neosporosis in cattle.

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