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SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *NEOSPORA CANINUM* IN DAIRY CATTLE OF WESTERN DAIRY POCKET AREA IN CHITWAN DISTRICT OF NEPAL

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ABSTRACT

Dairy industry is growing in Nepal and western dairy pocket area is the main milk producing area in Chitwan district. This study was carried out to determine the seroprevalence and risk factors associated with *N. caninum* in western dairy pocket area in Chitwan district of Nepal. A cross-sectional study was conducted from April 2014 to July 2014 among the small holder dairy farms, and out of 2188 animals from different sampling villages, 186 (8.5%) animals were randomly selected. Individual blood samples (5-10 ml) were collected and screened for *N. caninum* antibodies by ELISA test kit. The study showed that apparent overall prevalence of *N. caninum* to be 4.84% and true prevalence, 4.07%. The prevalence varied from a low of 0% to a high of 13.16% in various VDCs. The prevalence rates among Holstein-Friesian cross and Jersey cross Cattle were 6.94% and 3.51% respectively. The seroprevalence of *N. caninum* was found to be 16.13% and 2.5% in animals with and without history of abortion respectively, showed statistically significant different (p value <0.05). Similarly, prevalence were 13.64% and 3.66% in animals with and without presence of dog respectively and 8.5%, 3.39% and 0% in the animals of age group of 3-5 years, 1-3 years and above 5 years respectively, showed statistically non-significant association (p value <0.05). This study shows that *N. caninum* is associated with abortion in dairy cattle of Chitwan region. The study may contribute the base line data of *N. caninum* in Nepal for future preventive strategy for stake-holders and government.

Key words: Dairy cattle, *Neospora caninum*, Seroprevalence, Nepal

INTRODUCTION

Neospora caninum is an intracellular apicomplexan protozoan parasite, one of the major causes of abortion, neonatal morbidity and mortality in cattle, sheep, goats and horses (Dubey *et al.*, 2007). It is one of the most important causes of abortion in dairy cattle in many countries of the world (Dubey and Lindsay, 1996; Paré *et al.*, 1996; Anderson *et al.*, 2000; Trees and Williams, 2005; Dubey, 2003). It was first reported in dog in Norway (Bjerkas *et al.*, 1984) and in cattle in Mexico in 1987 (Trees and Williams, 2005; Sevgili and Altas, 2005). The sexual stage of life cycle of *N. caninum* occurs in dog; act as the definite host, whereas asexual stage of life cycle occurs in cattle, sheep, goats and horses; acts as the intermediate hosts of the parasite (Gondim *et al.*, 2002). Transmission of *N. caninum* occurs through vertically from infected pregnant dams to her offspring or horizontally between infected cows to cows or infected cows to dogs (Barber and Trees, 1998; Bergeron *et al.*, 2000; Davison *et al.*, 2001; Akca *et al.*, 2005; Bartels *et al.*, 2005). Experimental infection of *N. caninum* also occurs in pregnant cattle and abortion occurs on the basis of time of inoculation (Williams *et al.*, 2000), infection in early gestation results in fetal death (Macaldowie *et al.*, 2004), whereas in mid-gestation results in fetal infection and fetal survival (Maley *et al.*, 2003). The most common route of transmission of *N. caninum* infection in cattle is vertical (transplacental) transmission and occurs through infected dam to her offspring during successive pregnancies (Antony and Williamson, 2001; Frössling *et al.*, 2005). Neosporosis in dairy cattle has been reported in various countries of the world as in Senegal (Kamga *et al.*, 2010), Egypt (Ibrahim *et al.*, 2009), Sudan (Ibrahim *et al.*, 2012), Algeria (Ghalmi *et al.*, 2012), Pakistan (Nazir *et al.*, 2013), Italy (Otranto *et al.*, 2003), Iran (Nematollahi *et al.*, 2011), Paraguay (Osawa *et al.*, 2002), France (Ould *et al.*, 1999), Japan (Koiwai *et al.*, 2006) and China (Xu *et al.*, 2012) but not reported from Nepal so far. The aim of current study was to estimate, for the first time, the seroprevalence and risk factors associated with *N. caninum* in dairy cattle of the western dairy pocket area in Chitwan district of Nepal which helps to prevent or control neosporosis in dairy cattle.

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MATERIALS AND METHODS

Study area

A cross-sectional study was conducted from April 2014 to July 2014 among the smallholder dairy farms of the western dairy pocket area in Chitwan district of Nepal. The geographic locations of the five sampling sites of the western dairy pocket area in Chitwan district of Nepal are consisting of the following villages: Divyanagar, Gunjanagar, Shardanagar, Mangalpur and Gitanagar.

Sampling

Five villages were randomly selected from a sampling frame of total villages of western dairy pocket in Chitwan district of Nepal. Out of 2188 animals in these villages, 186 (8.5%) animals were randomly selected from various farms of the villages. Individual blood samples (5-10 ml) were collected by venipuncture from the jugular vein of dairy cattle using dry vacutainer tubes and labeled according to animal age, breed, herd holder and location. The samples were forwarded to the National Avian Disease Diagnostic Laboratory, Bharatpur, Chitwan (for antibody testing). After centrifugation at 1000 revolutions/minute for 20 minutes, sera were removed and stored at - 20˚C until testing.

Serological examination

Antibodies to *N. caninum* were screened in dairy cattle sera using commercially available IDEXX *Neospora* X2 Ab test kit (IDEXX Laboratories, Inc., Westbrook, Maine, USA) according to the manufacturer's instructions. The S/P (sample to positive) ratio was used to determine the seropositivity of *N. caninum* in dairy cattle where S is the difference between the optical density of the sample and optical density of the negative sample and P is the difference between the optical density of the positive control and the optical density of the negative control. The reading was made using ELISA reader having filter with optical density of 650 nm. The tested samples were interpreted as seropositive for *N. caninum* when S/P ratio was greater than or equal to 0.5.

Epidemiological data

Epidemiological data were collected through semi-structured questionnaires from the farmers or farm owners of the selected areas in order to obtain information about the risk factors such as: Farm location, cattle age, Cattle breed, presence of dog in the farm, abortion history of cattle and infertility problems in cattle.

Statistical analysis

The data were analysed by using the softwares Win Episcope 2.0, WinPepi (version 4.0) and Epi Info 2002 (Anderson *et al*., 2000). The assessment of Chi square and Fischer's exact tests at 95% confidence interval were used to compare selected risk factors: villages, ages, breeds, Dogs and history of Abortion. The true prevalence of the *N. caninum* was calculated at 95% confidence interval using computer software 'True Prevalence program of the Survey Toolbox' considering apparent prevalence, sample size, sensitivity and specificity of the ELISA test kit. The sensitivity and specificity of the ELISA test kit for *N. caninum* are 100% and 99.2% respectively.

RESULTS AND DISCUSSION

The overall seroprevalence of *N. caninum* in dairy cattle of western dairy pocket area in Chitwan district of Nepal was found to be 4.84%. Out of 186 samples, 9 samples were found to be positive by IDEXX *Neospora* X2 ELISA Test Kit. The Apparent Prevalence was found to be 4.84% and True prevalence was found to be 4.07% (CI 95%: 2.524-5.621). The data of all the risk factors and their association with seroprevalence included in the study were represented in Table 1.

Among the five VDCs, highest number of positive samples (five) were found in Gitanagar (13.16%) followed by two positive samples in Mangalpur (5.41%). Sharadanagar (2.70%) and Divyanagar(2.70%) showed one positive sample in each and no positive samples were found in Gunjanagar (0%). The result obtained was subjected for Chi-squared test to test significance of variation. The result showed that seroprevalence of *N. caninum* antibody in various locations was statistically non significant ($p > 0.05$).

There were 7 (8.33%) positive samples for age group 3-5 years, 2 (3.39%) for age group 1-3 years and no positive sample for age group above 5 years (0%). The result obtained was subjected to Chi-squared test to test. The result showed that seroprevalence of *N. caninum* antibody in various age groups was statistically non significant ($p > 0.05$). Odd ratio for 1-3 years and 3-5 years was 0.386 and for above 5 years and 3-5 years was 0. It indicates that animals of age group 3-5 years are at more risk to disease than age groups 1-3 years.

The breed wise seroprevalence breed were found to be 4 (3.51%) and 5 (6.94%) for Jersey Cross and Holstein Friesian respectively. The result obtained was subjected to Fisher's Exact test. The result showed that seroprevalence of *N. caninum* antibody in various breed was statistically non significant ($p > 0.05$). Odd ratio for Jersey Cross and Holstein Friesian was 1.311. It indicates that animals of Jersey Cross breed are at more risk than Holstein Friesian breed**.**

Out of 9 positive samples, 5(16.13%) were associated with history of abortion. Similarly, 4(7.55%) positive samples were associated with history of infertility (repeat breeding and anoestrus) and 3(13.64%) were associated with history of presence of dog. Number of samples with history of neonatal death were found to be zero. The result obtained was subjected to Fisher's Exact test. The result showed statistically significant ($p<0.05$) association of history of abortion with seroprevalence of *N. caninum*. Other risk factors as infertility and presence of dog were statistically non significant ($p > 0.05$). Odd ratio for animals with history of abortion and non-abortion was 7.26, for infertile and fertile animals were 2.09 and for presence and absence of dogs were 4.16. It indicates animals with history of abortion are at about 7.26 times more risk than those without abortion. Similarly, the animals with history of presence of dog in the farm are at 4.16 times more risk than without dogs in the farm. . Conversely, infertile animals are at 2.09 times less risk than fertile animals.

Table 1. Risk Factors wise distribution of *Neospora caninum* in the cattle sera

Risk Factors		Total Samples	Positive Sample	Apparent Prevalence (%)	True Prevalence (%)	Confidence Interval (CI) (95%)	Odd Ratio (95% CI)	Chi-squared P Value	Fisher's Exact Test P value
Location	Mangalpur	37	2	5.41	4.65	0.988-8.306		0.080	
	Gunjanagar	37	0	0.00	N/A	N/A			
	Divyanagar	37	1	2.70	1.92	0.000-4.537			
	Sharadanagar	37	1	2.70	1.92	0.000-4.357			
	Gitanagar	38	5	13.16	12.46	7.064-17.856			
Age	1-3 years	59	2	3.39	2.61	0.293-4.929	0.386*[a] (0.077-1.928)	0.401	
	3-5 years	84	7	8.33	7.59	4.624-10.557			
	>5 years	43	0	N/A	N/A	N/A			
Breed	Jersey Cross	114	4	3.51	2.73	0.000-5.493	2.052*[b] (0.322-5.329)		0.470
	Holstein Friesian	72	5	6.94	6.19	3.243-9.136			
Others	Abortion	31	5	16.13	15.45	8.954-21.954	7.26*[c] (1.828-28.831)		0.015
	Non-abortion	155	4	2.58	1.79	0.541-3.047			
	Infertility	53	4	7.55	6.80	3.234-10.375	2.09*[d] (0.538-8.104)		0.465
	Non-infertility	133	5	3.76	2.98	1.361-4.607			
	Presence of dog	22	3	13.64	12.94	5.744-20.143	4.158*[e] (0.960-18.001)		0.151
	Absence of dog	164	6	3.66	2.88	1.440-4.326			

The present study is the first report on the seroprevalence of *N. caninum* in dairy cattle of western dairy pocket area in Chitwan district of Nepal. Neosporosis has been reported in many countries (Kim *et al*., 2002; Kim *et al*., 1998; Gondim *et al*., 2004) with different prevalence rates since the disease was recognized in 1988. The seroprevalence of antibody to *N. caninum* in this study is 4.84%, in dairy cattle of western dairy pocket area in

Chitwan, is lower than that reported for cattle in Brazil (14.09%) (Gondim *et al.*, 2004), Romania (34.6%) (Gvavrea *et al.*, 2011), Pakistan (43%) (Nazir *et al.*, 2013), Jordan (35 %) (Talafha and Al-Majali, 2013), China (15.07%) (Xu *et al.*, 2012), India (12.61%) (Sengupta *et al.*, 2012), Iran (10.5%) (Nematollahi *et al.*, 2011), Sudan (10.7%) (Ibrahim *et al.*, 2012) but resembles that reported in Australia (3.2%) (Nasir *et al.*, 2012), Korea (4.1%) (Kim *et al.*, 2002), Czech Republic (5.83%) (Vaclavek *et al*., 2003), France (5.6%) (Ould *et al.*, 1999) and Japan (5.7%) (Koiwai *et al.*, 2006). This difference in seroprevalence may be due to the type of test used, their cut-off points, change in geographic area, variation in sample size and other associated risk factors.

The seroprevalence of *N. caninum* in aborted cattle was 16.13% which is higher than that of non-aborted cattle and the prevalence of *N. caninum* in samples with history of abortion is higher than samples without history of abortion (Voural *et al*., 2006). Our study showed similar results. Although the presence of antibodies to *N. caninum* in dairy cattle only indicates the exposure to the parasite, the probability of abortion in seropositive cattle is 7.26 times higher than in seronegative cattle. The results of this study showed that there was a significant relationship with abortion ($p<0.005$). The highest risk of abortion due to neosporosis was in the 3-5 years old of cattle however relationship between neosporosis and abortion is a speculative. Jensen *et al.* (1999) reported that seroprevalence increases with age. Razmi *et al.* (2006) reported the highest risk of abortion due to neosporosis in dairy cattle of 1-2 years of age. In contrast, Hajikolaei *et al.* (2008) and Sadrebazzaz *et al.* (2004), observed no significant difference between age and seropositivity to neosporosis. The presence of farm dog increases the seropositivity of *N. caninum* in the dairy cattle although it is non-significant but shows higher risk of *N. caninum* to dairy cattle. Bartels *et al.* (1999), Mainar *et al.* (1999) and Otranto *et al.* (2003) also reported that presence of farm dog increases the seropositivity to *N. caninum* to the cattle. The seropositivity of *N. caninum* in this study is higher in Holstein Friesian cross breed than Jersey cross breed and is non-significant. Sadrebazzaz *et al.* (2004) also observed non-significant relation between the breeds of cattle to the *N. caninum.* The percentage of seropositivity of *N. caninum* is higher in cattle with infertility problems than the cattle with normal fertility and observed non- significant relation to the *N. caninum.*

In conclusion, the present study shows that *N. caninum* is present in Chitwan district of Nepal and 4.84% dairy cattle are positive for *N. caninum* by ELISA test kit on selected sampling sera. The exposure to *N. caninum* was more frequently associated with history of abortions, infertility and presence of dogs respectively. This result suggests that *N. caninum* might be one of the major causes of abortion in dairy cattle in this region. As this is the first study of *N. caninum* in Nepal and it has been done only for Chitwan district with limited risk factors, it may contribute the baseline data of *N. caninum* in Nepal which will help for future preventive strategy for stake-holders and government authority to investigate further.

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