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Research Article

Seroprevalence and its associated risk factors of Bovine Neosporosis and Bovine Viral Diarrhea in cattle of Tilottama municipality, Rupandehi, Nepal

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Keywords: Abortion; Bovine Neosporosis; Bovine viral diarrhea virus; Mixed farming; Nepal

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Abstract

Background: Bovine Neosporosis (BN) and Bovine Viral Diarrhea (BVD) are economically significant livestock disease that causes reproductive and respiratory problems in cattle and buffalo caused by protozoan parasite *Neospora caninum* and Bovine Viral Diarrhea Virus (BVDV) respectively. The burden of production and economic loss due to infectious reproductive diseases are still unknown in the various location of Nepal.

Objectives: To overview the prevalence of bovine neosporosis and bovine viral diarrhea infection in a commercially important livestock herd from Tilottama Municipality, Rupandehi, Nepal.

Methods: A cross-sectional purposive study was conducted through a questionnaire survey to collect demographic and farm characteristics information. In total 92 serum samples from jugular veins were collected from a different herd of Tilottama Municipality of Rupandehi, Nepal. The antibody against BVDV was tested using the competitive Enzyme-Linked Immunosorbent Assay (c-ELISA).

Results: The individual animal-wise apparent prevalence was 9.78% with a true prevalence of 9.70% (5.09 - 17.57) and 3.26% with a true prevalence of 3.10%; CI (0.93 - 9.06) for BN and BVD respectively. The risk factor history of abortion was associated significantly with BN and BVD seropositivity ($p < 0.05$). Animals in the mixed farming system and contact with dogs were associated significantly with the occurrence of bovine viral diarrhea and bovine neosporosis respectively at ($p < 0.05$).

Conclusion and recommendation: The stuy indicated the circulation of *Neospora caninum* and bovine viral diarrhea in dairy herds of the study area. It is suggested that the stakeholders in the study area should be sensitized to the impact of *Neospora caninum* and bovine viral diarrhea virus infection in dairy herds and include both diseases in the regional and national surveillance system.

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Abbreviations

BN: Bovine Neosporosis; BVDV: Bovine Viral Diarrhoea Virus; GDP: Gross Domestic Product; ELISA: Enzyme-Linked Immunosorbent Assay; IBR: Infectious Bovine Rhinotracheitis

Introduction

Nepal has 7.46 million cattle, 5.16 million buffalo, 0.79 million sheep, and a 13.44 million goat population [1]. Livestock is an integral part of the agriculture system in Nepal which plays an important role in the economy and nutrition of the majority of the people. Cattle rearing plays an important role in the livelihood of a large percentage of poor, marginal farmers and landless migratory laborers in Nepal. Livestock sectors play an important role in an economy generation to the farmers and contribute about 6.23 % to the national Gross Domestic Product (GDP) [1]. Meanwhile, there is a big challenge in front to commercialize and gaining maximum profit by less cost of production. Diminishing land size along with increasing population has contributed to the difficulty in rearing large ruminants by the marginal farmers. The important challenge of dairy production is a shortage of food, diseases of animals, involvement of inexperienced people in the livestock field, and poor breed improvement programs [2]. The problems are also exaggerated by wrong AI bull selection, poor skills of inseminators, and the absence and unorganized policy for crossbreeding [3].

Rupandehi district comprises 98423 (1.32% of the total population) cattle population [1]. Tilottama Municipality of Rupandehi district comprises 8.86% of the total population of cattle in Rupandehi (8718 heads) [1]. The system of cattle rearing is still conventional and lots of efforts have been made to commercialize this enterprise. The shift of cattle rearing from conventional to commercial introduced a large number of problems/diseases. Certain diseases and parasites have been recognized as life-threatening problems for cattle. The main circumstances in cattle rearing are the occurrence of reproductive disorders by various biological and non-biological agents [4]. The fertility of cattle determines the whole economy of farmers. Various infectious and non-infectious agents hamper the fertility of cattle like *Brucella abortus* and agents like Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea (BVD), and leptospirosis causing an abortion along with metritis and placental retention [5].

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 [6]. It is one of the most important causes of abortion in dairy cattle in many countries of the world [7,8]. It was first reported in dogs in Norway and in cattle in Mexico in 1987 [8,9]. Bovine Viral Diarrhoea is caused by the bovine viral diarrhea virus of the Flaviviridae family which is a highly infectious disease that has great economic importance in dairy cattle all over the world and is listed as a notifiable disease by The World Organisation for Animal Health [10]. Infections with bovine viral diarrhea virus are distributed throughout the world and cause heavy economic losses by decreased milk production, reduction in conception rate, and incidence of abortion, malformation, and stillbirth [11].

Neosporosis and bovine viral diarrhea are economically important diseases in many countries, and problems related to the disease are thought to be increasing in some areas. Worldwide reviews of the economically assessed production losses and intervention programs (e.g. eradication programs, vaccination strategies, and biosecurity measures) incurred by both *Neospora caninum* and BVD infection have been published. The research focused on the overall seroprevalence of bovine neosporosis and bovine viral diarrhea and its associated risk factors in Tilottama Municipality, Rupandehi, Nepal. After the completion of the research, the farmers will gain updated information about neosporosis and BVD and its overall health and economic losses.

Materials and methods

Study populations

The research was conducted in cattle of Tilottama Municipality of Rupandehi district (Figure 1). Questionnaire survey design and sample collection were done. The municipality has an estimated cattle population of 8718 [1] and 7 Village Development Committee (VDC).

Research design and sampling methods

Cross-sectional purposive study design to identify seroprevalence consists of epidemiological data collection and laboratory examination. The purposive cross-sectional survey was performed in the pocket area aiming to determine seroprevalence in commercial and non-commercial herds not exceeding 5 samples from one herd. The animals with reproductive disorders are mainly included in the sampling.

Sample size determination

The sample size necessary for detection of BVDV and BN was calculated from EpiTools Epidemiological Calculators by Ausvet [12], with the assumption of an overall 3.4% of prevalence of BVDV infection [13] and 4.84% of seroprevalence of *Neospora caninum* [14] in dairy cattle of Tilottama Municipality computed

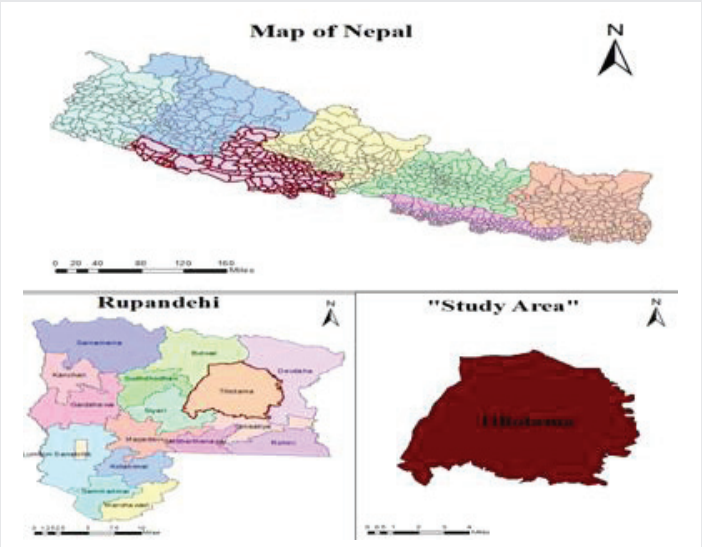

Figure 1: Research site.

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with expected precision of 5% and 95% of confidence level. The required sample size becomes 66 for bovine neosporosis and 51 for bovine viral diarrhea in Tilottama Municipality of Rupandehi Nepal. But all together 92 cattle serum samples were collected for study.

Epidemiological data collection and sample collection

In the following topic, individual animal data were recorded using a structured record-keeping sheet through physical inspection and face-face interviews with owners with the help of a questionnaire survey. A sample of blood of about 5 ml. was collected from the jugular vein and placed in a clot activator tube for serum separation. Thus collected serum is then transferred to a serum vial and stored at -20 °C.

Sample transportation and serological examination

The collected and stored sample will be transported in a cool ice box bag. The serological study will be done in Animal Health Research Division (AHRD), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, and ELISA will be done as per the principles and procedures of the manufacturer's protocol. A diagnostic kit is designed to detect specific antibodies directed against the p80-125 protein (also known as NSP2-3) of the BVD/ Mucosal Disease/ Border disease virus and anti-*Neospora caninum* antibody in serum sample by competitive ELISA, ID.vet FRANCE.

Data analysis

Data collected in the field using individual data sheets were entered in an MS Excel spreadsheet (MS Office 2013). Data from the Excel spreadsheet were imported into SPSS version 24.0 for statistical significance. All analysis was based on ELISA serological test results. Seroprevalence was calculated by dividing the number of seropositive samples from ELISA by the total number of samples tested. Association between the categorical variables and the outcome variable was assessed by Pearson's chi-square *p - value* and Fisher's exact *p-value*. *P – value* < 0.05 was considered statistically significant. Risk estimation was done through Odd's value in SPSS version 24. Finally, tables were used to present the results generated from SPSS and graphical presentations were done through MS Excel 2013.

Result and discussion

Characteristics of farm sample

Face-to-face interviews with farm owners or caretakers directly involved in livestock care were conducted using a semi-structured questionnaire to collect information on sampled farm characteristics. The questionnaire included questions on farm demographics, management practices, bio-security situations, and herd-health management. Management practices included herd structure, feeding, stall style, water source, veterinary service, and types of breeding methods used (natural or artificial insemination). Hygiene and biosecurity aspects include farm cleaning, other animals in contact with, disinfectant use, and quarantine and isolation facility. Herd health status includes the history of repeat breeding, retention of placenta, abortion, and the problem of mastitis on the farm. Production status on the farm is analyzed through the visual production losses on the farm. Farmer's knowledge about bovine neosporosis and bovine viral diarrhea with knowledge about the zoonotic disease is also assessed.

The overall seroprevalence of bovine neosporosis and bovine viral diarrhea antibodies

Out of 92 samples tested 9 samples were found positive for *Neospora caninum* and 3 samples positive for BVD (Figure 2). The apparent prevalence was 9.78% with a true prevalence of 9.70%; CI (5.09-17.57) and 3.26% with a true prevalence of 3.10%; CI (0.93-9.06) for bovine neosporosis and bovine viral diarrhea respectively (Table 1). None of the farms in the sampling location follows the vaccination procedure against *Neopsora caninum* and bovine viral diarrhea infection. So the appeared prevalence must be due to natural infection. The estimated prevalence of bovine neosporosis is higher than the previous research done in Nepal; 4.84% in the Chitwan district (Yadav, et al. 2017). The individual level prevalence of BVD in this location is comparable to the previous research done in Nepal; 2.6% in the Chitwan district [11] and 3.4% in Kavreplanchowk [13]. The findings are in agreement with the previous report on worldwide BVDV antibody prevalence in cattle, which ranges from 0 to 90% [15]. The dairy cattle in Nepal are mainly brought in from nearby Uttar Pradesh in India, where the prevalence is low, and from Punjab, where the prevalence of BVDV is zero [16].

Age-wise distribution of serum antibodies against BN and BVD

The result showed no significant effect of age in the occurrence of Bovine Neosporosis and BVD ($p > 0.05$). It was

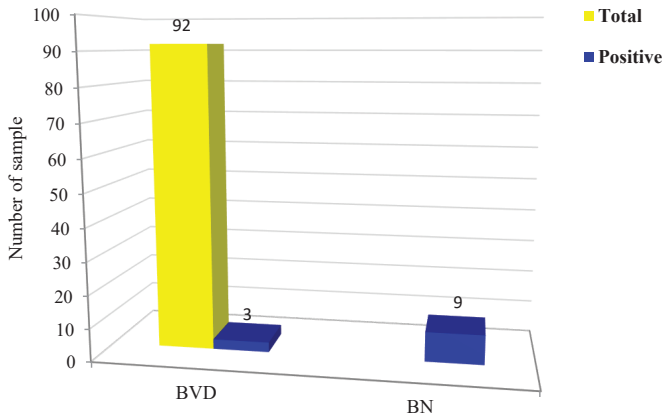

Figure 2: Total and positive sample for BVD and BN.

Table 1: Overall seroprevalence of *Neospora caninum* and BVDV antibodies in individual cattle sera.

Total sample	Positive sample		Apparent prevalence		True prevalence	
	Neospora	BVD	BN %	BVD %	BN % (CI)	BVD % (CI)
92	9	3	9.78	3.26	9.70 (5.09 - 17.57)	3.10 (0.93 - 9.06)

Note: figure in bracket indicates 95% confidence interval

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found that 10.2% (5/49), and 9.3% (4/43) samples were seropositive for *Neospora caninum* among 3 and less than 3 years (young) of age and above 3 years (adult) of age respectively. 2.04% (1/49), 4.65% (2/43) samples were seropositive for BVDV among 3 and less than 3 years (young) of age and above 3 years (adult) of age respectively (Table 2). The highest seroprevalence of *Neospora caninum* in young aged animals than older may be due to placental transmission from the infected dam to offspring and successive transmission occurs with the increase in age. No significant difference between various age groups in the occurrence of bovine neosporosis was also reported in Iran [17,18] and the previous report in Nepal [14].

The highest seroprevalence of BVD in older aged animals than in younger ones may be due to an increase in the animal risk of exposure to BVDV, this finding supports the finding of Nigussie, et al. 2010 [19] Similarly, the prevalence of BVDV antibodies has been reported lowest in cattle of 7 – 12 months old and highest in animals aged five years or over [20]. The findings are similar to the previous research done in Nepal as there was no significant association of age with the appearance of seropositivity [11].

Breed-wise distribution of serum antibodies against BN and BVD

The positive sample was compared among different breeds of cattle and showed a seroprevalence of 6.66% (3/45); 14.81% (4/27) and 10% (2/20) among jersey cross, Holstein Friesian cross, and local breed respectively for bovine neosporosis and seroprevalence of 4.44% (2/45); 3.70% (1/27) among jersey cross and Holstein Friesian cross respectively for BVD (Table 3). The odds ratio for the jersey cross is compared to Holstein-Friesian. The chi-squared *p - value* showed breeds are not associated significantly ($p > 0.05$) with seroprevalence of both bovine neosporosis and bovine viral diarrhea. No significant difference among breeds with the seroprevalence of *Neospora caninum* was also reported in Nepal [14]. The research shows jersey is more susceptible to BVDV infection than Holstein-Friesian. This finding supports the previous finding done in Nepal, they found jersey crosses are more susceptible than HF crosses; 2.8% (6/212) versus 2.2% (3/138), Odds ratio 1.311; (CI 0.3223–5.329) and there was no significant association of breed with antibody detection of BVDV [11] Figure 3.

Distribution of antibodies against BN and BVD in the different farming systems

Rural framing household cattle seropositivity counts to 10.52% (4/38) and intensive farming results in little less 9. 25% (5/54) for bovine neosporosis (Table 4). Rural household farms are apparently more free from antibodies against BVDV than Intensive farms (5.56%; 3/54). Fisher's exact test *p - value* ($p > 0.05$) showed the farming system is not significantly associated with the seroprevalence of Bovine neosporosis and BVDV. High infection of BVD in cattle of intensive farms due to purchased to exotic high yielding cattle from Utter Pradesh and Punjab in the study site. Ahmad, et al. 2013 [21] stated BVD is prevalent in farms of Panjab. BVD is also prevalent in cattle in Utter Pradesh (UP) [22]. Also maximum farmers in rural household rear local cattle which are found seronegative instead of exotic and cross breeds.

Distribution of antibodies against BN and BVD in different cattle origin

Out of 92 samples, 12.9% (4/31) were born on the farm and 8.19% (5/61) purchased were tested seropositive against *N. caninum*. Results showed cattle that are born on farms are apparently free from BVDV. Purchased cattle were found to be 4.92% (3/61) seropositive to BVDV. This variable doesn't have a significant association ($p > 0.05$) with seroprevalence (Table 5).

Risk factors related distribution of serum antibodies

In order to determine various risk factors like herd size, mixed farming, history of diarrhea, history of abortion, and the presence of a dog, Fisher's exact test and chi-square test were applied (Table 6). The odds ratio is also compared between variables. The result showed large herd i.e. above 25 was found to be positive (9.38%; 3/32) for BVDV. This finding

Table 2: Distribution of sera in different age groups.

Age group	Total sample	Positive sample		Apparent prevalence		True prevalence		Odds ratio		Fisher's exact p - value	
		BN	BVD	BN %	BVD %	BN % (CI)	BVD % (CI)	BN	BVD	BN	BVD
3 and less than 3 years (young)	49	5	1	10.20	2.04	10.13 (4.29 - 21.82)	1.86 (0.10 - 10.62)	1.108 (0.278 -4.420)	0.427 (0.037 - 4.882)	1.00	0.597
Above 3 years (adult)	43	4	2	9.30	4.65	9.21 (3.52 - 21.66)	4.51 (1.10 - 15.44)	N/A	N/A		

Note: The figures in brackets indicate the 95% confidence interval.

Table 3: Breed-wise seroprevalence of BVDV.

Breed	Total	Positive samples		Apparent prevalence		True prevalence		Odds ratio		Chi-squared p -value	
		BN	BVD	BN%	BVD%	BN% (CI)	BVD% (CI)	BN	BVD	BN	BVD
Jersey cross	45	3	2	6.66	4.44	6.55 (2.12 -17.87)	4.30 (1.04 - 14.81				
Holstein- Friesian cross	27	4	1	14.81	3.70	14.79 (5.79 - 32.67)	3.55 (0.15 -18.30)	0.411[a] (0.085 - 1.996)	1.209[b] (0.104 - 14.005)	0.708	0.641
Local	20	2	0	10.00	0.00	9.92 (2.62 - 30.27)	N/A	N/A			

Note: The figures in brackets indicate the 95% confidence interval.

[a]Odds ratio for Jersey cross is compared to Holstein-Friesian for BN

[b]Odds ratio for Jersey cross is compared to Holstein-Friesian for BVD

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supports the finding done by Talafha, et al. 2009 [23]. He found animals in large herds are more likely to be infected by BVDV than in small herds. Solis-Calderon, et al. 2005 [24] also found that large herds are more susceptible to BVDV infection. Herd size with 10-25 cattle population in number showed a higher prevalence of bovine neosporosis (13.79%; 4/29) with an odd ratio of 2.400 (0.405 - 14.209) between small and medium-sized herds. Cattle rearing with other animals (mixed farming) is significantly associated with the occurrence of bovine neosporosis ($p < 0.05$). All the seropositive sample for bovine viral diarrhoea were in a mixed farming system (10.00%; 3/30). 26.67% (8/30) samples were found positive for *Neospora caninum* infection in cattle with regular dog contact (presence of dog). The presence of a dog and regular contact with the dog is significantly associated with the seropositivity of *Neospora caninum* ($p < 0.05$). The result shows an animal with a history of abortion is significant with both *Neospora caninum* and bovine viral diarrhea infection ($p < 0.05$). Other factors were found non-significant with the seroprevalence of BN and BVD. A nonsignificant association between the occurrence of diarrhea in animals and BVD seropositivity supports the finding of Manandhar, et al. 2011 [11].

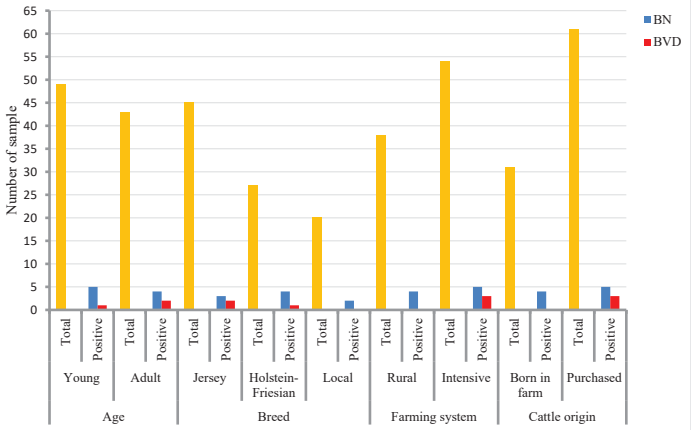

Figure 3: Total and positive sample for different categories of age, breed, farming system and cattle origin.

Conclusion

The overall seroprevalence of bovine neosporosis and BVD in the study site is found to be 9.78% and 3.26% within individual levels with a true prevalence of 9.70% (5.09 - 17.57) and 8.23%; CI (2.14 - 25.96) respectively. Since there was no vaccination

Table 4: Antibodies of BN and BVD in the different farming systems.

Farming system	Total sample	Positive sample		Apparent prevalence		True prevalence		Fisher's exact test *p* -value	
		BN	BVD	BN%	BVD%	BN% (CI)	BVD% (CI)	BN	BVD
Rural household	38	4	0	10.52	0.00	10.45 (4.02-24.22)	N/A	1.00	0.265
Intensive farming	54	5	3	9.25	5.56	9.17 (3.87-19.95)	5.42 (1.73-15.09)		

Note: The figures in brackets indicate the 95% confidence interval.

Table 5: Distribution of BN and BVD antibodies in different cattle origins.

Cattle origin	Total sample	Positive sample		Apparent prevalence		True prevalence		Fisher's exact test p - value	
		BN	BVD	BN%	BVD%	BN% (CI)	BVD% (CI)	BN	BVD
Born in farm	31	4	0	12.90	0.00	12.86 (4.99-29.00)	N/A	0.548	0.479
Purchased	61	5	3	8.19	4.92	8.09 (3.39-17.81)	4.78 (1.50-13.45)		

Note: The figures in brackets indicate the 95% confidence interval.

Table 6: Association of BN and BVD antibodies among various risk factors.

Risk factors		Total sample	Positive sample		Apparent prevalence		True prevalence		Odds ratio		Chi-square p -value		Fisher's exact test p - value	
			BN	BVD	BN%	BVD%	BN% (CI)	BVD% (CI)	BN	BVD	BN	BVD	BN	BVD
Herd size	Small (less than10)[c]	31	3	0	9.67	0.00	9.59 (3.18 -25.00)	N/A	N/A	N/A	0.612[a]	0.055[b]		
	Medium (10-25)[c]	29	4	0	13.79	0.00	13.7 (5.36-30.73)	N/A	2.400[c] (0.405-14.209)	N/A				
	Large (above 25)	32	2	3	6.25	9.38	6.12 (1.55-20.19)	9.2 (3.08-24.31)	N/A	N/A				
Mixed Farming	Yes	30	2	3	6.66	10.00	6.55 (1.67-21.38)	9.92 (3.30-25.73)	0.561 (0.109-2.882)	N/A			0.713	0.032*
	No	62	7	0	11.29	0.00	11.23 (5.44-21.58)	N/A						
Abortion History	Yes	8	3	2	37.50	25.00	37.40 (13.65-70.07)	24.25 (9.59-66.65)	10.50 (1.82-60.45)	24.333 (1.918-308.74)			0.024*	0.018*
	No	74	4	1	5.41	1.35	5.27 (1.95-13.05)	1.17 (0.14-5.17)						
Diarrhea History	Yes	19		2		10.52		10.45 (2.77- 31.57)		8.471 (0.725-98.943)			0.107[a]	
	No	73		1		1.36		1.13 (0.13- 7.25)						
Presence of dog	Yes	30	8		26.67		26.6 (14.15-44.79)		10.50 (1.82-60.45)				0.000[b]**	
	No	62	1		1.61		1.43 (0.12-8.49)							

Note: [a]Fisher exact test *p* - value for BVD.
[b]Fisher's exact test *p* - value for BN.
[c]Odds ratio of small herd size is compared with medium herd size.
*Significant at $p < 0.05$.
**Highly significant at $p < 0.01$.

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is applied against *Neospora caninum* and bovine viral diarrhea virus infection, the seropositivity results from the natural infection. There is no significant association between different locations, ages, breeds, farming systems, and cattle origin with the occurrence of *Neospora caninum* and BVDV antibody in serum ($p > 0.05$). Animal with a history of abortion associates significantly with seroprevalence of Bovine Neosporosis and BVD within the individual level ($p < 0.05$). Risk factor presence and regular contact with dogs and mixed farming practices are associated significantly with the seroprevalence of BN and BVD respectively. Despite some limitations, seroprevalence study in the study provides a basis for the future monitoring and surveillance of disease. Previous study and this study provides a basis for the future to control of reproductive disorders at the regional and national level to protect our dairy industry and animal health.

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