



## Research Article

# Seroprevalence and Associated Risk Factors of Bovine Viral Diarrhea in the Dairy Cattle of Rupandehi District of Nepal

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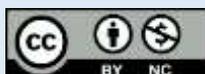
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**Keywords:** Bovine Viral Diarrhea, Bovine Viral Diarrhea Virus, seroprevalence, risk factors, cattle, Nepal.

### Abstract

Bovine Viral Diarrhea (BVD) is a contagious disease caused by Bovine Viral Diarrhea Virus (BVDV), which significantly impacts cattle health and productivity, leading to substantial economic losses for farmers. The Rupandehi district in Nepal, which shares an open border with India, is particularly vulnerable to the introduction and spread of BVD. Consequently, the objective of this research was to examine the prevalence of Bovine Viral Diarrhea Virus (BVDV) infections and the associated risk factors that contribute to it in the Rupandehi district of Nepal. A two-phase random sampling method was used, where a total of 92 blood samples were obtained from female cattle from Devdaha, Sainamaina, Tilottama, and Suddhodhan Municipality. Serological testing using ID-Screen® BVD p80 Antibody Competition ELISA revealed that 10 samples (10.86%) tested positive for BVDV infection. Cattle aged over 5 years exhibited a higher seroprevalence rate in comparison to other age groups of 3-5 years and < 3 years. Notably, Holstein (26.7%) and Holstein cross (15.4%) cattle exhibited a significantly higher prevalence compared to jersey, jersey cross, and indigenous cattle ( $p < 0.05$ ). None of the assessed risk factors, including repeat breeding (infertility), history of abortion, history of retained placenta, diarrhea, and neonatal death, were found to be statistically significant in relation to the seroprevalence. The findings of this study indicate a comparatively higher seroprevalence rate than previous studies conducted in Nepal. Furthermore, the farmers of the affected herds displayed a lack of awareness regarding BVD and its presence on their farms. These results emphasize the urgent need for the relevant authorities to recognize BVD as a serious issue and take necessary nationwide measures for its control and eradication.

## Introduction

Bovine Viral Diarrhea (BVD) is an infectious disease that affects cattle globally and is predominantly caused by the Bovine Viral Diarrhea Virus (BVDV) and it is classified into two species, BVDV1 and BVDV2, which have distinct genetic and antigenic differences (Neill *et al.*, 2019). These species further consist of several sub-genotypes differentiated at the genomic level While cattle are the principal species susceptible to this pathogen, it is noteworthy that BVDV can also be detected in other

animals, including sheep, goats, and wild ruminants, serving as potential reservoirs for the virus (Sudharshana *et al.*, 1999). Bovine Viral Diarrhea Virus (BVDV) belongs to the Flaviviridae family and is classified as a pestivirus, characterized by its positive single-stranded RNA genome. BVD is a highly prevalent and economically significant disease in cattle populations. Clinically, the infection manifests in three distinct forms: (1) a persistently infected form (PI), (2) an acute transient form that can either be subclinical or exhibit symptoms such as fever, diarrhea, and

short-term immunosuppression, which can complicate concurrent illnesses like pneumonia (Kabongo and Van Vuuren, 2004); and (3) mucosal disease characterized by severe erosive lesions in the oral and intestinal mucosa, diarrhea and death (Houe, 1995). Bovine Viral Diarrhea Virus (BVDV) can be transmitted through vertical means, resulting in the congenital infection of the fetus, or through horizontal transmission after birth. Based on the timing of infection, fetal infection during the first trimester can result in fetal or embryonic death, leading to absorption, abortion, or mummification, infections during the second trimester can cause inflammatory responses and organ defects, while infections in the last trimester usually have less severe consequences for the fetus or the birth of persistently infected (PI) calves (Bielefeldt-Ohmann, 1995). These persistently infected individuals, which comprise approximately 1.0% to 2.0% of the cattle population, continuously shed the infectious virus (Fray *et al.*, 2000). Upon the introduction of a persistently infected (PI) animal into a herd, it is commonly assumed that the herd will remain infected over time. This is primarily attributed to the continuous production of PI animals, as susceptible cows become infected during pregnancy (Handel *et al.*, 2011). The availability of reliable diagnostic techniques, including ELISA and PCR, for detecting BVDV antibodies in milk or serum has significantly improved BVDV surveillance and the potential for disease control (Fray *et al.*, 2000).

In Nepal, with the contribution of 11.17% on the National gross domestic product by Livestock production (LIVESTOCK STATISTICS OF NEPAL, 2022), Livestock plays a pivotal role in farmers' livelihoods, contributing significantly to their earnings. Previous studies conducted in Nepal, specifically in the districts of Chitwan and Kavreplanchowk, have reported diverse seroprevalence rates of Bovine Viral Diarrhea Virus (BVDV) antibodies in cattle. These studies found higher prevalence rates among Holstein Friesian cross cattle, older age groups (above 5 years), and farms with larger populations of cattle (Gaire *et al.*, 2016; Manandhar *et al.*, 2018). In Bangladesh, a study found an overall prevalence of BVDV antibodies to be 51.1%, with significant seropositivity observed in cows with a history of abortion and retained placenta (Uddin *et al.*, 2017). BVDV prevalence varies globally, with the UK at 62%, Denmark at 78% and 64%, Sweden at 41% and 46%, Norway at 19%, the US at >10%, and India at 15.29% (Houe, 1995; Sudharshana *et al.*, 1999; Scharnböck *et al.*, 2018). The productivity and overall output of livestock are contingent upon their health status and the prevalence of diseases within their population. The introduction of the Bovine Viral Diarrhea (BVD) virus into a herd has detrimental consequences, including reduced milk production, impaired reproductive performance,

stunted growth, heightened susceptibility to other diseases, decreased overall vigor, premature culling, and elevated mortality rates among young animals. These repercussions lead to substantial economic losses for farmers, underscoring the critical impact of BVD on the profitability and sustainability of livestock operations (Houe, 2003). A meta-analysis study revealed that BVDV vaccination can reduce abortion by nearly 45% and fetal infection rate by about 85% in field trials. However, it also increases the pregnancy risk by 5% (Newcomer *et al.*, 2015). The Rupandehi district, encompassing a sizable cattle population of approximately 98,423 (LIVESTOCK STATISTICS OF NEPAL, 2022), is of particular concern due to its open border adjacency with India, posing potential threats of Bovine Viral Diarrhea (BVD). However, to date, there are no published reports addressing the prevalence of the BVD virus in this specific district. Consequently, the absence of comprehensive data underscores the need for research and surveillance efforts to evaluate the extent and impact of BVD within the region. Hence, the primary aim of this study was to examine the seroprevalence of Bovine Viral Diarrhea Virus (BVDV) infections and assess the related risk factors within the confines of the Rupandehi district in Nepal. Through rigorous investigation, this research endeavor sought to gain valuable insights into the prevalence and potential determinants of BVDV infections within the aforementioned district and will enable stakeholders to make informed decisions to mitigate the risks associated with BVD, safeguard livestock health, and protect the economic interests of farmers in the district.

## **Methods and Methodology**

### ***Survey Design***

The study was conducted in Rupandehi district, which spans approximately 1360 Sq. km and has a total cattle population of 98,423 (LIVESTOCK STATISTICS OF NEPAL, 2022) (Fig. 1). The survey design employed a two-stage random sampling procedure, with the selection of municipalities as the first stage and randomly selected animals within those municipalities as the second stage. Blood samples were collected from the municipalities of Devdaha, Sainamaina, Tilottama, and Suddhodhan.

### ***Sample Size and Selection of Sample***

The sample size for this study was determined using OpenEpi version 3, considering a confidence level of 95%, a desired precision of 5, a population size of 98,423 cattle (LIVESTOCK STATISTICS OF NEPAL, 2022), and an expected prevalence of 2.57% (Manandhar *et al.*, 2018). Based on this calculation, the required sample size was 39. However, a total of 92 female cattle were randomly selected from 17 different farms across four municipalities for blood collection (Table 1).



Fig. 1: Map of Study area

Table 1: Place and number of samples collected

S.N.	Place of blood collection (Rupandehi)	Sample numbers
1.	Sainamaina Municipality	25
2.	Suddhodhan Municipality	23
3.	Devdaha Municipality	26
4.	Tilottama Municipality	18
	<b>Total</b>	<b>92</b>

### Collection of sample and data

Blood samples were collected aseptically from the jugular vein of the cattle between January 28th and February 4th, 2021. Approximately 5-6 ml of blood was drawn into sterile 10ml tubes containing clot activator and labelled for identification. The tubes were then kept at room temperature overnight to allow for serum separation. The extracted serum was subsequently stored at -20 °C until further laboratory testing at the haematology lab of AHRD. To gather information on associated risk factors of BVD, a specially designed short questionnaire was prepared and administered to the farmers.

### Laboratory Procedures

The laboratory work was carried out at the Animal Health Research Division (AHRD), NARC, Khumaltar. To detect Bovine Viral Diarrhea Virus (BVDV) antibodies, a serological test was conducted using the ID-Screen® BVD p80 Antibody Competition enzyme-linked immunosorbent assay kit. This particular diagnostic kit is specifically designed to identify the presence of antibodies targeting the p80-125 protein (also known as NSP2-3) of the Bovine Viral Diarrhea virus through a competitive ELISA method. It is noteworthy that the kit exhibited a high level of both specificity and sensitivity, with a value of 100% for each. The test was performed following the guidelines provided in the manufacturer's manual "Thermo Fisher Scientific". The test is considered validated if the mean value of the

Negative control OD (ODnc) exceeds 0.7, and the mean value of the Positive control (ODpc) is below 30% of the ODnc (ODpc / ODnc < 0.3).

### Interpretation

For each sample, calculate the competition percentage (S/N %)

$$S/N \% = \left( \frac{OD_{\text{sample}}}{OD_{\text{nc}}} \right) \times 100$$

If the S/N % is equal to or less than 40%, it is classified as POSITIVE; if the S/N % is greater than 40% but equal to or less than 50%, it is categorized as DOUBTFUL; and if the S/N % exceeds 50%, it is considered NEGATIVE.

### Analysis

The data entry regarding the questionnaire survey and results of serological test were performed in Microsoft Office Excel and analyzed using SPSS. The relationship between sero-positivity and risk factors was examined using statistical tests including chi-square and Fisher's Exact Test.

### Results

#### Overall Prevalence

Among the 92 serum samples analyzed using the ID-Screen® BVD p80 Antibody Competition ELISA kit, 10 samples tested positive, 82 samples tested negative, and there were no samples classified as doubtful. The overall seroprevalence, determined on an individual animal basis,

was calculated to be 10.86%. This information is presented in Table 2.

**Table 2:** Overall sero-prevalence of BVD antibody in cattle Sera

Total Samples	Positive Samples	Negative Samples	Prevalence %
92	10	82	10.86

Similarly, among the total 17 farms, 6 of these farms had either one or two cattle infected with BVDV. Moreover, 4 of these 6 farms had 2 cattle infected and the other 2 farms had single animal infected. The farm wise prevalence was found to be 35.29% i.e 6 out of 17.

**Location wise Prevalence**

The seroprevalence of BVDV antibodies was higher in Sainamaina Municipality (12%) and lower in Suddhodhan Rural Municipality (8.69%). However, there was no significant association between seroprevalence and location based on the statistical analysis ( $p > 0.05$ ). The detailed location-wise prevalence can be found in Table 3.

**Age wise Prevalence**

The seroprevalence of Bovine Viral Diarrhea Virus (BVDV) antibodies was observed to be higher in cattle aged over 5 years compared to those in the age groups of 3-5 years and less than 3 years. However, the differences in prevalence among these age groups did not reach statistical significance ( $p > 0.05$ ). The detailed age-wise prevalence can be found in Table 4.

**Breed Wise Prevalence**

The study revealed that Holstein and Holstein cross cattle had a higher risk compared to jersey, jersey cross and local (indigenous). The seroprevalence of BVDV antibodies was found to be 15.4% among Holstein cross cattle and 26.7% among Holstein cattle. Furthermore, a statistically significant difference in the prevalence of BVDV antibodies was observed among different cattle breeds. Detailed information regarding these findings can be found in Table 5.

**Risk Factors Wise Prevalence**

Out of 10 positive samples, 2 (20%) of them were reported with the history of abortion, 4 (40%) of them reported with the history of repeat breeding and 1(10%) of them reported with the history of uterine prolapse. None of the samples with the history of diarrhea, still birth and neonatal death was found to be positive. Risk factors like history of abortion, repeat breeding, neonatal death, diarrhea and still birth were not found to be statistically significant with positive samples. Although the statistical significance was not observed with positive samples, the odds ratio for animals with a history of abortion compared to those without abortion was determined to be 3.85. Similarly, the odds ratio for infertile (repeat breeding) animals compared to fertile animals was found to be 1.71. These results indicate that animals with a history of abortion are at a 3.85 times higher risk than those without abortion, and animals exhibiting signs of repeat breeding are at a 1.71 times higher risk compared to animals without such signs. These are presented in the Table 6.

**Table 3:** Location wise prevalence of BVDV antibody in the sera of cattle

S.N.	Location	Sample taken	Sample Positive	Prevalence	Fisher's Exact Test (p value)
1	Sainamaina Municipality	25	3	12%	
2	Suddhodhan Rural Municipality	23	2	8.69%	$p > 0.05^*$
3	Devdaha Municipality	26	3	11.53%	
4	Tilottama Municipality	18	2	11.11%	

\*Confidence interval at 95%

**Table 4:** Age wise Prevalence of BVDV antibody in the sera of cattle

Age group	Total	Positive	Prevalence	Fisher's Exact Test (p value)
Less than 3 yrs	24	2	9.09%	0.271
3 to 5 yrs	35	2	6.06%	$(p > 0.05)^*$
5 yrs and above	33	6	22.22%	

\*Confidence interval at 95%

**Table 5:** Breed wise prevalence of BVDV antibody in the sera of cattle

Breed	Total	Positive	Prevalence	Fisher's Exact Test (p value)
Local (indigenous)	7	0	0.0%	
Jersey	19	1	5.3%	0.015
Jersey Cross	25	1	4.0%	$(p < 0.05)^*$
Holstein	15	4	26.7%	
Holstein Cross	26	4	15.4%	

\*Confidence interval at 95%

**Table 6:** Risk factors prevalence and odd ration of risk factors

Risk Factors	Total samples	Positive samples	Prevalence	Odd ratio	Fisher's Exact Test (P value)
Repeat Breeding	27	4	14.81%	1.71	0.6547*
No Repeat breeding	65	6	9.23%		
Abortion History	7	2	28.57%	3.85	0.3327*
No abortion history	85	8	9.41%		
History of Retained Placenta	3	0	0.00%	N/A	N/A
Diarrhea	6	0	0.00%	N/A	N/A

\*Confidence interval at 95%

There was no statistically significant difference in the seroprevalence between pregnant and non-pregnant cattle (Fishers exact test,  $P > 0.05$ ), as well as between lactating and non-lactating cattle (Fishers exact test,  $P > 0.05$ ). The seroprevalence result also did not show any statistically significant association with farm size or farmer training. Although not reaching statistical significance, the odds ratio between farmers with training and those without training was 6.857, indicating that farmers without training are at 6.8 times higher risk of BVD presence on their farms compared to farmers with training.

## Discussions

The current study revealed that out of 92 serum samples collected from cattle in Rupandehi district, 10.86% ( $n=10$ ) tested positive for BVDV antibodies using ELISA test. These findings indicate a relatively higher seroprevalence compared to previous studies conducted in Nepal (Gaire *et al.* 2016; Manandhar *et al.*, 2018), but lower compared to studies conducted in India (Sudharshana *et al.*, 1999), Malaysia (Daves *et al.*, 2016), Ethiopia (Aragaw *et al.*, 2018), Bangladesh (Uddin *et al.*, 2017), and Cameroon (Handel *et al.*, 2011). The seroprevalence observed in this study was higher than the findings reported by (Kabongo and Van Vuuren, 2004), where BVD viruses were detected using virus isolation in cell cultures. This variation in seroprevalence among previous studies and the present study could be attributed to geographical differences, variations in ELISA kit types, different methodologies employed, and time intervals between the studies, among other factors. It is worth noting that Rupandehi district, located in the southern part of Nepal, shares an open border with India, which may contribute to inadequate quarantine measures both at the cross-border and farm levels. Considering the high prevalence of BVDV in India (Sudharshana *et al.*, 1999), it is plausible to suggest that viral pathogens could have been transmitted between the countries during the transportation of cattle. This cross-border movement may have contributed to an increase in the seroprevalence of BVDV antibodies compared to previous studies conducted in the Nepal in other regions. The higher seroprevalence in other countries might be due to the production of antibodies triggered by the vaccination. Since there is no provision of BVD vaccination in the farms

of our study area, the seroprevalence may due to the natural infection between the animals.

There was no statistically significant association found between location and the occurrence of BVDV ( $p > 0.05$ ). However, Sainamaina Municipality (12%), Devdaha Municipality (11.53%), and Tilottama Municipality (11.11%) exhibited higher prevalence percentages compared to Suddhodhan Municipality (8.69%). These variations in prevalence could be attributed to factors such as geographical barriers, cattle market and trade activities, and management practices, which could have facilitated or prevented the transmission of the virus among farms. Furthermore, it is worth noting that Sainamaina, Devdaha, and Tilottama municipalities are closely connected to highways, and the transportation of animals from the open border with India may contribute to the higher prevalence rates of BVD. Further research is needed to fully understand the reasons behind these differences. The study did not observe a significant difference in BVD prevalence based on age ( $p > 0.05$ ), indicating that all age groups have similar chances of contracting BVD. However, the seroprevalence of Bovine Viral Diarrhea Virus (BVDV) antibodies was observed to be higher in cattle aged over 5 years compared to those in the age groups of 3-5 years and less than 3 years. This finding aligns with previous research by Daves *et al.* (2016); Demil *et al.* (2021) & Hashemi *et al.* (2022), which also reported an increase in BVD positivity with age whereas study of Manandhar *et al.* (2018) and Uddin *et al.* (2017) showed the prevalence to be higher in age group 3 to 5 years. The higher prevalence of BVD in older age groups might be attributed to factors such as cumulative exposure to the virus over time throughout the animals life, especially when detected via antibody- based assays (Demil *et al.*, 2021; Hashemi *et al.*, 2022).

The present study observed a significantly ( $P < 0.05$ ) higher seroprevalence of BVD among Holstein and Holstein cross cattle compared to Jersey, jersey cross and indigenous breed. These findings align with a previous study by Aryal (2017) but contradict the reports of higher prevalence among jersey cross cattle Manandhar *et al.* (2018) and jersey cattle followed by Friesian Daves *et al.*, (2016). Similarly, a recent study in Ethiopia by Aragaw *et al.* (2018) and Southern Ecuador by Herrera-Yunga *et al.* (2018) found

a higher seroprevalence among HF cattle compared to jersey cattle. Holstein and Holstein cross cattle might possess certain genetic factors that make them more vulnerable to BVDV and, therefore, more likely to exhibit higher seroprevalence compared to other breeds such as Jersey and jersey cross. However, further research is needed to understand the underlying mechanisms behind these breed-specific differences in BVD prevalence.

The current study revealed no significant associations between the seroprevalence of BVDV antibodies and various risk factors such as abortion, repeat breeding, stillbirth, diarrhea, and neonatal death. This finding is consistent with the findings of a previous study conducted by Manandhar *et al.* (2018). Similarly, reproductive problems were not found to be associated with BVDV seroprevalence except repeat breeding in the study of Aragaw *et al.* (2018).

### Conclusions and Recommendations

The higher prevalence of BVDV antibody in this study compared to previous studies in Nepal highlights a significant economic threat at the farmer level, indicating the absence of effective control measures for BVD prevention and eradication. Furthermore, the lack of awareness among farmers about BVD and its presence in their herds increases the risk of transmission between farms through animal contact and movements. Limited studies on BVD have been conducted in only a few districts of Nepal, making this study an important baseline for further research on BVD in the country. It is crucial for the relevant authorities to recognize and address this issue seriously, implementing necessary nationwide measures for the control and eradication of BVD.

### Authors' Contribution

Padam Tandan conceived and designed the study, collected the samples, performed the laboratory analysis, interpreted the results, drafted the final version of the manuscript. Madhav Paudel contributed in sample collection, laboratory work, statistical analysis and writing & revision of the manuscript. Both the authors reviewed and revised it for important intellectual content, approved the final version of the manuscript for submission and take responsibility for the accuracy and integrity of the work.

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### Ethical Concern

As a researcher, I am deeply concerned about animal welfare and the need for informed consent from cattle

owners in my study. Throughout the research process, I prioritize the well-being of the animals, minimizing health risks and stress during sample collection and testing. Obtaining informed consent from cattle owners is crucial, respecting their rights as stakeholders. I am fully committed to following proper protocols and guidelines, prioritizing animal welfare and ensuring informed consent to address this ethical concern responsibly.

### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

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