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Case Report
Prevalence of Bovine Viral Diarrhea Virus Infection in Dairy Herds of Nepal

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Abstract

Background: The Bovine Viral Diarrhea Virus (BVDV) infection causes reproductive and respiratory disease in cattle and buffaloes and is an economically important livestock disease across the world. In Nepal, cattle and buffalo farming is an important source of income for a majority of farmers. Infectious reproductive and respiratory diseases are common in Nepalese dairy herds and a large proportion of these cases remains undiagnosed. The burden of BVDV in Nepal is unknown. The objective of this pilot study was to determine the prevalence of persistent BVDV infection in dairy cattle in two commercially important livestock districts of Nepal. **Materials and Methods:** In total, 240 ear notch samples (153 samples from Chitwan and 87 samples from Kavrepalanchowk) from 60 dairy herds (30 herds in each district) were collected from cattle and buffaloes from December, 2014 through April, 2015. Questionnaire surveys were conducted to collect information on demographics and farm characteristics. **Results:** The farm-wise prevalence was 3.3% (95% CI: 0.1-17.2%) in Chitwan and 10.0% (95% CI: 2.1-26.5%) in Kavrepalanchowk, whereas individual animal prevalence was 0.7% (95% CI: 0.0-3.6%) in Chitwan and 3.4% (95% CI: 1.2-9.7%) in Kavrepalanchowk. Questionnaire survey indicated overall poor biosecurity in dairy farms and use of natural insemination for breeding with bulls of unknown origin, which may contribute to the spread of BVDV infection. **Conclusion:** This pilot study indicates circulation of BVDV and the presence of persistent infections in dairy herds in Nepal. It is suggested that the Nepal government and commercial dairy farmers include BVDV in regular surveillance and diagnostic activities.

Key words: Bovine viral diarrhea, biosecurity, dairy herds, prevalence

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bovine Viral Diarrhea Virus (BVDV) belongs to the genus *Pestivirus* within family Flaviviridae and is one of the most important pathogens of ruminants worldwide, particularly in cattle herds where it causes substantial economic losses[1]. The BVDV has a global distribution and is responsible for a variety of consequences in cattle, including reproduction dysfunctions (abortion, teratogenesis, embryonic resorption, fetal mummification and still birth), reduced milk yield, diarrhea and hemorrhagic syndrome[2-4]. Pregnant cattle undergoing infection with a non-cytopathogenic biotype of BVDV are able to transmit BVDV infection to their fetuses, resulting in the development of Persistently Infected (PI) carrier calves. These PI cattle potentially shed the virus for life, thus playing an important role in the epidemiology of BVDV infection[5,6]. Semen produced by PI bulls may contain the virus that can be transmitted to cows either by natural or artificial insemination[7,8]. Collectively, both acute and persistent infections with BVDV are responsible for causing substantial economic damage in infected herds and severe consequences to the health and productivity of individual animals. Proper biosecurity in animal houses, testing of new animals before introducing to the herd and removal of positive PI animals from herds are argued to be effective measures for control of BVDV infection[9]. Diagnosis of BVDV infection, particularly in PI animals, is critical to the establishment of control practices in dairy herds to minimize the losses caused by respiratory and reproductive problems associated with BVDV infection.

The prevalence of persistent BVDV infections ranges from 0.5-2% in cattle populations from different countries of the world[10,11]. In India, 15.3% cattle and 23.2% buffaloes were reported to be BVDV positive[12] in 1999. Similarly, in 2014, 24% of yaks (*Bos gruniens*) in Qinghai, China[1] and 26% of cattle in Saudi Arabia[13] were identified as being infected with BVDV.

Reproductive problems including abortion and infertility are major issues in the dairy herds of Nepal. There is scarce information on the causes of bovine reproductive problems in Nepal. District veterinary reports imply that the majority of reproductive problems among dairy herds are due to various types of infections and poor nutrition. Though BVDV has not been reported in Nepal, government surveillance activities for livestock diseases has revealed livestock showing clinical signs similar to BVDV infection such as respiratory illnesses, diarrhea and abortion. In addition, 91% of livestock are bred through natural service by bulls having an unknown origin[14]. The lack of baseline data of BVDV in domestic ruminants has resulted in a poor overall understanding of the epidemiology of this disease in Nepal.

The purpose of this study was to screen for persistent BVDV infection in dairy cattle of Nepal and characterize the factors associated with its occurrence. The study included two districts; Chitwan and Kavrepalanchowk, both of which have higher than average livestock population and milk production and have commercial livestock farms. The objectives were to estimate the prevalence of persistent BVDV infection in commercial dairy herds and, characterize factors associated with the positivity of BVDV infection.

MATERIALS AND METHODS

Study design: This study was conducted in the Chitwan and Kavrepalanchowk districts of Nepal (Fig. 1). Chitwan, a southern district of Nepal, represents Terai low land and Kavrepalanchowk, in the North, represents mid-hill of Nepal. These two districts are the major supplier of milk to the capital Kathmandu as well as to other nearby districts.

Sampling framework: The unit of interest in this study was the dairy herd. Herds that had more than 5 individual cattle and/or buffaloes were included in this study. In Nepal, a large proportion of farmers keeps only one or two cattle and/or buffaloes to support their livelihood. There is no legal definition for commercial versus subsistence farms in Nepal, but generally commercial farmers sell their milk to the nearby collection centers, which in turn are transported to the dairy industry in the cities, whereas, subsistence farmers basically consumes milk in their home and sell extra milk to the local teashops and individual houses. Based on the experience of field veterinarians, a cut-off value of five or more cattle and/or buffaloes was chosen and considered them as commercial dairy farms. The estimated number of dairy herds that meets these criteria in Chitwan and Kavrepalanchowk districts were 140 and 90, respectively, with the average number of animals per herd ranging from 5-50 (Personal communication: District Livestock Service Office). Assuming 25 animals on an average, approximately 5,000 cattle and/or buffaloes in commercial dairy herds in both Chitwan and Kavrepalanchowk were estimated. This estimation was done as District Livestock Service Office did not have official data on commercial dairy herds. Since baseline data on BVDV infections in Nepal is not available, expected prevalence of 17% based on the prevalence of BVDV in India[12] was estimated.

The sample size was calculated using an online software program Epitools[15]. The assumptions were population size of 5,000 cattle and buffaloes, expected prevalence of 17%, test sensitivity and specificity of 95 and 99%, respectively, 5% precision and 95% confidence interval. The required sample size calculated was 235.

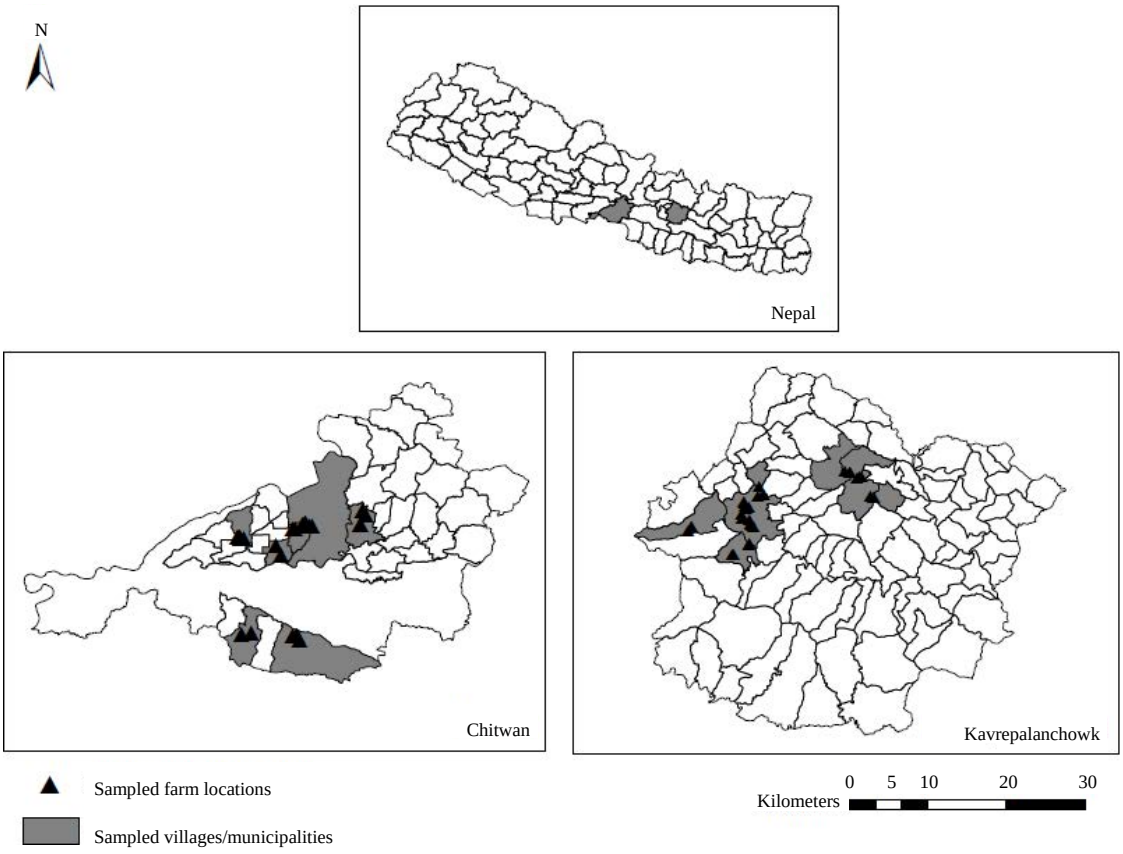

Fig. 1: Map of Nepal (top) and study districts showing sampling locations (black triangles) and villages (gray shaded areas)

A list of dairy herds in the districts was prepared after communicating with local field veterinarians and veterinary technicians and individual herd owners from that list were contacted. Once the herds were selected, the number of individual animals to be sampled in each herd was determined depending upon the herd size as well as owner's permission. Best effort was made to collect samples of different age group from each herd. In total, 240 ear notch samples from 60 herds (153 samples from 30 herds in Chitwan district and 87 samples from 30 herds in Kavrepalanchowk district) were sampled. Out of 153 samples in Chitwan, 109 samples were from cattle and 44 samples were from buffaloes. All the samples from Kavrepalanchowk were from cattle. Samples were collected from six village development committees and two municipalities in Kavrepalanchowk and five village development committee and two municipalities in Chitwan.

Sample analysis: Ear notch samples were obtained from calves and their mother and other adults from dairy herds constituting both cattle and buffalo and transported to the laboratory of Himalayan College of Agricultural Sciences and Technology (HICAST) in a cooler box containing ice packs. Before ear notch samples were collected, permission was obtained from farm owners or managers. Trained veterinarians collected the sample and communicated test results to the farmers. The samples were tested for the presence of antigen using commercial BVDV antigen detection assay (SNAP BVDV Antigen Test, IDEXX Laboratories, Westbrooke, ME USA) per manufacturer instructions.

Questionnaire survey: Face to face interview with farm owners or caretaker 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 situation and herd-health management. Management practices included feeding, veterinary services they receive from and types of breeding methods used (natural or artificial insemination). Bio-security questions included contact history of animals, fencing status of the farm, provision of footbaths and quarantine measures (how do they handle when new animals were brought to their

Table 1: Characteristics of farms sampled for BVDV in Chitwan and Kavrepalanchowk

Variable and category	District	
	Chitwan percentage (95% CI)	Kavrepalanchowk percentage (95% CI)
Management measure		
Feeding		
Stall feeding	83.3 (65.3-94.4)	60 (40.6-77.3)
Mixed (grazing and stall feeding)	16.7 (5.6-34.7)	40 (22.7-59.4)
Type of reproduction		
Artificial insemination	40 (22.7-59.4)	10 (2.1-26.5)
Natural insemination	10 (2.1-26.5)	66.7 (47.2-82.7)
Both	50 (31.3-68.7)	23.3 (9.9-42.3)
Veterinary services		
Animal health technicians	93.3 (77.9-99.2)	73.3 (54.1-87.7)
Veterinarians	6.7 (0.8-22.1)	26.7 (12.3-45.9)
Bio-security		
Contact with animal from other herds	10 (2.1-26.5)	33.3 (17.3-52.8)
Farm fenced	43.3 (25.5-62.6)	46.7 (28.3-65.7)
Provision of footbath	10 (2.1-26.5)	0
Quarantine measures	6.6 (0.8-22.1)	16.7 (5.6-34.7)
Distance to nearest farms (m)		
<100	53.3 (34.3-71.7)	16.7 (5.6-34.7)
100-500	36.7 (19.9-56.1)	46.7 (28.3-65.7)
>500	10 (2.1-26.5)	36.7 (19.9-56.1)
Herd health status		
History of diarrhea	23.3 (9.9-42.3)	10 (2.1-26.5)
History of respiratory illness	66.7 (47.2-82.7)	30 (14.7-49.4)
History of abortion	30 (14.7-49.4)	30 (14.7-49.4)

farm). Herd health status included questions on the history of diarrhea, abortion, respiratory illnesses, any unusual signs exhibited by their animals and vaccination status.

Data analysis: For statistical analysis, the raw data were managed in Microsoft® Excel. The prevalence of BVDV infection at herd level and individual animal level were calculated for each district by dividing the number of positive herds and individual animals by total herds and individuals tested in that district, respectively. Herds were considered positive if at least one individual from that herd tested positive. Descriptive statistics of farm characteristics were performed. Risk factor analysis was not conducted as there were very few positive herds and individuals. Descriptive statistical analysis was performed using SAS 9.4 software (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

The result showed that one farm out of 30 in Chitwan and three farms out of 30 in Kavrepalanchowk were positive for persistent BVDV infection. The farm-wise prevalence was 3.3% (95% CI: 0.1-17.2%) in Chitwan and 10% (95% CI: 2.1-26.5%) in Kavrepalanchowk. Among individual animals, only one animal among 153 animals tested positive in Chitwan while three animals among 87 animals tested positive for BVDV infection in Kavrepalanchowk. Individual animal-wise prevalence was 0.7% (95% CI: 0.0-3.6%) in Chitwan and 3.4% (95% CI: 1.2-9.7%) in Kavrepalanchowk.

Overall, the farm-wise prevalence was 6.7% (95% CI: 2.6-15.9%) and individual animal-wise prevalence was 1.7% (95% CI: 0.6-4.2%). Out of four positive samples, all were cattle and none of the samples from buffalo was positive for BVDV. Of 60 herds, none of the herds were vaccinated against the BVDV. The details of farm characteristic were collected and analyzed to describe the farm characteristics (Table 1).

Farm characteristics: Average herd size among our sampled farms was 12.9 (95% CI: 10.0-15.8) and median herd size was 10 animals (cattle and buffaloes) (range: 5-32 animals) in Chitwan, whereas average herd size was 9.2 (95% CI: 7.2-11.2) and median herd size was 8 animals (cattle and buffaloes) (range: 5-31 animals) in Kavrepalanchowk. The questionnaire survey indicated that none of the farmers had heard about BVDV in Chitwan while only one out of 30 farmers had heard about the disease in Kavrepalanchowk. The detail characteristics of sampled farm for each district on management practices such as feeding and breeding, bio-security situation and herd health condition are presented in Table 1.

DISCUSSION

Four cattle (two calves, one heifer and one adult cow) were found positive for BVDV antigen while none of the buffalo were positive. There is no history that animals selected for this pilot study were vaccinated against BVDV as vaccines against BVDV are not available in Nepal. Antigens detected in sampled ruminants indicate a natural exposure to BVDV infection.

In this study, individual animal prevalence was 1.7% and farm prevalence was 6.7% when combined for both districts. A recent study from China, which share border with Nepal, found similar rates, with an average prevalence of persistent BVDV infection of 1.4% (14/1,010) for four species (dairy cows, beef cattle, yak and water buffalo) using the same testing platform that we used[16]. Another epidemiological investigation revealed the prevalence of persistent BVDV infection ranging from 0.5-2.0% in cattle populations globally[11].

Nepal shares open borders with India to the east, west and south and livestock movement between the two countries is frequent. Although, there is little formal livestock trade through official quarantine routes, the clandestine trade makes up a larger proportion of trade in-movements. Even through official quarantine, animals in incubation period can easily pass through, as apparently healthy animals are frequently not quarantined in Nepal. When the disease is present in one country, there is a higher probability of disease introduction to adjacent countries when the border biosecurity measures are porous. The seroprevalence of BVDV was 24.7% among 385 dairy cattle reared under a small-holder system in Trichur district of Kerala State in India[17] and in India seroprevalence of BVDV[12] was 17.3% whereas, seroprevalence of yaks was reportedly 53.65% in Tibet[18] and movement of animals has great impact on the epidemiology of many economically important diseases including BVDV[19]. Moreover, farmers of infected herds are unaware of their own animal's BVDV status in farms[20] and this situation leads to the risk of BVDV through movement of animal and neighboring contact[21].

This survey indicated that the bio-security and quarantine measures practices in both districts were poor. Less than 50% farms were fenced in both districts and only 10% of the farms had the provision of footbath at the entrance of the farm in Chitwan and interestingly, none of the farms had the provision of footbath at the entrance of the farm in Kavrepalnchowk district. The majority of the farms were within 100-500 m distance which suggests a higher density of farms. Similarly, only 6.6 and 17.6% of the farms in Chitwan and Kavrepalanchowk districts practiced isolation of animals when new animals were introduced in the farm (quarantine measures). Lack of quarantine measures adopted by farmers poses a risk of BVDV introductions in the farms through the direct purchase of infected animals[22]. The risk further increases if the purchased animal is seropositive pregnant dams as it gives birth to Persistent Infected (PI) calves[8]. This poor bio-security and quarantine related measures may have contributed to the introduction of the disease, however, we don't have evidence for that. This hypothesis is also supported by Garoussi *et al.*[23] who indicated that prevalence of BVDV is influenced by cattle density.

Maintaining open breeding on farms is a primary risk factor for BVDV introduction[24,25]. Results show that 10% of the farms in Chitwan and more than 50% of the farms in Kavrepalanchowk practice natural insemination. In Nepal, one or two private or community bulls for entire villages are maintained for natural insemination and these bulls provide service to the cow of the entire village. This may be one risk factor that could be associated with the introduction of BVDV in these farms and account for the higher prevalence of BVDV in herds in Kavrepalanchowk district. In Nepal, 91% of livestock are bred through natural service by bulls having an unknown origin[14]. Use of bulls without testing for BVDV could transmit the virus by natural or artificial insemination[7,8]. This study found that greater than 30% of farms in both districts had a history of abortion and calf mortality. The BVD is one of the most important infectious diseases associated with infertility and abortion[4,26] and deserves great attention in Nepal.

CONCLUSION

This is the first report to characterize the status of persistent BVDV infection and important farm characteristics in Nepal. Of 60 dairy herds, in two commercially important livestock districts of Nepal, nearly seven percent had animals with persistent BVDV infection, which could be significant sources of infection to other susceptible cattles and buffaloes. Studies to obtain baseline prevalence and epidemiological features of a disease are necessary to the rational formulation of disease control programs. Surveillance plans should be developed and awareness programs strengthened to better understand BVDV in Nepal, to minimize economic losses, increase milk production and enhance dairy herd health.

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STATEMENT OF ANIMAL RIGHTS

The objective of the study was explained to the farmers before the sample collection and the consent was obtained to collect the ear notch samples from their animals. Trained government veterinarians were involved in sample collection.

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