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Epidemiological survey of bovine viral diarrhoea in dairy cattle in Nepal

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Summary

A random survey was conducted to study the seroprevalence and associated risk factors of bovine viral diarrhoea virus (BVDV) in the Western Chitwan district of Nepal, using the 'Survey Toolbox' sampling software. A two-stage sampling procedure was adopted. In the first stage, Village Development Committees (VDCs) were selected, and in the second stage animals were selected. A total of 350 animals from five selected VDCs were screened for BVDV antibodies by an indirect enzyme-linked immunosorbent assay (indirect ELISA). The study showed the apparent overall prevalence of BVDV to be 2.6% and the true prevalence to be 2.2%, with the highest prevalence of 2.7% in Gitanagar VDC, followed by Sharadanagar (2.4%), Mangalpur (2.1%), Gunjanagar (0%) and Divyanagar (0%). The prevalence of BVDV antibodies in Jersey cross cattle was 2.4% and in Holstein–Friesian crosses it was 1.7%. In cattle of one to three years of age the prevalence was 1.2%, in those of three to five years it was 3% and in those above five years it was 2.1%. Similarly, the prevalence values in cattle with a history of abortion, infertility, diarrhoea and neonatal death were 9%, 0.95%, 5.8% and 0%, respectively. None of the risk factors studied was associated significantly with BVDV ($p > 0.05$). The study revealed a very low prevalence of antibodies to BVDV, which suggests that Nepal is virtually free from BVDV. To the authors' knowledge this is the first study of bovine viral diarrhoea (BVD) in Nepal, and it took place only in the Chitwan district, therefore this study has produced baseline data on BVD in Nepal which will help the authorities to investigate further.

Keywords

Bovine viral diarrhoea (BVD) – Bovine viral diarrhoea virus (BVDV) – Chitwan – Nepal – Risk factor – Seroprevalence.

INTRODUCTION

Bovine viral diarrhoea (BVD) is an infectious disease of cattle caused by bovine viral diarrhoea virus (BVDV), which is one of the most important viral pathogens of cattle worldwide [1]; BVDV is a pestivirus in the Flaviviridae family. Infections with BVDV are endemic in most cattle-producing countries throughout the world, and cause significant economic losses to the cattle industry [2]. There are two species of BVDV, BVDV-1 and BVDV-2, which are discernible by antigenic and genetic analysis [3]. The prevalence of BVDV varies across the world: BVDV-2 historically represented around 50% of the isolates in North America, although an increasing percentage of BVDV-1b has accounted for 75–100% of the samples collected after 2001, while BVDV-1 dominates in Europe, comprising more than 90% of the isolates [4, 5, 6]. In addition, an atypical bovine pestivirus, BVDV-3, has recently been detected in cattle in South America, Asia and Europe and in contaminated Madin–Darby bovine kidney (MDBK) cells [7]. Transmission of BVDV may occur either vertically, leading to congenital infection of the fetus, or horizontally after birth. Depending on the timing of infection, there may be a significant reduction in conception rate and an increased number of abortions, malformations, stillbirths or births of persistently infected (PI) calves [8].

In Nepal, especially in the Chitwan district, the majority of dairy farms are small, rearing two to three dairy cattle with their calves. However, some farmers keep more than three dairy cattle. According to the Department of Livestock Services of the Government of Nepal there are large commercial dairy farms in Gitanagar, Sharadanagar and Mangalpur Village Development Committees (VDCs). However, there is no difference in animal management practices between the small and large herds. Cattle are fed the same type of roughage and concentrate feeds, undergo the same vaccination protocols, deworming and artificial insemination (AI) practices, and are visited by the same veterinarians. Reproductive problems including infertility, anoestrus, repeat breeding and abortion are major economic burdens in cross-bred dairy cattle in Nepal [9, 10]. Various infectious causes of infertility in dairy cattle have been investigated, but not BVDV [11]. In the Chitwan district of Nepal, which shares a border with India, most of the cross-bred dairy cattle are brought in from India, where BVDV is prevalent [12, 13, 14, 15]. Various clinical signs, including diarrhoea, abortion and mouth lesions, and post-mortem lesions, such as petechial and ecchymotic haemorrhage on serosal surfaces and abomasal ulceration, similar to mild acute infection with BVDV, have been reported (personal communication). However, there has been no report of an investigation of BVDV infection to date. Therefore, the objectives of this study were to investigate the seroprevalence of BVDV infections and associated risk factors in the Chitwan district of Nepal.

MATERIALS AND METHODS

Survey design

Village Development Committees (VDCs) form the basic geographical and administrative units in the Chitwan district. Most of the farms in the VDCs are small. The animals are kept in close contact and tend to mingle freely while grazing or drinking water. Cattle from different farms, which are reared using similar husbandry techniques, are thus exposed to the same infectious diseases. Animal health camps are organised several times a year, during which animals from most of the VDCs come together and susceptible animals may be exposed to infection. Some VDCs are very near to national park areas, thus sporadic exposure to wildlife cannot be excluded. In Nepal, 91% of livestock are bred through natural service by bulls of unknown origin [16]. In the study area, however, most of the dairy cattle are either Holstein–Friesian crosses or Jersey crosses, and breeding is done with the help of AI. Most of animals in the study area are vaccinated against foot and mouth disease (FMD), haemorrhagic septicaemia and 'black quarter' (*Clostridium chauvoei* infection), but there is no history of vaccination against BVDV, and farmers have no knowledge about BVDV. Most of the dairy animals in this area are brought in from India, and movement of animals between VDCs is frequent because of the purchase and sale of animals. The western dairy pocket area in the Chitwan district is divided into ten VDCs according to existing political boundaries, and this division was applied during the design of the survey. The VDCs are displayed in Figure 1, and a two-stage sampling survey design was used [13].

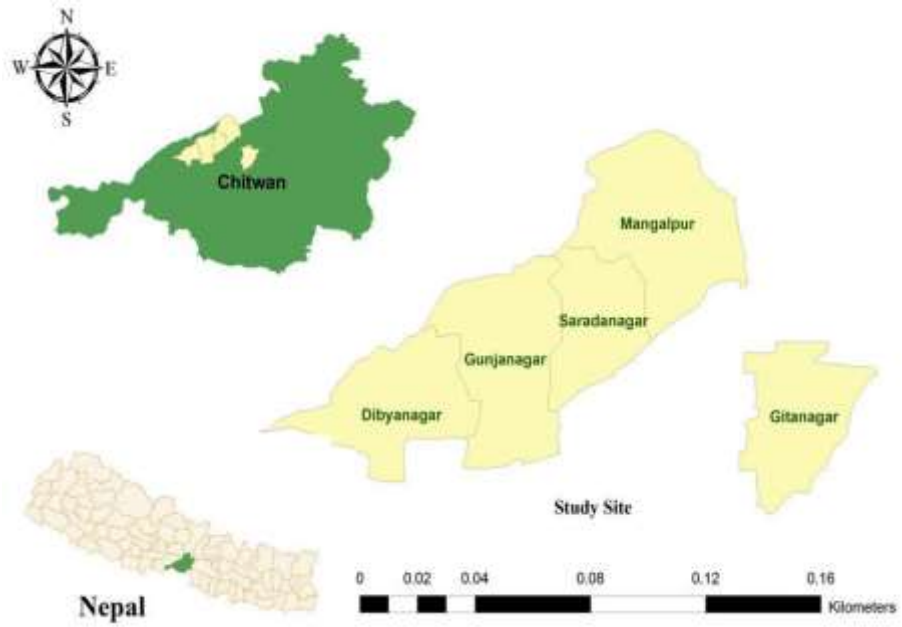

Fig. 1
Map of the study site

Sample size and selection of sample

Given that a good sampling frame containing all the VDCs in the study area was available, including reliable livestock population data, a probability proportional to size (PPS) design was used in the first stage. The VDC was the primary sampling unit and was selected on a random basis from all ten VDCs in the western 'dairy pocket area' of Chitwan. In this design, VDCs with a larger cattle population had a greater chance of being selected. In the second stage, a fixed proportion of animals, i.e. 350 (16%) of the total population, was chosen from the selected VDCs, using simple random sampling. Each of the animals in the VDCs was numbered individually. The required number of animals from each VDC was selected by lottery for all five selected VDCs. A map showing the selected VDCs is shown in Figure 1, which was developed using Arc Map 9.1 (Esri India Technologies Ltd., India). The sample size was calculated using the computer programme Survey Toolbox, a practical manual and software package for active surveillance of livestock diseases in developing countries (Australian Centre for International Agricultural Research, Bruce Act 2617, Australia). The programme used a two-stage sampling survey design [17] considering the total size of the population, an estimated prevalence of 50%, a within-VDC variance of 0.15, between-VDC variance of 0.03, fixed width confidence interval of ± 10% and a confidence level of 95%.

The required sample size was calculated as 350 from the five selected VDCs in western Chitwan. Table I shows the selected VDCs with the number of samples collected. The selected farmers were surveyed and information regarding the age and breed of their cattle, abortions, infertility and the presence of diarrhoea was collected from the farmers at interview.

Table I
Selected VDCs and calculated number of samples

Selected VDCs	Total cattle	No. of samples
Gunjanagar	110	17
Divyanagar	100	16
Sharadanagar	450	72
Mangalpur	504	81
Gitanagar	1,024	164
Total	2,188	350

Collection of data

The study was conducted from November 2013 to April 2014. Of the 350 samples collected, 212 were from Jersey cross and 138 were from Holstein–Friesian cross cattle. Blood samples were collected in sterile 10 ml vacutainer tubes and centrifuged. The separated serum was removed and stored at –20 °C until testing.

Laboratory procedures

Laboratory work was performed at the National Avian Disease Investigation Laboratory (NADIL), Bharatpur, Chitwan. The IDEXX BVDV Total Ab Test Kit (IDEXX Laboratories Inc., Westbrook, Maine, USA) is an indirect enzyme-linked immunosorbent assay (indirect ELISA) and was used for the detection of BVDV antibodies in individual serum samples. The test was run in accordance with the procedure detailed in the manufacturer's manual. The sensitivity and specificity of the kit are 96.3% and 99.5%, respectively.

Statistical analysis

Data entry, management and analysis were completed using Microsoft® Office Excel 2007. The associations between BVDV seropositivity and different risk factors for disease, such as location, age, breed, history of abortion, history of infertility (repeat breeding and anoestrus) and history of diarrhoea, were compared and analysed statistically using chi-square (χ^2) analysis and the Fisher exact test in the computer software OpenEpi version 2.3 (Open Source Epidemiologic Statistics for Public Health; The OpenEpi Project, Atlanta, Georgia, USA) [18], with the significance level defined at $p < 0.05$. True prevalence was calculated with a 95% confidence interval (CI) using the 'True Prevalence' programme in Survey Toolbox, in which sensitivity, specificity and sample size were taken into consideration. Odds ratios (OR) for breed, age group and different risk factors were calculated using OpenEpi version 2.3.

RESULTS

The overall and location-specific seroprevalence values for BVDV in cattle in the study area are shown in Table II and Table III. The overall true prevalence of BVDV antibodies found was 2.2% (CI 1.31–3.01). The highest proportion of positive samples was found in Gitanagar (3.1%; 5/164), followed by Sharadanagar (2.8%; 2/72) and Magalpur (2.5%; 2/81). There were no detectable antibodies against BVDV in samples collected from Divyanagar and Gunjanagar. The three VDCs with positive samples accounted for a higher number of samples tested than the two VDCs with no positive samples.

Table II
Overall seroprevalence of BVDV antibodies in cattle sera

Total samples	Positive samples	Negative samples	Apparent prevalence	True prevalence
350	9	341	2.60%	2.16% (1.314–3.007)

Note: The figure in brackets indicates the 95% confidence interval

Table III
Location-wise distribution of serum antibodies against BVDV

Location	Total sample	Positive sample	Apparent prevalence	True prevalence	Chi-squared *p* value
Mangalpur	81	2	2.47%	2.056% (0.330–3.783)	0.9669
Gunjanagar	17	0	0.00%	N/A	
Divyanagar	16	0	0.00%	N/A	
Sharadanagar	72	2	2.78%	2.38% (1.318–4.006)	
Gitanagar	164	5	3.05%	2.662% (1.318–4.006)	

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

The age-wise and breed-wise seroprevalence values of BVDV in cattle in the study area are shown in Table IV and Table V. The results showed no significant effect of age and breed on the seroprevalence of BVDV. It was found that 1.6% (2/123), 3.4% (5/147) and 2.5% (2/80) animals were seropositive for BVDV among those aged one to three years, three to five years and above five years, respectively. Similarly, the results in Table V show that 2.8% (6/212) of Jersey cross and 2.2% (3/138) of Holstein–Friesian cross cattle were seropositive for BVDV.

Table IV
Age-wise distribution of serum antibodies against BVDV

Age group	Total	Positive samples	Apparent prevalence	True prevalence	Odds ratio	Chi-squared *p* value
1–3 years	123	2	1.63%	1.18% (0.037–2.322)	0.469 [a] (0.0894–2.463)	0.655
3–5 years	147	5	3.40%	3.03% (1.531–4.523)	N/A	
Above 5 years	80	2	2.50%	2.09% (0.340–3.835)	0.7282[b] (0.1381–5.316)	

Note: The figures in brackets indicate the 95% confidence interval.
[a] Odds ratio for 1–3 years compared with 3–5 years age group.
[b] Odds ratio for above 5 years compared with 3–5 years age group.

Table V
Breed-wise distribution of serum antibodies against BVDV

Breed	Total	Positive samples	Apparent prevalence	True prevalence	Odds ratio	Fisher's exact test *p* value
Jersey cross	212	6	2.83%	2.432% (1.292–3.572)	1.311 [c] (0.3223–5.329)	0.9928
Holstein–Friesian	138	3	2.17%	1.743% (0.502–2.985)	N/A	

Note: The figures in brackets indicate the 95% confidence interval.
[c] Odds ratio for Jersey cross compared with Holstein–Friesian cross.

In order to identify risk factors, for example a history of abortion, infertility, diarrhoea or neonatal death, associated with the prevalence of BVDV, Fisher's exact test was applied. The results in Table VI show that 9.1% (3/33) of animals had a history of abortion (OR=5.18; CI 1.23–21.78); 1.4% (1/71) had a history of infertility (OR=0.5; CI 0.06–3.93) and 6.1% (2/22) had signs of diarrhoea (OR=3.0; 0.57–14.35); these factors were not significantly associated ($p > 0.05$) with the seroprevalence of BVDV. Higher prevalence and OR were seen for cattle with a history of abortion or diarrhoea but the CI for the prevalence and OR in both cases were very wide. There were no detectable antibodies against BVDV in animals with a history of neonatal death in their offspring.

Table VI
Risk factor related distribution of serum antibodies against BVDV

Risk factors	Total samples	Positive samples	Apparent prevalence	True prevalence	Odds ratio	Fisher's exact test *p* value
Abortion	33	3	9.09%	8.97% (3.957–13.976)	5.183 (1.234–21.78)	0.087
No abortion	317	6	1.89%	1.45% (0.685–2.217)		
Infertility	71	1	1.41%	0.95% (0.000–2.351)	0.5 (0.05954–3.933)	0.85
No infertility	279	8	2.87%	2.47% (1.473–3.475)		
Signs of diarrhoea	22	2	6.06%	5.80% (1.646–9.961)	3 (0.5687–14.35)	0.41
No signs of diarrhoea	317	7	2.21%	1.79% (0.958–2.611)		
Neonatal death	14	0	0.00%	N/A	N/A	N/A

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

DISCUSSION

The overall seroprevalence of BVDV infection in cattle found in this study was 2.6%, which is very low when compared with reports from the Republic of Korea (58%) [19], southern Vietnam (18%–79%) [20], Saudi Arabia (26%) [21], Ireland (98.7%) [22], Mexico (14%) [23], southern Chile (73.8%) [24], Sweden (40%) [25], Denmark (43–87%) [26] and India (0–40.9%), where the prevalence varies according to the state [13, 14]. The findings are in agreement with previous reports of worldwide BVDV antibody prevalence in cattle, which range from 0% to 90% [15]. The differences may be due to the different antigens of BVDV used in serological kits and their cut-off values. The nature of the antigen used in the BVDV serological kit chosen for this study is not mentioned. It has been shown that the humoral immune response develops against structural glycoproteins E2 and Erns (which are known to show antigenic variation) or the antigenically conserved NS2-3 protein (p80 in non-cytopathic and p125 in cytopathic types) [27]. Among these antigens, NS2-3 protein is considered the antigen of choice and it is used in most commercial kits for the detection of BVDV antibodies in cattle [12]. This protein has shown high sensitivity and specificity for the detection of BVDV infection in comparison with whole virus antigen [28]. Most of the dairy cattle in Nepal are brought in from nearby Uttar Pradesh in India, where the prevalence is low [14], and from Punjab, where the prevalence of BVDV is zero [14]. This may be the one of the reasons for the low prevalence seen in the study.

The difference in prevalence among VDCs might be attributed to differences in management of the farms, the source of the animals, and the type of production. According to the Department of Livestock Services of the Government of Nepal there are large commercial dairy cattle farms in Gitanagar, Sharadanagar and Mangalpur VDCs. These farms keep high-producing animals that were imported from India, where BVDV is already prevalent [11, 13, 14, 15]. Higher numbers of seropositive animals have been reported in larger herds [29]; thus, farm size could also be related to the differences in prevalence. Additionally, most of the dairies and dairy herd replacement cattle are concentrated in these VDCs. Therefore, the farmers in these VDCs are more likely to be involved in the purchase of animals, and to have more visitors, such as AI technicians and veterinarians, and more workers. All of these factors pose a risk for lower biosecurity and the introduction and maintenance of disease [29].

Higher seroprevalence was found in older age groups when compared with younger cattle. This may be due to an increase in an animal's risk of exposure over time. A higher prevalence of BVDV antibodies in cattle above 270 days old age, when compared with younger calves, has been reported in Ireland [29]. Similarly, the prevalence of BVDV

antibodies has been reported to be lowest in cattle of 7–12 months old and highest in animals aged five years or over [30].

The breed of cattle was shown to have no significant association with the prevalence of BVDV in the current study. There is no known breed susceptibility for BVD seen elsewhere, but different breeds of cattle sometimes are managed with different husbandry practices, and this may explain the slightly higher prevalence in Jersey cross-bred cattle. However, the husbandry practices in the study area are generally the same for both breeds of cattle. In addition, risk factors including a history of abortion, infertility or diarrhoea were not significantly associated with the seroprevalence of BVDV. However, the seroprevalence was higher in animals with a history of diarrhoea or abortion. It has been reported that the severe economic impact due to BVDV occurs as a result of repeat breeding, abortion and neonatal mortality [21, 31]. However, in this study the CI for the seroprevalence and OR were very wide and thus the associations of risk factors with seroprevalence could not be confidently established.

CONCLUSIONS

This study revealed a very low prevalence of antibodies to BVDV (2.6%), which suggests that Nepal is virtually free from BVD. Further studies are required to determine the prevalence of BVDV in different districts of Nepal, which will help in evaluating the true impact of BVDV in the country. This study does not support age and breed as factors contributing to the prevalence of BVDV. Also, in this study no correlation was found between the seroprevalence of BVDV and diarrhoea or reproductive problems such as abortion, repeat breeding and anoestrus. Variation in the seroprevalence of BVDV with location, demonstrated by a higher prevalence among dairy cattle from larger herds and locations having a higher density of cattle, which are imported mainly from India, may reflect the significance of importation as a route of introduction of the disease to the country.

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