Research Article

Seroprevalence of Bluetongue Disease among Domestic Ruminants Raised in International Border Areas of Nepal

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Abstract | This study was aimed to assess the seroprevalence of Bluetongue disease and understand its associated risk factors from the international border areas of Nepal. A total of 220 blood samples were collected randomly from apparently healthy ruminants (cattle, buffalo, sheep and goat) and screened for Bluetongue virus (BTV) antibodies in sera using a cELISA kit. Out of 220 sera samples, 92 were positive for BTV, accounting for 41.8% prevalence in ruminants. Seroprevalence rate was the highest in Buffaloes (58.3%) followed by sheep and goats (each 40%), and cattle (37.5%). The BTV seropositivity varied significantly ($p<0.001$) among ruminants in different sampling areas with the highest prevalence in Gokuleshwor (66.6%). Interestingly, the BTV was detected higher ($p<0.01$) in females (48.36%) and older ruminants (49.12%). The seroprevalence of BTV showed a significant association ($p<0.01$) with vector density and resulted in 46.5% of seropositivity. It was concluded that bluetongue disease exists in the international border areas of Nepal and its prevalence was widespread among cattle, buffalo, sheep and goat. The present study showed sex, age and vector population as the main influential risk factors for BTV infection. Further studies are imperative to identify the vector from different agro-climatic zones at the species level and to serotype the BTV prevalent in the study areas.

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Introduction

Bluetongue disease is an emerging, economically important vector-borne viral disease of domestic and wild ruminants. It is caused by the Bluetongue virus (BTV) belonging to the *Orbivirus Genus* of the *Reoviridae family* (Mozaffari and Khalili, 2012). So far, 26 distinct serotypes of BTV have been identified (Khair *et al.*, 2014). BTV infects animals throughout the world, lying between latitude 40°N and 35°S. However, the virus has spread far beyond the aforementioned range (Sperlova and Zendulkova, 2011). The prevalence of bluetongue disease correlates with the distribution of competent culicoides vectors and appropriate climatic conditions (Raut *et al.*, 2013). Bluetongue disease has been included in "List A" disease of Office of International Epizootic (OIE) due to its enormous potential for rapid spread and substantial economic losses arising from poor weight gain, production loss, increase in reproductive prob-

lems and subsequent death of the infected animals. Moreover, the international trade of infected animals and its product is banned (Najarnezhad and Rajae, 2013).

Nepal is sustaining around 0.8 million sheep and 9 million goats, which together contribute to 25% of total meat production and 582 metric ton wool productions, whereas, 30% milk comes from 7.2 million cattle and 70% of milk comes from 5 million buffaloes (MoAC, 2011). Almost every household raises livestock and its products are valuable sources of protein and the main source of cash income for livestock producers in Nepal. Thus, the outbreak of bluetongue disease may deteriorate the human health and results in serious socio-economic effect.

The bluetongue is considered as an important Transboundary Animal Diseases (TADs) in Nepal but there is an unrestricted movement of livestock from the neighboring countries, *i.e.*, India and China, where bluetongue disease is endemic. So, the disease may enter Nepal along with imported animals. Because of the high density of the vector, the disease spreads rapidly. Moreover, Nepal being a member country of WTO, it has to follow the sanitary and phytosanitary (SPS) measures and regulations on technical barriers of trade. Therefore, the control of this BTV infection is a necessity in Nepal, given a large number of animals and their importance in the national economy. Thus, to unravel the prevalence of bluetongue disease in ruminants, random serosurveillance is an utmost in the particular region. The present study was mainly aimed to detect antibodies against BTV in ruminants raised in international border areas of Nepal.

Materials and Methods

Site selection

The study was conducted in the Darchula district, the remote mountainous district of Far Western Nepal, expanding between latitude 29°01' to 30°15' N and Longitude 80°03' to 810°09' E. This district has a diverse geographical variation with altitude varying from 357 to 7132 meters high from the sea level and has subtropical to temperate climatic conditions. It shares a national border with Bhajangonon the east, Baitadi on the south, and international border with Uttarakhand (India) and Taklakot (China) on the west and north, respectively. The study was conducted in September, 2013 in 6 villages (Shankarpur, Mallikarjun, Gokuleshwor, Sikhar, Latinath and Bulgar) of Darchula District (Figure 1).

Sample design

A two-stage sampling survey was performed in the present study. The villages, taken as the primary sampling unit, were selected on a random basis. At the second stage, a fixed proportion of animals were selected from the population using simple random sampling. The sample size was calculated using a computer program which uses two-stage prevalence surveys as described earlier (Cameron, 1999). The questionnaire was prepared and a field visit was made to the research site. Information related to age, gender, breed, presence or absence of vector and history of abortion were collected and recorded from the owner in an interactive manner. Before visiting the site, consent was obtained from the Department of Livestock, District livestock service office, Darchula regarding the intention to conduct study in the area.

Sample collection and testing

Approximately, 5 ml of blood samples were collected aseptically by jugular vein puncture. The serum was separated after 3-4 hours of clotting in slanting position at sites and preserved in the icebox. Afterwards, it was dispatched to the laboratory, where further refineries of sera were carried out by centrifugation at 2000 rpm for 15 minutes. The separated serum samples were stored properly at -20°C until testing. Competitive ELISA test kit (ID-Vet, Montpellier, France) was used for detecting the antibody to BTV in sera. The serological test was carried out according to the manufacturer's guidelines and results were reported positive or negative based on optical density reading (450 nm) when compared with the positive and negative control.

Statistical analysis

The data related to age, gender, breed, presence or absence of vectors and history of abortion were entered into Excel. Firstly, associations between the BTV infection and its potential risk factors were calculated using Pearson's Chi-square test (PH STAT 2) and Chi-squared with Yates' correction was used when at least 20% of expected frequencies are less than five. Fisher's exact test was used for the estimation of the level of significance and association of that factor was expressed as highly significant, significant and non-significant based on the p-value being less than 0.01, between 0.05 and 0.01 and greater than 0.05 respectively.

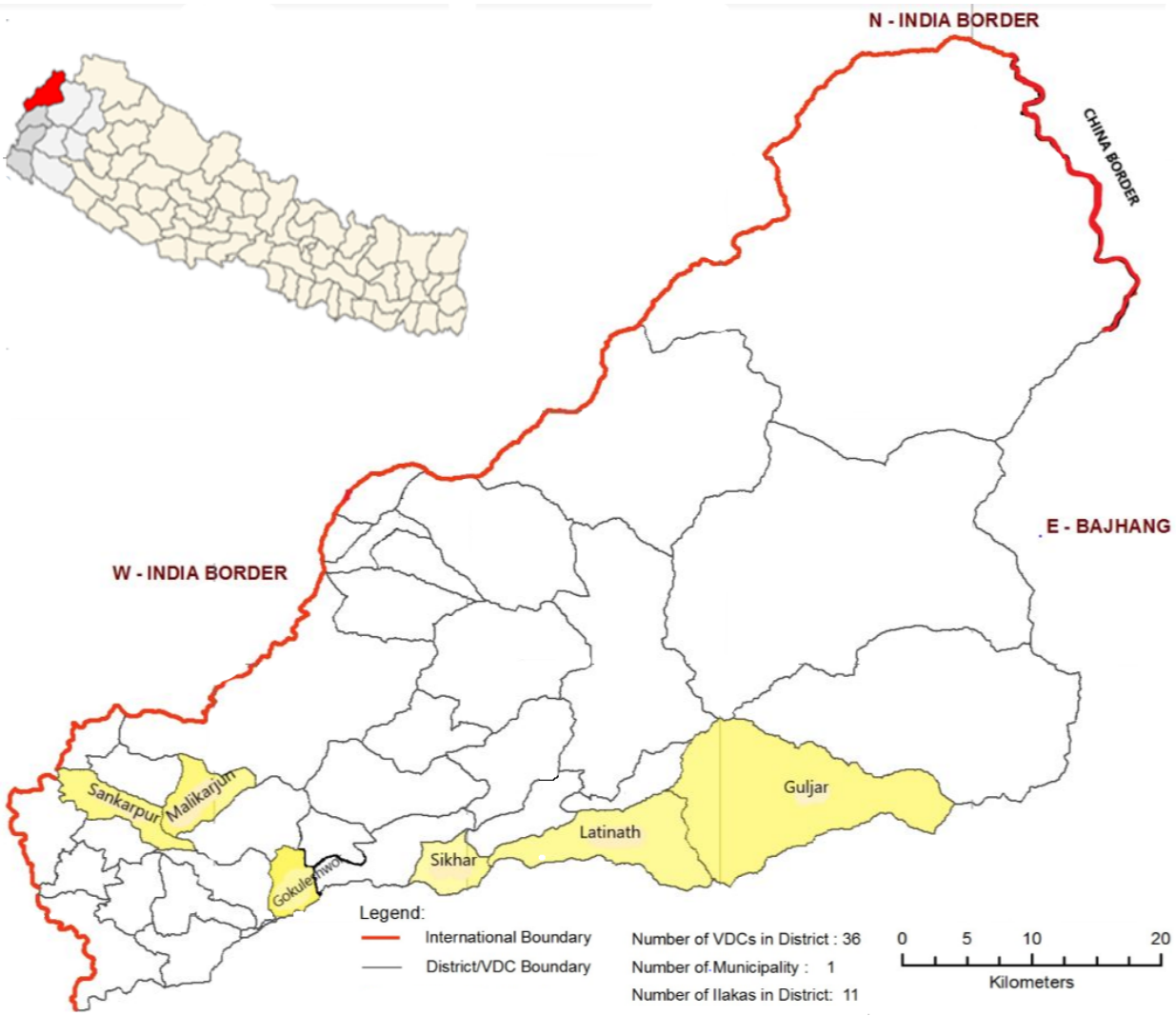

Figure 1: *Map of Darchula district showing the study location. The region marked with yellow color shows the sample collection areas. The thick red demarcation indicates the international borders with India and China.*

Results and Discussion

Seroprevalence of BTV antibody among domestic ruminants

Overall, the seroprevalence of bluetongue was 41.8% among ruminants. Out of 220 serra samples tested for antibody to BTV (Table 1), 92 samples were found positive for BTV comprising 37.5% (39) cattles, 58.3% (21) buffaloes, 40% (12) goats and 40% (20) sheep. Furthermore, the seropositivity was highest in buffaloes (58.3%) but no significant association ($p>0.05$) was noted among species. Such a high seroprevalence might be due to the huge vector population following monsoon. However, an earlier study of Gaire (2013) reported 28.16% seroprevalence of BTV in sheep, goat and cattle in two bio-diverse landscapes of Nepal. Similarly, Jha *et al.* (2008) examined 401 sera samples of sheep from 11 districts using c-ELISA and reported 28.4% seropositivity for BTV antibodies. As per our knowledge, this was the first seroprevalence survey conducted in buffalo from Nepal to detect the antibody against the BTV. The result showed 58.9% seropositivity against BTV antibodies in the buffalo population, which was in agreement with earlier researchers from India who reported 58.33% (Chauhan *et al.*, 2004) and 60.12% (Patel *et al.*, 2007), seropositivity of BTV. This higher seroprevalence in buffalo might be due to greater exposure to the vector and their larger body surface area as compared to sheep and goat. Moreover, the skin of buffalo is quite softer while cattle have tough skin making culicoides difficult to suck blood. Further, previous exposure to the virus may also produce a more marked antibody response (Uhaa *et al.*, 1990).

Similarly, 40% (12) goats were seropositive to BTV antibodies (Table 1) which were in agreement with the result of Sreenivasulu *et al.* (2004) who reported 43.6% seropositivity in goat in Andhra Pradesh and Shlash *et al.* (2012) observed 39.47% positivity in

goats in Iraq. The greater prevalence of bluetongue in goats, i.e., 58.01% and 54.5% has also been reported from Goa (Barbuddhe *et al.*, 2005) and Uttar Pradesh (Bitew *et al.*, 2013), respectively of India. This higher seroprevalence in small ruminants may reflect their involvement in basic ecology of the virus (Sreenivasulu *et al.*, 2004). Further, the goat was assumed to be infected with serotypes of the virus which causes disease without overt clinical signs (Chand *et al.*, 2009).

Table 1: *Seroprevalence of BTV antibody in domestic ruminants.*

Category	Risk factors	Total samples	Positive (%)	df	χ2	p- value
Species	Cattle	104	39(37.5%)	3	4.941	0.1761
	Buffalo	36	21(58.3%)			
	Goat	30	12(40%)			
	Sheep	50	20(40%)			
VDCs	Shankarpur	40	7(17.5%)	5	19.291[a]	**p= 0.0017
	Malikarjun	31	16(51.6%)			
	Gokuleshwor	21	14(66.6%)			
	Shikhar	36	18(50%)			
	Latinath	32	16(50%)			
	Guljar	60	21(35%)			

*[a,b] Indicate significant differences (p<0.05) between the means of the groups. * and **= significance level at p<0.05 and p<0.01 respectively, NS= not significant. Figures in parenthesis are percentages.*

In this study, the c-ELISA test exhibited BTV seropositivity in 40% of sheep. However, Jha *et al.* (2008) reported the prevalence rate of 28.4% in a large population of sheep in Nepal. Similarly, Yadav (2012) and Gaire (2013) reported 19.3 % and 25%, respectively in sheep. The seropositivity rate seems quite higher in the present study than those reported previously in Nepal. However, the higher seroprevalence of BTV was reported in sheep as 58.82% in Assam (Joardar *et al.*, 2013) and 57.66% in West Bengal (Panda *et al.*, 2001) of India. The both higher as well as lower prevalence rate of BTV antibodies was detected in sheep populations of several countries. Specifically, it was reported as 54.10% in Saudi Arabia (Yousef *et al.*, 2012), 48.8% in Pakistan (Akhtar *et al.*, 1997), 45.7% in India (Sreenivasulu *et al.*, 2004), 29.59% in Southeast Turkey (Gur, 2008) and 21.40% in Kazakhstan (Lundervold *et al.*, 2003).

The present study showed lower seropositivity in cattle (37.5%) than those of sheep (40%) and goats (40%). Similar findings have been reported in earlier study, *i.e.*, in cattle (33.4%) and sheep (45.71%) conducted in Indian subcontinents (Sreenivasulu *et al.*, 2004). The prevalence rate of blue tongue disease observed in the present study was remarkably higher than that observed as 29.32% in Chitwan and Lamjung (Gaire, 2013). These discrepancies in rates might be due to spatial and temporal variation. On the contrary, the findings of the present study were notably lower than the earlier prevalence rates in cattle, i.e., 70% in Assam (Joardar *et al.*, 2013) and 58.82% in Punjab (Oberoi *et al.*, 1988) state of India. Similarly, earlier researchers reported 93.5%, 88% and 84.57% of cattle sera positive for BTV antibodies in Iran (Jafari-Shoorijeh *et al.*, 2010), Turkey (Gur, 2008) and Khartoum and Gezira state of Sudan (Elhassan *et al.*, 2014), respectively. Cattle are considered as the main amplifying host and thereby play a vital role in the maintenance of viruses in nature (Khezri and Bakhshes, 2014).

However, the sera samples from different location of the Darchula showed seropositivity of Shankarpur (17.5%), Mallikarjun (51.61%), Gokuleshwor (66.67%), Shikhar (50%), Latinath (50%) and Guljar (35%). Overall, samples from Gokuleshwor areas showed a higher seroprevalence for BTV (Table 1). The result showed a remarkable difference (p<0.01) in the seroprevalence of BTV antibodies at different locations of the study areas. This might be due to diverse climatic condition of Darchula including hot and humid temperature following monsoon that favours for the vector growth and activity (Kumar *et al.*, 2018). Moreover, the district share open boarder with India and China, where several outbreaks of bluetongue disease have been reported (Joardar *et al.*, 2013; Bitew *et al.*, 2013; Zhang *et al.*, 2004). Hence, the outbreak of disease in one country increases probability of disease transmission into another country. Similarly, poor husbandry practice and transhumance system of rearing in high hills might also contribute for higher prevalence of circulating BTV antibodies among ruminants in Darchula District.

Age wise seroprevalence of BTV antibodies

The age-wise seroprevalence of BTV antibodies has been listed in Table 2. The seroprevalence of antibodies to BTV among different age groups of ruminants revealed higher seropositivity in older (49.12%) age (greater than 6 months) as compared to younger (16.32%) ages (less than 6 months). Interestingly, age-wise differences were observed only in cattles and goats. Higher prevalence of BTV antibodies was

Table 2: *Age-wise seroprevalence of BTV antibody.*

Species	Age	Animal tested	Positive (%)	df	χ^2	p-value
Cattle	Younger	24	4 (16.66%)	1	Fisher's Exact test	* p=0.02
	Older	80	35 (43.75%)			
Buffalo	Younger	7	2 (28.57%)	1	Fisher's Exact test	NS p=0.10
	Older	29	19 (31.03%)			
Goat	Younger	7	0	1	Fisher's Exact test	* p=0.02
	Older	23	12 (52.17%)			
Sheep	Younger	11	2 (18.18%)	1	Fisher's Exact test	NS p=0.16
	Older	39	18 (37%)			
Total	Younger	49	8 (16.32%)	1	16.837[a]	*** P=0.000030
	Older	171	84 (49.12%)			

*[a,b] Indicate significant differences (p<0.05) between the means of the groups. * and **= significance level at p<0.05, p<0.01 and p<0.001 respectively, NS= not significant. Figures in parenthesis are percentages.*

Table 3: *Sex wise seroprevalence of BTV antibody in cattle, buffalo, goat and sheep.*

Species	Sex	Animal tested	Positive (%)	df	χ^2	p-value
Cattle	Male	30	9 (30%)	1	Fisher's Exact test	NS P=0.37
	Female	74	30 (40.5%)			
Buffalo	Male	14	3 (21%)	1	Fisher's Exact test	** p=0.0005
	Female	22	18 (81.8%)			
Goat	Male	9	2 (22.2%)	1	Fisher's Exact test	NS p=0.24
	Female	21	10 (47.6%)			
Sheep	Male	14	4 (28.6%)	1	Fisher's Exact test	NS p=0.35
	Female	36	16 (44.4%)			
Total	Male	67	18 (26.86%)	1	8.853[a]	** P=0.0030
	Female	153	74 (48.36%)			

*[a,b] Indicate significant differences (p<0.05) between the means of the groups. * and **= significance level at p<0.05 and p<0.01 respectively, NS= not significant. Figures in parenthesis are percentages.*

found in older ruminants (49.12%). This may correlate with increased duration of exposure, rather than increased age susceptibility to infection per species (Ward *et al.*, 1994). The older animals are more exposed to the midges because of their larger total body surface area (Uhaa *et al.*, 1990). Besides, some culicoides have more affinity towards ruminant derived carbon dioxide production which greatly fluctuates during their life (Kline *et al.*, 1994). Normally, young animals are kept indoors and are well-taken care by the owner until 6 months of age. Afterwards, they are released into pasture for grazing, where vector exposure and subsequent BTV infection is high. The findings of the present study are consistent with the previous findings (Mohammadi *et al.*, 2012; Uhaa *et al.*, 1990; Ward *et al.*, 1994; Lundervold *et al.*, 2003; Gaire, 2013; Khair *et al.*, 2014).

Sex wise seroprevalence of BTV antibodies

The seroprevalence of BTV antibody varied (p<0.01) among various sex groups (Table 3). It showed higher seropositivity in females (48.36%) than males (26.86%) The sex-wise difference was observed only in buffaloes, with higher seroprevalence in females (81.8%) than males (21%). This study also revealed the sex wise differences in prevalence rate of BTV. Sex was highly significant (p=0.002) risk factor and females were more prone to BTV infection compared to males. The reason might be due to bias in the sample size as more female ruminants were included in present study as similar to earlier study (Elhassan *et al.*, 2014). In agreement with the present finding, Yadav (2012) also found higher seroprevalence in females (15%) than males (9.8%) in Lalitpur district. Likewise, Patel *et al.* (2007) reported 65.14%

Table 4: *Relationship with abortion history and BTV antibody in ruminants.*

Species	Abortion	Animal tested	Positive (%)	df	χ^2	p-value
Cattle	Yes	2	0	1	Fisher's Exact test	NS p=0.52
	No	102	39 (38.23%)			
Buffalo	Yes	1	0	1	Fisher's Exact test	NS p=0.41
	No	35	21 (60%)			
Goat	Yes	7	3 (42.85%)	1	Fisher's Exact test	NS p=1
	No	23	9 (31.13%)			
Sheep	Yes	15	9 (60%)	1	Fisher's Exact test	NS P=0.11
	No	35	11 (31.42%)			
Overall	Yes	25	12 (48%)	1	0.443[a]	NS P=0.524
	No	195	80 (41.02%)			

*[a,b] Indicate significant differences (p<0.05) between the means of the groups. * and **= significance level at p<0.05 and p<0.01 respectively, NS= not significant. Figures in parenthesis are percentages.*

and 51.56% seropositivity in females and males, respectively by c-ELISA in Gujrat. Also, Elhassan *et al*. (2014) recorded higher seroprevalence in females (74%) than males (9.8%). However, Gaire (2013) reported higher seropositivity in males (31.31%) than females (27.27%), indicating both males and females are equally susceptible to BTV infection.

Association of BTV seroprevalence with abortion history

The seropositivity of BTV with and without abortion history was 48% and 41.02% respectively. However, no significant differences (p>0.05) in BTV antibodies were found among ruminants with or without a history of abortion (Table 4). Regarding abortion history, 48% and 41.02% seropositivity was found in ruminants with and without abortion history, respectively. But, no significant association (p>0.05) was observed between prevalence rate and abortion because some serotypes cause subclinical infection or a prolonged period of past endemic infection (Mohammadi *et al*., 2012). However, the previous study conducted in Chitwan and Lamjung district of Nepal showed higher (65.75%) seropositivity of BTV in animals with abortion history (Gaire, 2013). The other factors may be involved for abortion in animals. Elhassan *et al*. (2014) described that different viral and protozoan diseases causes abortion among cattle in Sudan.

Conclusions and Recommendations

Vaccination against bluetongue was not in practice hence antibody detected in ruminants of international border areas of Nepal might be from natural infection. Interestingly, no clinical case of bluetongue has been reported from the study area till date indicates there no absence of disease but necessitates the intensive surveillance with improved diagnostic technique. Further study is also required to characterize the serotypes of specific viruses and their vector present in the study area.

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Novelty Statement

The present study detected antibodies against BTV for the first time in ruminants raised in international bordered areas of Nepal. The findings may be useful to plan the prevention strategies of bluetongue diseases and likely to be of great interest to the related researchers, clinicians and epidemiologists who read this popular journal.

Authors' Contribution

Bikash Puri: Designed the study, collected and processed samples, analysed data and wrote the manuscript.

Anil Kumar Tiwary: Analysed the data and r eviewed the manuscript.

Bharata Regmi: Processed the samples.
Dinesh Kumar Singh: Designed and supervised the research.
Doj Raj Khanal: Designed and supervised the laboratory work.
Manoj Kumar Shah: Revised the manuscript.

Conflict of interest
The authors declare that there is no conflict of interest with this research.

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