Detection of antibodies against Bluetongue virus among domestic ruminants in the highlands of Nepal

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Biting midges,
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c-ELISA,
Highlands,
Nepal,
Seroprevalence.

Summary
Bluetongue (BT) is one of the most economically important transboundary animal diseases. In recent years, it has been considered a disease related to climate change. A study was undertaken in 2013 in Nepal to measure the prevalence of Bluetongue virus (BTV) infection among domestic ruminants inhabiting the 3 agro-climatic zones with altitudes ranging from 150 to 2,400 metres above sea level. Twelve clusters representing the 3 altitudes were selected. The presence of antibodies against BTV was demonstrated in serum samples of sheep, goats, cattle, buffaloes, yaks/chauries, and chyangra goats (Himalayan goat) of Nepal. For this purpose, a total of 2,084 sera were collected from a population of 202 sheep, 739 goats, 590 cattle, 379 buffaloes, 105 yaks/chauries, and 69 chyangra goats between February 2013 and January 2014. The presence of antibodies against BTV was investigated using competitive enzyme-linked immunosorbent assay (c-ELISA). Of the 2,084 collected sera, 45.20% were positive for BTV antibodies. Species-wise prevalence was 17.82%, 47.50%, 53.05%, 58.05%, 7.62%, and 20.29% in sheep, goats, cattle, buffaloes, yak, and chyangra goats, respectively. Contrary to the general belief, maximum numbers of seropositive cases were recorded in buffaloes followed by cattle, goats, chyangra goats, sheep, and yak/chauries. The samples collected in the post-monsoon period (July-August is the monsoon period) show a seroprevalence higher than the pre-monsoon samples. This study shows the seroprevalence of BT in domestic ruminant population of Nepal at all altitudes. The highest prevalence has been reported in the plains of Terai followed by gradual decline in the mid-hills, and in the high mountains. Furthermore, detection of antibodies against BTV in both small and large ruminants (chyangra goats and yak/chauries) dwelling in high altitudes in the absence of BT vaccination is suggesting vector movement to the highlands as a consequence of warmer climate. These findings suggest that the climatic conditions, even at the higher elevation, are suitable for the survival of biting midges responsible for the transmission of BTV.

Anticorpi contro il virus della Bluetongue in ruminanti domestici degli altopiani del Nepal

Parole chiave
Bluetongue,
c-ELISA,
Culicoides,
Nepal,
Sieroprevalenza,
Virus della Buetongue.

Riassunto
La Bluetongue (BT), recentemente correlata ai cambiamenti climatici, è una malattia transfrontaliera che ha importanti ricadute economiche. Nel 2013 è stato condotto uno studio per misurare la prevalenza del virus della BT (BTV) in ruminanti domestici di 3 aree del Nepal con diverse caratteristiche agro-climatiche e altitudini da 150 a 2.400 metri sopra il livello del mare. In queste aree è stata verificata la presenza di anticorpi contro BTV in sieri prelevati da pecore, capre, bovini, bufali, yaks/chauries e capre himalayane (capre chyangra). Tra febbraio 2013 e gennaio 2014 sono stati prelevati 2.084 campioni di siero da una popolazione di 202 pecore, 739 capre, 590 bovini, 379 bufali 105 yaks/chauries e 69 capre himalayane. Sono state selezionate 12 aree rappresentative delle 3 zone agroclimatiche. La presenza di anticorpi

verso BTV è stata rilevata mediante *competitive enzyme-linked immunosorbent assay* (cELISA). Il 45,2% dei 2.084 campioni prelevanti è risultato positivo per la presenza di anticorpi anti BTV con prevalenza pari a: 17,82%, 47,50%, 53,05%, 58,05%, 7,62% e 20,29% rispettivamente in pecore, capre, bovini, bufali, yaks/chauries, e capre himalayane. Contrariamente a quanto generalmente ritenuto, il più elevato numero di casi sieropositivi è stato registrato nei bufali e a seguire in bovini, capre, capre himalayane, pecore e yaks/chauries. I campioni prelevati nel periodo post-monsonico (periodo monsonico = luglio-agosto) hanno mostrato valori di sieroprevalenza più alta rispetto a quelli raccolti nel periodo pre-monsonico. Lo studio ha permesso di rilevare la sieroprevalenza della BT nei ruminanti domestici del Nepal a tutte le altitudini con valori più alti di prevalenza nella piana di Terai, seguita da un graduale declino a metà collina e in alta montagna. Il rilevamento di anticorpi anti BTV nei piccoli e grandi ruminanti (capre himalayane e yaks/chauries) non vaccinati e presenti ad alte quote, suggerisce che, a causa del clima più caldo, i vettori si stiano spostando verso gli altopiani. Questi risultati indicano che le condizioni climatiche, anche a quote elevate, permettono la sopravvivenza dei *Culicoides*, responsabili della trasmissione del BTV.

Introduction

Bluetongue (BT) is an insect vector borne, viral disease of domestic and wild ruminants. It is seen primarily a disease of sheep with severe clinical manifestations. Occasionally goats, cattle, buffaloes, and other ruminants are also affected. Although cattle rarely suffer from severe clinical disease, BT has a strong economic impact on trade of cattle due to restrictions on movement, because cattle and buffaloes can act as carriers of the virus without showing any clinical forms of the disease. Bluetongue was responsible for the loss of $125 million annually in sheep and cattle industry in USA (Mayo *et al.* 2014, UCDAVIS 2014).

Nepal's neighbouring countries, India and China, have reported clinical cases of BT (Joardar *et al.* 2013, Li *et al.* 1996, Panda *et al.* 2011, Prasad *et al.* 1992, Sapres 1964, Zhang *et al.* 2004). Ever since the first reported outbreak of BT in sheep and goats in India during I964 in Maharashtra State, several outbreaks of BT have been reported in sheep. Bluetongue was first recognised in China in 1979 in Yunnan Province, and both the clinical disease and antibodies have since been recorded in other provinces in China (Bi *et al.* 1996). In Nepal, Jha and Tamang (Jha and Tamang 2008) first reported the presence of antibodies to BT in ovine sera from Nawalparasi and Rupandehi districts, which are contiguous to the state of Uttar Pradhesh, India. Subsequent to their report, few other investigators performed seromonitoring in animal population in different parts of the country (Gaire *et al.* 2014, Jha *et al.* 2009, Yadav 2012) and reported the presence of circulating antibodies against Bluetongue virus (BTV) in both small and large ruminants. The present work spans over 3 climatic zones (Southern plain of Terai with an elevation < 500 metres above sea level (masl), mid-hills with 1,000-2,200 masl, and high mountains with 2,200-2,400 masl), and includes samples collected in different seasons (pre-and post-monsoon) and from all species of domestic ruminants of Nepal.

Materials and methods

Site and animal selections

In consultation with Regional Agricultural Research Stations and District Livestock Services Office of all the sampled regions, 12 clusters representing 3 altitudes with climatic variations (warm-subtropical Southern Terai, temperate mid hills and mild-alpine Northern high mountains) were selected (Figure 1). Twelve local veterinary technicians (invigilators) were hired for animal selection and serum collection. Blood was collected in a plain vacuum tube from the individual ruminant after getting informed consent from the farmers under the supervision of veterinary scientist of the Animal Health Research Division (AHRD). Blood samples were collected in 2 seasons: before and after monsoon (July and August are the rainy months) from sheep, goats, cattle, buffaloes, chyangra goats (Himalayan goat), and yak/chauries (*Bos grunniens*). Sera were harvested after 3-4 hours of clotting in the slanting position at the field sites and were transported cold to AHRD in cool box with ice cubes and stored at -80°C until competitive ELISA (c-ELISA) test was carried out.

Serological assay

Competitive ELISA test kits for measuring anti-viral protein 7 (VP7) antibodies to BTV in sera were procured from ID Vet (Innovative Diagnostics, 310 rue Louis Pasteur, 34790 Grabels, France, Lot No 517,

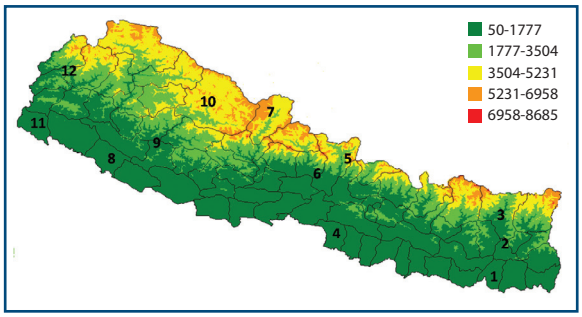

Figure 1. *Map showing 12 cluster sites (1-12) for serum collection representing 3 agro-climatic zones with altitudinal variations ranging from 150-2,400 masl.* 1, 4, 8 &11 = Plain Terai with < 500 masl; 2, 6, 9 & 12 = Mid hills with 1,000-2,200 masl; 3, 5, 7 &10 = High mountains with > 2,200 masl; 1 = Sunsari, 2 = Dhankuta, 3 = Murtidhunga, 4 = Bara, 5 = Gatlang, Rasuwa; 6 = Lamjung; 7 = Mustang; 8 = Bardia; 9 = Surkhet; 10 = Jumla, 11 = Kanchanpur, 12 = Baitadi. Coordinates of Nepal are 28.3949° N, 84.1240° E.

Exp. 05/2015). The serological test was carried out at AHRD as per instructions provided by manufacturer. Positive and negative control sera provided in the kits were included in each assay. The optical density was read at 450 nm.

Results and discussion

Of 2,084 sera screened for the presence of anti-VP7 antibodies against BTV, 942 (45.20%) sera were positive. The overall prevalence in sheep, goats, cattle, buffaloes, yak/chauries, and chyangra goats was 17.82%, 47.50%, 53.05%, 58.05%, 7.62% and 20.29%, respectively (Figure 2). Contrary to the general belief, maximum numbers of seropositive cases were recorded in buffaloes followed by cattle, goats, chyangra goats, sheep, and yak/chauries in decreasing order compared to primary host sheep. Due to large body surface area with thin hair coverings and soft skin, buffaloes have been considered of significance for harbouring and serving as the reservoir of the virus. As such they seem to favour an increased incidence of BT disease. The geographic areas of BT appear to have been expanded from the warm Southern plain region to the alpine Northern high mountains. The ruminants of rugged high mountain areas generally do not get vaccinated for any disease, so antibodies detected against BTV must have been derived from natural infection, and not from vaccination. Due to warmer climate in the recent years, it has been noticed that the insect vectors are moving up in the highlands. They are believed to be responsible for the circulation of antibodies against BTV in these areas.

However, the species of biting midges transmitting BTV and the circulating serotypes of virus have not been studied so far in Nepal. Furthermore, clinical outbreaks of BT disease have not yet been reported in Nepal. Non-reporting of BT outbreak does not mean the absence of the disease, it rather shows the need for an improvement in surveillance and diagnostic methods. Due to reporting of clinical outbreaks of BT in both India and China, Nepal needs to increase its surveillance mechanism for the detection and containment of BT, as this could have a great impact on trade. In the absence of BT vaccination in Nepal, the detection of antibodies in the sera is attributable for the on-going process of active infection. This study was able to demonstrate only the presence of antibodies against BTV but not the serotypes responsible for the development of antibodies. And also, the species of *Culicoides* responsible for transmitting BTV is yet to be identified. The detection of more viral antibodies during post-monsoon season might be due to higher activity of biting midges arisen from warmer climate (Figure 3) suggesting the sparing effect of seasonality (relative risk ratio of 0.87). The reasons for lowered levels of antibodies in goats are unknown.

Likewise, seroprevalence of BTV antibodies was highest at plain of Terai, followed by mid-hills and the high mountains in descending order (Figure 4). This result could be due to more warmer and conducive climate prevailing in Terai for propagation of biting midges. It is noteworthy that the seroprevalence of BTV antibodies in sheep flocks has been reported from 1 of the bordering district of India (Jha *et al.* 2008). The findings of highest prevalence of BTV antibodies in animals of plain Terai described in the present study, in addition to the one described by Jha and colleagues (Jha *et al.* 2008), indicate the continued circulation of antibodies arisen from the on-going process of sub clinical infection of BT in the endemic form.

The present study documented the presence of

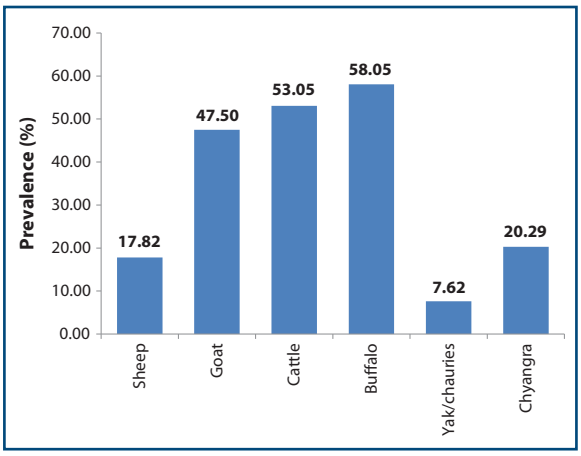

Figure 2. *Prevalence of antibodies against Bluetongue virus in different ruminant livestock species in Nepal.*

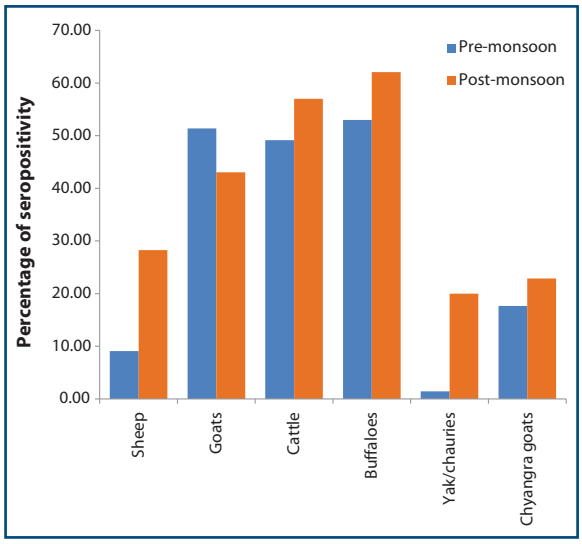

Figure 3. *Presence of antibodies against Bluetongue virus in domestic ruminants in 2 seasons (pre-monsoon and post-monsoon) during the year 2013 (July-August is the monsoon period) in Nepal.*

highest levels of circulating antibodies against BTV in buffaloes followed by cattle, goats, and sheep in descending order, as well as in yaks/chauries and chyangra goats (high mountain goat) inhabiting the highlands of Nepal, where the presence of biting midges was not considered to occur before.

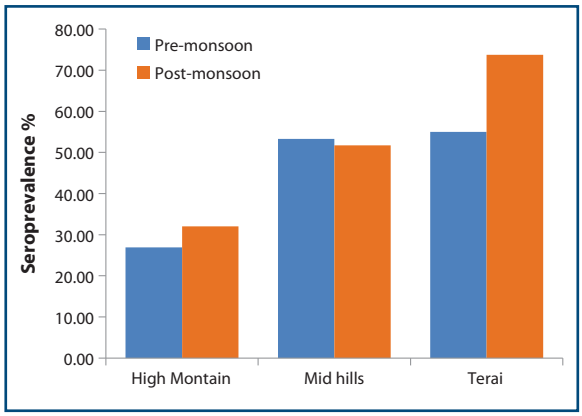

Figure 4. *Seroprevalence of antibodies against Bluetongue virus in domestic ruminants in 2 seasons at three different altitudes of Nepal.*

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