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**Research Article**

# DETECTION OF BOVINE HERPES VIRUS-1 ANTIBODIES IN BOVINES IN THREE DISTRICTS OF UTTARAKHAND BY COMPETITIVE ELISA

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## ABSTRACT

The present study was aimed to obtain epidemiological information regarding exposure of bovines in Uttarakhand, India to bovine herpes virus type-1 (BHV-1). Total 200 random serum samples obtained from unvaccinated bovines in three districts of Uttarakhand (Udham Singh Nagar, Dehradun and Pithoragarh) were tested for presence of antibodies against BHV-1 using competitive ELISA. An overall prevalence of 18.15% was observed for BHV-1 antibodies. Buffaloes were found to have 25.00% prevalence as compared to 17.44% in cattle. Further higher prevalence was seen in females (19.02%) than males (16.22%). Of the three districts, highest prevalence was observed in Pithoragarh district. These results indicated that exposure to BHV-1 is common within the study area. Therefore, preventive and control measures against this infectious agent should be adopted.

**Key words:** BHV-1, cELISA, IBR, seroprevalence

Bovine herpes virus-1 (BHV-1) belongs to the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae* (MacLachlan and Dubovi, 2011). BHV−1 is the etiological agent of a variety of disease conditions in bovines such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB), conjunctivitis, abortion, enteritis etc. BHV-1 has many subtypes but all are antigenically similar. Even though mortality is low, the disease with its severe impact on growth, milk production and international livestock trade may jeopardize the agro-economy of a country.

BHV-1 infection in a farm is deleterious and if established cannot be eradicated easily since virus persists for the lifetime in the host and transmit the infection in the population if proper preventive measures are not taken (Muylkens *et al.,* 2007; Biswas *et al.,* 2013). The disease is prevalent throughout the country and has been reported in India in bovine population by various workers (Singh and Yadav, 2010; Verma *et al.,* 2014). The present study was carried out to determine BHV-1 infection in three districts of Uttarakhand using competitive ELISA.

## MATERIALS AND METHODS

**Sample Collection:** Total 200 blood samples were collected from three districts of Uttarakhand viz. Udham Singh (U.S.) Nagar (50), Dehradun (115) and Pithoragarh (35). The data related to area, species and sex were also collected. Serum was separated from these samples and stored till analysed.

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**Competitive ELISA**: cELISA was performed at the Virology laboratory, CADRAD, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. Commercial competitive ELISA kit procured from Institut Pourquier, France was used for the detection of antibodies to BHV-1 gB glycoprotein. The test was performed according to the manufacturer's instruction**.** Percentage inhibition was calculated using following formula:

$$\text{Inhibition \%} = \frac{\text{OD 450 of analyzed serum}}{\text{Mean OD 450 of negative control}} \times 100$$

Then it was interpreted as: ≤50 % (positive), 50- 55 % (suspect) and ≥55 % (negative)

Serum with percentage of inhibition ≤50% was considered to have specific antibodies to the gB protein of BHV-1 (positive). Serum with percentage of inhibition between 50%-55% was considered as doubtful while serum with percent inhibition ≥55% was considered as negative.

**Statistical Analysis:** Statistical analysis of data was carried out as per the method described by Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

Of the 200 serum samples tested by cELISA, 37 (18.50%) were found positive for BHV-1 antibodies

(Table 1). Species-wise non-significantly higher prevalence was seen in buffaloes than cattle (Table 1). Prevalence of BHV-1 antibodies was higher in females (19.02%) than males (16.22%). Higher prevalence in females than males was illustrated in cattle population, howver, lower prevalence in females compared to males was observed among buffaloes (Table 1). Dependency of disease on sex was non-significant at $p<0.05$.

In Udham Singh Nagar district, overall 20.00% prevalence was recorded and within species also, 20.00% samples each, from both cattle and buffaloes were positive. In Dehradun district, 12.15% cattle and 37.50% buffaloes were positive for BHV-1 antibodies with an overall prevalence of 13.91%. Prevalence was highest in cattle (31.42%) of Pithoragarh district as compared to overall prevalence of 17.44% in cattle. No buffalo serum sample was collected from Pithoragarh (Table 2).

Since none of the herds included in the study were vaccinated against BHV-1, the seroprevalence studies indicated that the study herds might had been exposed to the virus, assuming that the presence of antibodies can only be caused by exposure to the pathogen (van Schaik *et al.,* 1998; Kampa *et al.,* 2004). In Uttarakhand, Jain *et al*. (2006) observed a seropositivity of 10.39% for IBR antibodies which was lower than the present study. However, higher seropositivity of 22.30% and 40.71%, in cattle population was reported by Nandi *et al.* (2011) and Kollannur *et al.* (2014), respectively. Much higher prevalence rates from different parts of India and world were reported by various workers (Nandi *et al.*, 2004; Singh and Yadav, 2010; Trangadia *et al.,* 2010; Romero-Salas *et al.*, 2013; Verma *et al.,* 2014). Compared to present study lower prevalence rates were observed by Singh and Sinha (2006) and Das *et al.* (2014) from different parts of India.

The prevalence in present study in the state could be attributed to the fact that there is no vaccination policy regarding IBR, disproportionate cross breeding practices and lack of availability of specific infrastructure for bovines. Gonzalez-Garcia *et al.* (2009) indicated lack of specific cattle infrastructure and beef cross breeding as important risk factors associated with BHV-1 infection in Spain along with herd size, history of reproductive disorders, purchase of replacements and proximity to an urban area. Natural breeding with bulls without knowing their disease status could also be responsible for the rapid spread of the disease as also opined by Romero-Salas *et al.* (2013). In species wise study, similar to our observation Nandi *et al.* (2004) and Trangadia *et al.* (2010) also observed lower seropositivity in cattle than buffaloes in India. Contrary to the present observation, Dwivedi (2005) and Jain *et al.* (2006) reported higher prevalence in cattle than buffaloes in Uttarakhand. Likewise, Verma *et al.* (2014) in Uttar Pradesh reported greater prevalence rate in cattle than buffalo population.

Observations regarding sex-wise prevalence indicated that BHV-1 antibodies were more prevalent in females than males and this was evident even at species level for cattle. Jain *et al.* (2006) in Uttarakhand, Singh and Sinha (2006) in Bihar and Sharma *et al.* (2009) in Uttar Pradesh also reported greater percent prevalence in females than males. In the present study, however within species, there was deviation in buffalo population as more males were positive for BHV-1 antibodies, which was comparable with the study of Singh and Sinha (2006) where buffalo bulls have higher prevalence than females. Males, older age and large herd size are also considered as risk factors for higher sero-positivity of BHV-1 (Boelaert *et al.,* 2005). Hence, continuous sero-surveillance of bulls and the exclusion of BHV-1 positive bulls should be done on priority basis for maintaining the BHV-1 free status of bulls at a frozen semen bank.

Overall low prevalence in the Dehradun district might be due to the reason that most of the samples in this area were collected from organized dairies which are regularly getting their animals screened for various diseases. High prevalence in district Pithoragarh might be attributed to transit of unscreened new milch cattle from different regions of India like Uttar Pradesh where high seroprevalence had been reported. There are several other factors which might be responsible for the spread of disease like unrestricted movement of animals, procurement of animals without prior testing, absence of quarantine before introduction in to the main herd, lack of preventive measures, etc.

**Table 1**

**Species and sex-wise prevalence of BHV-1 antibodies**

| Sex | Cattle | | Buffalo | | Overall | |
|---|---|---|---|---|---|---|
| | ST | SP (%) | ST | SP (%) | ST | SP (%) |
| Male | 29 | 03 (10.34) | 08 | 03 (37.50) | 37 | 06 (16.22) |
| Female | 143 | 27 (18.88) | 20 | 04 (20.00) | 163 | 31 (19.02) |
| Total | 172 | 30 (17.44) | 28 | 07 (25.00) | 200 | 37 (18.50) |

**Table 2**

**Species and district-wise prevalence of BHV-1 antibodies**

| District | Cattle | | Buffalo | | Total | |
|---|---|---|---|---|---|---|
| | ST | SP (%) | ST | SP (%) | ST | SP (%) |
| U.S. Nagar | 30 | 6 (20.00) | 20 | 4 (20.00) | 50 | 10 (20.00) |
| Dehradun | 107 | 13 (12.15) | 08 | 3 (37.50) | 115 | 16 (13.91) |
| Pithoragarh | 35 | 11 (31.42) | - | - | 35 | 11 (31.42) |
| Total | 172 | 30 (17.44) | 28 | 25.00 | 200 | 37 (18.50) |

ST=Sample tested; SP=Sample positive

The present study suggests that the disease is endemic in Uttarakhand and warrants concerted efforts for its control. The results of this pilot study warrant future studies with an aim to effectively screen the larger bovine population of cattle and buffaloes as well as characterization of the isolates to understand the molecular mechanism of pathogenesis and development of suitable vaccine. There is no vaccination programme at present but such a programme should be implemented.

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