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A serological survey of antibodies to infectious bovine rhinotracheitis by SNT

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Abstract
The present study was conducted to know the status of Bovine herpes virus-1(BHV-1) antibodies in the bovine population of the selected districts of Uttarakhand. Total 489 serum samples, 392 of cattle and 97 of buffaloes were collected from the unvaccinated bovine population with history of respiratory/reproductive disorders from five districts *viz*., Dehradun, Haridwar, Nainital, Pithoragarh and U.S. Nagar were tested by micro Serum Neutralization Test (mSNT). The overall prevalence observed was25.15%. At district level, highest prevalence was recorded in Nainital (32.37%) while it was lowest in U.S. Nagar (18.00%). The unorganized dairy units had higher prevalence of BHV-1 antibodies (27.00%) as compared to organized farms (22.79%). At species level, buffaloes were found to have greater prevalence (36.08%) compared to that in cattle (22.44%). Overall, 26.32% females and 10.81% males respectively were found to harbour antibodies to the virus. It was concluded that the bovine population of the state have been exposed to BHV-1 and hence effective prevention and control strategies must be implemented to counter the loss incurred by the virus.

Keywords: Bovine herpes virus -1, infectious bovine rhinotracheitis, prevalence, antibodies, SNT, cattle, buffalo

1. Introduction
Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV), caused by bovine herpesvirus 1 (BHV-1), is a disease of domestic and wild cattle. BHV-1 is a member of the genus Varicellovirus in the subfamily Alphaherpesvirinae, which belongs to the Herpesviridae family, order Herpesvirales. The viral genome consists of double-stranded DNA [23].

Based on restriction endonuclease analysis, isolates of BHV-1 can be subdivided into three subtypes. BHV-1 type 1 (BHV-1.1) isolates are typically associated with respiratory disease and abortion, BHV-1.2 are typically associated with venereal infection. BHV 1.2 can be further subdivided into BHV-1.2a and −1.2b, being clinically distinguished by the association of the former with abortions, although these distinctions between subtypes are not absolute [7, 8, 10, 15, 16]. IBR has proved to be a serious threat to the bovine population, associating itself with variety of clinical syndromes involving ocular, respiratory and genital tract. Secondary bacterial infections, latency and poor prevention measures further adds to the sequelae of the disease, thus complicating recovery and generating an eminent threat of spontaneous reoccurrence. The productivity and re-productivity of the animals is greatly decreased as an outcome of the disease [3]. The productivity and reproductivity of the animals is greatly decreased as an outcome of the disease [4, 23]. Because virus latency is a normal sequel to BHV-1 infection, the identification of serologically positive animals provides a useful and reliable indicator of infection status. Any animal with antibodies to the virus is considered to be a carrier and potential intermittent spreader of the virus [19].

For conventional serology, VNT, BoHV1-antibody blocking ELISAs or indirect ELISAs may be used [19]. The mSNT is considered to be the 'gold standard' and an OIE recommended test [20]. The VNT has been widely used and is considered to be the 'gold standard' test [21].

The present investigation was carried out to make out the prevalence status of BHV-1 antibodies in bovines of Uttarakhand with history reproductive/ respiratory disorders by using micro-Serum Neutralization Test (mSNT) and to determine the significance of risk factors like management, species, sex etc. associated with the BHV-1 prevalence.

2. Materials and Methods

2.1 Approval of Animal Ethics Committee

The samples were collected from animals with history of respiratory/ reproductive disorders. As per CPCSEA guidelines, a study involving clinical samples does not require approval of Institute Animal Ethics Committee.

2.2 Sample Collection

Total 489 serum samples were collected from cattle and buffaloes of five districts of Uttarakhand *viz*., Dehradun, Haridwar, Nainital, Pithoragarh and Udham Singh Nagar with history of respiratory/ reproductive disorders (table 1). Total 216 serum samples were collected from organized herd, of which 208 were of cattle, (178 of cows and 30 of breeding bulls) and 8 from buffalo breeding bulls. From unorganized sector, total 274 serum samples were collected out of which 185 were of cattle and 89 from buffaloes. Serum samples were collected as per standard procedure. Serum samples were stored at −20°C till assay procedure.

The serum samples were tested by mSNTat Virology laboratory, CADRAD, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India to detect BHV-1 antibodies.

2.3 Microserum Neutralization test (mSNT)

2.3.1 Madin Darby bovine kidney (MDBK) cell line

Madin Darby bovine kidney cell line was obtained from the National Centre for Cell Science, Pune. It was maintained in the Virus laboratory by using Dulbecco's modified Eagle's medium (Life technologies, Carlsbad, CA, USA) with 10% foetal calf serum (FCS) (Life technologies) as growth medium. Gentamicin was added in the medium at the rate of 50 mg/l (Life technologies).

2.3.2 Reference virus

The BHV-1 virus isolate (216 IBR II) maintained at the virus laboratory of CADRAD, IVRI was used in this study. In the mSNT, 100 $TCID_{50}$ of BHV-1 was used.

2.3.3 Protocol for micro Serum Neutralization Test

All the serum samples were inactivated at 56^0C for 30 min. Then 1:2 and 1:4 dilutions of serum samples were made in maintenance media (MM) containing 2% FCS. For this, 150µl of maintenance media was taken in each well of 24-well plates then 150 µl serum sample was added to the one well, mixed properly and 150 µl from this well was transferred to second well. After proper mixing, 150 µl was discarded from second well leaving final volume to be 150 µl. Now to each well of 24-well plate, 150 µl of BHV-1 containing 100 $TCID_{50}$ was added. Plates were incubated at 37^0C for 2 hours. To each well of 96 well flat bottom plate 100 µl of MDBK cell suspension containing $3x10^4$ cells/well was added.100 µl of each dilution of serum was added to two wells of 96 well plate.

All dilutions were tested in duplicate. The positive serum control was set up by adding 50 µl of 1:2 dilutions of known positive serum, 50 µl BHV-1 virus and 100 µl cells suspension. In negative serum control well, 50 µl of 1:2 dilution of known negative serum, 50 µl of BHV-1 virus and 100 µl of cell suspensions were added. In virus control well, 50 µl of MM, 50 µl of BHV-1 virus and 100 µl cell suspensions were added. In the cell control well, 100 µl of MM, 50 µl and 100 µl cell suspensions were added. The plate was incubated for 48-72 hr at 37^0C in CO_2 incubator.

2.3.4 Interpretation of result

Cell control wells should show intact monolayer, i.e. no CPE. Virus control wells should show CPE. Positive serum control wells should not show CPE. In negative serum control, there should be appearance of CPE. If the 1:2 dilution serum (final dilution 1:4) does not show CPE, but the 1:4 dilution serum show CPE, the serum was considered positive and the titer is 2.If all wells (1:2 and 1:4 dilution) show CPE, the serum was considered to be negative. If cytotoxicity was observed in the wells, the sample is reported to be toxic (no result) unless neutralization of the virus without cytotoxicity is observed at higher dilutions and a titre can be read without ambiguity.

3. Results and Discussion

3.1 Overall Seroprevalence

Out of 489 samples screened, 123 (25.15%) were found to be sero-positive (Table 1).The findings were in accord to the observations of [22]who found 26.85% samples positive by SNT in Bulgaria. Samrath *et al.* [28] have reported an overall seroprevalence of 34.69% from Chhattisgarh while Kathiriya *et al.* [12] from Gujarat have recorded 35.19% seroprevalence. In other independent studies, Jain *et al.* [11] have observed lower seroprevalence, (10.39%) whereas much higher prevalence (40.71%) was reported by Kollannur *et al.* [13] with respect to the present study. The difference in the seroprevalence might be due to difference in year of study, area/district selected for sample collection, variation in sample size and test employed.

3.2 Prevalence in organized and unorganized herds

Overall seroprevalence was recorded higher in unorganized herds (27.00%) with respect to organized herds (22.79%). Similar to our observation Rajesh *et al.* [25] also reported higher prevalence in unorganized herds (18.75%) than organized herds (13.13%). However, Singh and Yadav [30] observed much lower prevalence in unorganized herds (13.2%) as compared to organized herds (43.3%) of Kerala. The possible reason for higher prevalence in unorganized dairy herds might due practice of natural breeding with bulls whose disease status is not known. The natural breeding with bulls without knowing their disease status could be responsible for the rapid spread of the disease as also opined by Romero-Salas *et al.* [27]. Gonzalez-Garcia *et al.* [9] indicated lack of specific cattle infrastructure and beef crossbreeding 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.

3.3 Species-wise seroprevalence

At species level prevalence was higher in buffalo population (36.08%), as compared to cattle (22.44%). The observation of the present study also concurred with those of Krishnamoorthy *et al.;* Renukaradhya *et al.;* Trangadia *et al.* [14, 26, 31]; have also observed lower sero-positivity in cattle than buffaloes in India. On the contrary, Dwivedi *et al.,* Jain *et al.,* Kathiriya *et al.* [6, 11, 12] in independent studies have reported higher prevalence in cattle compared to buffaloes from different parts of India.

3.4 Sex-wise seroprevalence

Data related to overall sex-wise prevalence revealed that higher percentage off males (26.32%) were positive IBR virus than males (10.81%). At species higher percentage of females were found to be positive compared to males among both the

species (Table 1).

Durham and Hassard [5] also noticed that seroprevalence of antibodies against IBR/IPV was lower among males. Results of the present study were in agreement with Jain *et al.* (2006) [11] who also observed that BHV-1 antibodies were more prevalent in females (12.35%) than males (5.80%) in Uttarakhand and this was evident even at species level for both cattle and buffaloes. Similarly, Nandi *et al.* [18] in Uttarakhand, Sharma *et al.* [29] in Uttar Pradesh and Krishnamoorthy *et al.* [14] in southern India; also reported greater percent prevalence in females than males. The possible reason for higher seropositivity in females might be due to use of infected semen/seropositive bull for insemination/breeding Romero-Salas *et al.* [27]. Afshar and Eaglesome [1] mentioned that BHV-1 could spread through AI due to the use of contaminated semen resulting in a variety of genital tract disorders. However, low prevalence in bulls in present investigation is attributed to the fact that the samples were collected from the bulls used for breeding purpose where regular screening of bulls for various diseases was being practiced and affected bulls are consequently eliminated from the herd.

3.5 District-wise seroprevalence

District-wise data analysis revealed, highest seroprevalence in Nainital district (32.37%); while lowest prevalence was recorded in Udham Singh Nagar district (18.00%). The higher sero-prevalence in the animals of district Nainital may be due to the fact that new animals are being regularly introduced from different areas. This area is predominantly catering to slaughterhouse requirements especially in Haldwani. It was reported that cattle in transit to slaughter areas posed a serious threat to other cattle with which they came into contact, as these transit cattle could easily spread the virus upon reactivation caused by the stress of trekking [17]. Most of the untreated animals are sent to the slaughter houses, which were mostly old aged buffaloes and high correlation between age and seropositivity has been reported by many workers. Aruna and Babu [2] and Kathiriya *et al.* [12] had observed the increased prevalence rate with subsequent increase in age of animal. The increase in the prevalence of IBR with higher age could be due to the fact that as animals grow older, they are more likely to come into contact with other animals which have recovered from the disease but remain carriers [24]. In district-wise analysis lowest seroprevalence (18.00%) was observed in area of U.S. Nagar (Pantnagar) which may be due to the fact that there is regular screening and treatment of animals in this area in the teaching veterinary hospital present in the university campus. Also, in this area, artificial insemination is done from the semen of tested sero-negative bulls so there are lesser chances of venereal transmission of organisms.

Table 1: Details of sample collected and categorization of seroprevalence by mSNT

	Cattle		Buffalo		Overall	
	ST	**SP (%)**	**ST**	**SP (%)**	**ST**	**SP (%)**
District-wise						
Udham Singh Nagar	30	6 (20.00)	20	3 (15.00)	50	9 (18.00)
Nainital	70	15 (21.42)	69	30 (43.47)	139	45 (32.37)
Dehradun	207	47 (22.70)	8	2 (25.00)	215	49 (22.79)
Haridwar	50	11 (22.00)	-	-	50	11 (22.00)
Pithoragarh	35	09 (25.71)	-	-	35	09 (25.71)
Total	392	88 (22.44)	97	35 (36.08)	489	123 (25.15)
Sex-wise						
Male	29	2 (6.89)	8	2(25.00)	37	4 (10.81)
Female	363	86 (23.69)	89	33(37.07)	452	119 (26.32)
Total	392	88 (22.44)	97	35(36.08)	489	123 (25.15)
Management System						
Organized	207	47 (22.70)	8	2 (25.00)	215	49 (22.79)
Unorganized	185	41 (22.16)	89	33 (37.07)	274	74 (27.00)
OVERALL	392	88 (22.44)	97	35 (36.08)	489	123(25.15)

ST= Samples Tested SP= Samples found Positive PP= Per cent Positive

4. Conclusion

From this study, it is clear that BHV-1 infection is widely prevalent in the state of Uttarakhand. The probability of disease occurrence increases with advancement of age. The unorganized herds were having higher percentage sero-positive animals giving an indication that managemental practices may play role in spread of virus. The present epidemiological investigation was undertaken with the prime objectives to collect data and correlate it with disease precipitating factors relating to the host and environment, for rational decision making to design effective preventive and control strategies for this socio economically important infectious diseases of bovines.

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6. Conflict of interest

We declare that we have no conflict of interest.

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