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SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) IN NORTH EASTERN (NE) STATES OF INDIA

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ABSTRACT

Infectious bovine rhinotracheitis (IBR) is an infectious disease caused by BoHV-1 and belongs to the Herpesviridae family. IBR is endemic in India including north eastern states of the country. Hence the study was undertaken to understand the seroprevalence of IBR in north eastern parts of the country. A total of 3125 cattle (Holstein Friesian crossbred) serum samples from 35 districts of five north eastern states (Assam, Manipur, Meghalaya, Mizoram, and Sikkim) of India were screened for infectious bovine rhinotracheitis (IBR) virus antibodies using Avidin biotin ELISA. A two-stage random sampling methodology was followed for the collection of samples. Results from the present study revealed that the overall seropositivity was reported around 29.50% while the highest and lowest seropositivity of 43.39% and 16.66% were reported in the states of Sikkim and Assam respectively, followed by Mizoram (42.16%), Manipur (29.86%) and Meghalaya (27.40%). Cattle of higher age groups showed the highest seropositivity compared to younger ones. A higher percent of IBR antibodies in cattle of NE states is a cause of concern and a detailed study on IBR prevalence comprising of a large number of the bovine population need to be undertaken.

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1 Introduction

Infectious bovine rhinotracheitis (IBR) is a highly contagious infectious disease caused by bovine herpesvirus type 1 (BoHV1) belonging to the genus *Varicellovirus*, subfamily *Alphaherpesvirinae* family *Herpesviridae* (Mc Lachlan & Dubovi, 2011; Farooq et al., 2019). In India, the endemic IBR has caused a huge economic loss in the dairy industry due to a drop in milk production, repeat breeding, and abortions. The disease is characterized by respiratory symptoms (rhinotracheitis), abortions during the second and third trimester of pregnancy, repeat breeding, conjunctivitis, infectious pustular vulvovaginitis (IPV), and infectious balanoposthitis (IBP) (Raaperi et al., 2012). The virus gets transmitted by contact with infected animals, aerosol route, and virus-contaminated semen from BoHV-1 infected bulls. BoHV-1 infection often leads to latency, and such animals can act as a source of infection. Stress conditions, corticosteroid treatment, and transportation can activate the virus in latently infected animals, resulting inre-excretion of the virus (Jones et al., 2000).

Presently, four subtypes of bovine herpesvirus are known i.e. 1.1 and 1.2a (associated with infectious bovine rhinotracheitis), 1.2b (associated with infectious pustular vulvovaginitis and infectious balanoposthitis (IBP) and 1.3 (encephalitis) (Patil et al., 2012; Biswas et al., 2013). More often these serotypes cannot be differentiated by common serological tests and hence the studies describe them as IBR infected ones. Latent and subclinical infections are common in IBR (Ranganatha et al., 2013; Patil et al., 2017) which can be identified through the detection of antibodies against BoHV-1 in serum (Lemaire et al., 2000). Bovine herpesvirus-1 infection was first reported in India in 1976 (Mehrotra et al. 1976). Screening, surveillance, and monitoring are the three most important aspects to be considered to maintain the herd health status and to decrease the economic losses caused by this disease (Raizman et al., 2011). Information regarding the seroprevalence of IBR in the north eastern region of India are in scanty and only three reports viz., Suresh et al. (1999) those who reported 13.64% prevalence in Assam; Chettri et al. (2016) those who recorded 20.34% prevalence of IBR in different organized farms in Guwahati, Assam and Nandi et al. (2011) showed the highest seroprevalence of IBR in Yaks in Assam. Keeping this in mind current study was planned to screen the cattle serum samples from the states of the northeast region of India.

2 Materials and Methods

A two-stage random sampling methodology was followed wherein the number of random and representative villages and the number of animals in each village were selected using a survey toolbox (Sergeant, 2018). A total of 3125 cattle (Holstein Friesian crossbred) serum samples representing 35 districts from five states viz., Assam, Manipur, Meghalaya, Mizoram, and Sikkim (Table 1a&b) were collected (Figure 1). The states have a hilly terrain except for Assam and the cattle population is remotely distributed, hence the sampling number was limited.

Serum samples were screened using Avidin Biotin ELISA (ICAR-NIVEDI) as per the described protocol. Briefly, all the controls (positive, negative, and conjugate controls), test samples, and other reagents were used and dissolved in blocking buffer (1% bovine gelatin and 0.05% Tween 20) and dispensed in 100 ul of volume. The 1:100 diluted controls and test samples were dispensed to BoHV-1 antigen-coated plates. Later on, plates were incubated on a shaker at 37^0C for 1 hr. Afterwards, the plates were washed 3 times with washing buffer (1X PBS with 0.05% Tween 20). Then, biotinylated anti-bovine IgG (1: 10,000 diluted in blocking buffer) raised in goats was added to all wells and incubated on a shaker at 37^0C for 1 hr. Again, wash the plate as described earlier. Then add HRPO conjugated Avidin (1:10,000) to all wells and incubated at 37^0C for 20 min and followed by the washing of the plate as described prior.

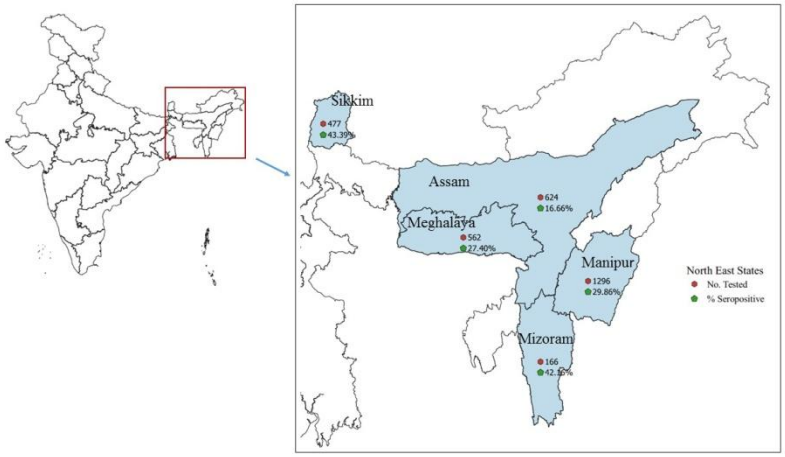

Figure 1 Location of bovine serum samples collected in five states of NE region of India

Table 1a Details of cattle serum samples used in the study

S. N.	State	2014-15			2015-16			2016-17				Grand Total		
		Total samples screened	No of Positive	Percent positivity	Total samples screened	No of Positive	Percent positivity	Total samples screened	No of Positive	Percent positivity	Total number of districts	Total samples screened	No of Positive	Percent positivity
1	Assam	80	5	6.25	228	17	7.46	316	82	25.95	11	624	104	16.66
2	Manipur	169	67	39.64	412	102	24.76	715	218	30.49	9	1296	387	29.86
3	Meghalaya	394	131	33.25	129	21	16.28	39	2	5.13	4	562	154	27.40
4	Mizoram	31	10	32.26	55	23	41.81	80	37	46.25	7	166	70	42.16
5	Sikkim	60	8	13.33	79	14	17.72	338	177	52.37	4	477	207	43.39
	Total	734	221	30.10	903	177	19.60	1488	516	34.67	35	3125	922	29.50

Table 1b State-wise seropositivity of IBR in north eastern states of India

Sl No	State	Grand Total				
		Total number of districts	Total samples screened	No of Positive	Percent positivity	True seropositivity at 95% CI
1	Assam	11	624	104	16.66	13.41 (10.29-17.00)
2	Manipur	9	1296	387	29.86	28.58 (25.78-31.50)
3	Meghalaya	4	562	154	27.40	25.75 (21.70-30.15)
4	Mizoram	7	166	70	42.16	42.72 (34.39-51.47)
5	Sikkim	4	477	207	43.39	44.13 (39.10-49.29)
	Total	35	3125	922	29.50	28.17 (26.36-30.03)
				$\chi2$=108	p=0.001	

Later on, 100 ul of TMB was added to all wells, incubate at 37^0C for 6-8 min, keep observing for colour development. This was followed by the addition of 50 ul of 1M stop solution (H_2SO_4) to all wells, and OD was measured at 450 nm (reference at 620 nm) (Annual Report, 2018). The sensitivity and specificity of the assay were found to be 92% and 95% respectively. There was no cross-reactivity between the samples as the antigen was precipitated and purified with polyethylene glycol (PEG). Results were interpreted as below,

'X'= Average OD of Strong Positive x 0.64

The test sample is positive if its OD values are greater than 'X'; the Test sample is negative if its OD values are less than 'X'

Appropriate and applicable statistical analysis such as chi-square test and confidence interval were calculated.

3 Results and Discussion

A total of 3125 cattle serum samples representing 35 districts from five states viz., Assam, Manipur, Mizoram, Meghalaya, and Sikkim were collected. Except for Assam, all the other states have hilly terrain, and cattle were housed remotely in different parts of the location. Cumulative seropositivity of 29.50% (922/3125) [28.17 (95%CI: 26.36-30.03)] was found to be on the higher side and was significant ($\chi2$=108, p=0.001).

Assam showed IBR seropositivity of 16.66% (Table 2). In Assam, earlier studies have reported seroprevalence of 13.64% in cattle (Suresh et al., 1999), 71.10% in Yak (Nandi et al., 2011), and 20.34% in cattle (Chettri et al., 2016). When the seroprevalence was compared between different ages of cattle, it was found that the cattle of 4 years of age showed the highest seropositivity of 21.46% followed by 14.47% in cattle of 3 years of age and 12.89% in cattle of 5 years of age. Although the cattle of 4 years of age in their peak of productivity showed the highest seropositivity, with not that significant values of $\chi2$= 6.92, p=0.031. Assam is the focal point for all dairy farming-related activities to cater to the needs of other states of north eastern states. Moreover, Assam shares a border with Bhutan which is very porous wherein there is no ban on the movement of man and animals.

Seropositivity of 29.86% was recorded in Manipur (Table 3). Cattle of 5 years of age showed seropositivity of 39.79%, followed by 24.20% seropositivity in cattle of 3 years of age, and cattle of 4 years of age revealed seropositivity of 20.65%. The highest seropositivity was recorded in cattle of 5 years of age which may be attributed to their peak productivity. Seropositivity amongst different age groups of cattle was insignificance with $\chi2$=50.80 and p=0.001. Suresh et al. (1999) showed a seroprevalence of 51.11% in Manipur which was found to be very high. Manipur also shares a border with other states Myanmar wherein the influx of materials into the state is very high.

Table 2 Age wise seropositivity of IBR in Assam

	Age in Years	Total tested	Total positive	Percent positivity	True seropositivity at 95% CI
Assam	3	152	22	14.47	10.89 (5.47-18.32)
	4	247	53	21.46	18.92 (13.56-25.28)
	5	225	29	12.89	9.07 (4.74-14.82)
		624	104	16.67	13.41 (10.29-17.00)
$\chi2$= 6.92 p=0.031					

Table 3 Age wise seropositivity of IBR in Manipur

	Age in Years	Total tested	Total positive	Percent positivity	True seropositivity at 95% CI
Manipur	3	219	53	24.20	22.07 (16.10-29.07)
	4	494	102	20.65	17.99 (14.15-22.34)
	5	583	232	39.79	39.99 (35.52-44.62)
		1296	387	29.86	28.58 (25.78-31.50)
$\chi2$=50.80 p=0.001					

In Meghalaya seropositivity was recorded at 27.40% (Table 4). However, seropositivity of 19.56% was reported during 2001-2009 (ICAR, 2010: http://www.kiran.nic.in/sero-prevalence.html) which might be attributed to easy movement of men and material as along with animals since they do not have demarcated boundaries. Cattle of 4 years of age showed seropositivity of 34.08%, followed by 30.56% seropositivity in cattle of 5 years of age and 19.19% seropositivity in cattle of 3 years of age with not so significant values of $\chi2$= 8.06 and p=0.018. There were not many reports on IBR seroprevalence from Meghalaya state.

A significant difference was seen in the seropositivity of IBR among the different age groups of cattle ($\chi2$= 27.0, p=0.001) of Mizoram. Suresh et al. (1999) showed a seroprevalence of 13.64% in Mizoram which was less compared to that shown in the present study (42.17%) (Table 5). Cattle of 5 years of age showed 75% seropositivity, which was in the peak productivity period and 27.27% seropositivity was recorded in cattle of 3 years of age. Mizoram shares a border with Myanmar and Bangladesh wherein there is no restriction on the movement of animals.

Sikkim is a small state that depends on Assam and West Bengal for its dairy needs. From this state, 43.39% seropositivity was recorded (Table 6). The highest seropositivity was observed among the 5 years age group cattle's while the lowest seropositivity was recorded in 3 years of age cattle's which was found to be highly significant ($\chi2$= 27.2, p=0.001). In general, it was reported that the animals of a higher age group of 9 years showed the highest seropositivity of 45.9% when compared with the younger ones of 2 years of age (6.89%) (Samrath et al., 2016).

Table 4 Age wise seropositivity of IBR in Meghalaya

	Age in Years	Total tested	Total positive	Percent positivity	True seropositivity at 95% CI
Meghalaya	3	99	19	19.19	16.31 (8.79-26.48)
	4	355	121	34.08	33.43 (27.99-39.27)
	5	108	33	30.56	29.37 (20.30-39.98)
		562	154	27.40	25.75 (21.70-30.15)
$\chi2$= 8.06 p=0.018					

Table 5 Age wise seropositivity of IBR in Mizoram

	Age in Years	Total tested	Total positive	Percent positivity	True seropositivity at 95% CI
Mizoram	3	66	18	27.27	25.60 (14.95-39.13)
	4	56	19	33.93	33.25 (20.60-48.28)
	5	44	33	75.00	80.46 (63.86-92.44)
		166	70	42.17	42.72 (34.39-51.47)
$\chi2$= 27.0 p=0.001					

Table 6 Age wise seropositivity of IBR in Sikkim

	Age in Years	Total tested	Total positive	Percent positivity	True seropositivity at 95% CI
Sikkim	3	87	27	31.03	29.92 (19.87-41.82)
	4	169	56	33.14	32.34 (24.69-40.85)
	5	221	124	56.11	58.75 (51.17-66.08)
		477	207	43.40	44.13 (39.10-49.29)
$\chi2$= 27.2 p=0.001					

Conclusion

IBR is endemic in India especially in the five states of north eastern states of the country. Seroprevalence of IBR showed the presence of IBR antibodies in animals with the indication of the presence of disease in the herd. Very few reports on the seroprevalence of IBR in the NE region are available; hence efforts were made to detect IBR antibodies in the cattle population in the NE region of India. Sikkim showed the highest seroprevalence while Assam revealed the lowest seropositivity of IBR. NE region is strategically located and has porous international borders with Myanmar, Bangladesh, and Bhutan wherein there is no restriction of movement of animals. Therefore, there should be strict vigilance on the movement of animals especially considering the health status of animals alongside international borders.

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Conflict of Interest

The authors declare that there is no conflict of interest

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