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Short Communication

SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) IN THE ANDAMAN AND NICOBAR ISLANDS, INDIA

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ABSTRACT: Infectious Bovine Rhinotracheitis (IBR) is caused by Bovine herpesvirus-1 (BoHV-1), an infectious disease-causing huge economic loss in dairy cattle. To keep dairy farming healthy and highly productive, there should be an IBR surveillance programme in dairy cattle, hence a study on the seroprevalence of IBR was undertaken in the Andaman and Nicobar Islands where the density of livestock population was more. A total of 418 cattle serum samples during 2019-20 from 11 villages of two districts of Andaman and Nicobar Islands were screened for IBR antibodies using ICAR-NIVEDI Avidin Biotin ELISA, of which 107 were found positive revealing 25.60% of seropositivity. HF crossbred and Jersey breed showed seropositivity of 24.06% and 33.33% respectively. Cattle of 5-6 years of age showed high seropositivity of 27.65-38.98%. Hence, it is recommended to test raw fresh semen/frozen semen straws, dairy animals regularly for IBR antigen/ antibodies to avoid the spread of infection.

Key words: Andaman and Nicobar Islands, Cattle, IBR, Seropositivity, Surveillance.

Infectious Bovine Rhinotracheitis (IBR) is a contagious disease of cattle and buffaloes caused by Bovine Herpesvirus type 1 (BoHV-1) belonging to genus *Varicellovirus,* subfamily *Alphaherpesvirinae,* family *Herpesviridae* (Mc Lachlan and Dubovi 2011). The virus is responsible for causing severe economic losses to the dairy industry worldwide due to abortions leading to increased calving interval, reduced milk yield, weight loss, and restrictions on international livestock trade (Nandi *et al*. 2009). Clinically the disease manifests as conjunctivitis, red nose, abortions, and reduction in milk yield, Infectious Pustular Vulvovaginitis (IPV) /Infectious Balanoposthitis (IBP) (Raaperi *et al*. 2012). IBR is endemic in India and the seroprevalence of IBR in organized dairy farms was ranged between 36.50-84.5% (Patil *et al.* 2017). Singh and Yadav (2010) reported a seroprevalence of 13.2% in an unorganized cattle herd in Uttar Pradesh. Samrath *et al*. (2016) reported a seroprevalence of 34.69% in different districts of Chhattisgarh. 29.03% seroprevalence was reported in five districts of Uttarakhand (Thakur *et al.* 2017).

The study

The Andaman and Nicobar (A&N) Islands, a Union Territory, is a unique ecosystem having both hot and humid climates with a bovine population of 40,138 (DAHD 2019). A&N Islands are the remotest islands located approximately 1400 km away from mainland India. The Andaman and Nicobar group of Islands are blessed with one of the unique and diversified ecosystems of the world. Being away from the mainland and population pressure, the area is still maintaining an almost pollution-free virgin environment, harbouring pure and rich germplasm resources. It is situated in the southern part of the Bay of Bengal between 92^{o} 12' E and 93^{o}57'E longitude and between 6^{o}45'N and 13^{o}41'N latitude with 10^{o}N channel separating the Andaman group of Island from the Nicobar group of Islands. It is a group of 572 Islands, Islets, and rocks covering a geographical area of 8293 km^{2} (Sunder *et al*. 2014). The majority of the materials including animals and their products are procured from the mainland either from Tamil Nadu or West Bengal (www.andaman.gov.in). The dairy industry in the Islands is in its infancy and animal health

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surveillance is important for effective control of infectious diseases of livestock. Hence, the diseases affecting dairy animals like IBR need to be investigated for better management and productivity. IBR was first reported in India in 1976 (Mehrotra *et al.* 1976). It causes huge economic losses due to a drop in milk production, repeat breeding, and abortions. Screening, surveillance, and monitoring are important to maintain the herd health status and to decrease the economic losses caused by this disease (Raizman *et al.* 2011). A&N Island procures frozen semen straws/animals from Tamil Nadu and West Bengal (Unpublished data). Suresh (1992) reported an overall seropositivity of 33.97% in Tamil Nadu after screening 156 sera samples of buffaloes. A seroprevalence of 20.16% was reported in cattle in Tamil Nadu in 1999 (Suresh *et al.* 1999). A seroprevalence of 48.15% was recorded in cattle in West Bengal (Ganguly *et al.* 2008). Not much data on the seroprevalence of IBR in A&N Islands is available except for two reports which revealed17.68% (Sunder *et al.* 2005) and 20.58%seroprevalence (Sunder *et al.* 2014). The study was undertaken to assess the seroprevalence of IBR in two districts of A&N Islands which are having more bovine population.

Backyard dairy farming is most common in India and husbandry practices remain similar in most of the households having bovines. Two-stage random sampling methodology was followed in the present study. The number of random and representative villages and the number of animals in each village was selected using a survey toolbox (Seargent *et al.* 2018). Randomness in selecting the number of villages (primary unit) and the number of animals (secondary unit) within the village was calculated using epi calculator (*(https://nivedi.res.in/Nadres_v2/Epical/herd_level sample_size.php)*. The prevalence of IBR was fixed at 30% (Annual Report 2019). A total of 418 cattle serum samples (345 from HF cross bred, 75 from Jersey, and one from Tharparkar crossbred) from 11 villages of two districts *viz*., South Andaman and North and Middle Andaman were collected during 2019-20. Four villages (Ferrargunj block), seven villages (Port Blair block), and one village (Rangat block) were randomly selected for sampling.

Serum samples were screened using Avidin-Biotin ELISA (ICAR-NIVEDI) as per the described protocol. Briefly, all the controls (positive and negative sera), test

Table 1. Details of seroprevalence of IBR in South Andaman, A&N Islands.

District	Block	Village	Total tested	Total Positive	Percent Positivity	True Prevalence at 95% CI
South Andaman	Ferrargunj	Elephant Point	60	19	31.67	30.65 (18.74-45.10)
		Manglutan	40	11	27.50	25.86 (12.77-43.49)
		Port Mourt	40	9	22.50	20.11 (8.41-37.36)
		Wimberlygunj	52	11	21.15	18.57 (8.33-33.37)
		Total *	192	50	26.04	24.19 (17.64-31.81)
	Port Blair	Dollygunj	41	10	24.39	22.29 (10.14-39.48)
		Garacharma	71	21	29.58	28.25 (17.51-41.40)
		Hutbay	7	3	42.86	43.51 (12.44-80.41)
		Indira Nagar	2	1	50.00	51.72 (2.80-1.0625)
		Junglighat	40	11	27.50	25.86 (12.77-43.49)
		Nimbu Bagicha	3	2	66.67	70.88 (18.12-1.072)
		Rangachang	40	5	12.50	8.62 (0.0053-24.27)
		Total #	204	53	25.98	24.12 (17.75-31.50)
North & Middle Andaman		Rangat	22	4	18.18	15.15 (2.65-38.52)
		Grand Total	418	107	25.60	23.68 (19.14-28.73)

*Significance: χ^2=1.94; p=0.586; # Significance: χ^2= 8.58; p=0.199.

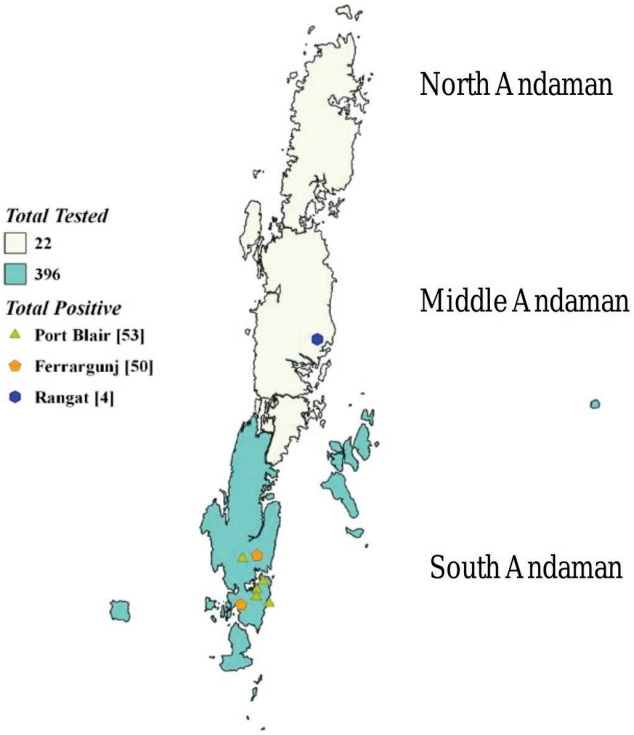

Fig. 1. Map of Andaman and Nicobar Islands showing the number of cattle serum samples tested and number of positive samples.

samples, and other reagents used were dissolved in blocking buffer (1% bovine gelatine and 0.05% Tween 20) and dispensed in 100 µl 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^{0}C for 1 hr. And washing of the plate 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^{0}C for 1 hr. Again, washing of the plate was done as described earlier. Then HRPO conjugated Avidin (1:10,000) was added to all wells and incubated at 37^{0}C for 20 min. And, washing of the plate as described above. Later on, 100 µl of TMB was added to all wells, incubated at 37^{0}C for 6-8 min, observing for colour development. Then, 50 µl of 1M stop solution (H_2SO_4) was added to all wells, measured OD 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 of the samples as the antigen was polyethylene glycol (PEG) precipitated and purified. The controls like positive, negative, and conjugate controls were included in the test. Results were interpreted as below,

'X'= Average OD of Strong Positive X 0.64

Test sample is positive if its OD values are greater than 'X'

Test sample is negative if its OD values are less than 'X'

Appropriate statistical analysis such as chi-square test, confidence interval, association of prevalence to particular risk factors such as age, gender and breed were

Table 2. Age wise seroprevalence of IBR in Andaman and Nicobar Islands.

District	Blocks	Age (years)	No Tested	No Positive	Percent Positive	OR	95%CI	Chi-Square	p value
South Andaman	Ferrargunj	1 to 4	53	12	22.64	-	-	4.05	0.256
		5	94	26	27.65	1.31	0.59-2.87		
		>6	45	12	32.43	1.64	0.64-4.21		
		Total	192	50	26.04	-	-		
	Portblair	1 to 4	130	29	22.30	-	-	9.01	0.011
		5	59	23	38.98	2.22	1.14-4.33		
		>6	15	1	6.66	0.25	0.03-1.97		
		Total	204	53	25.98	-	-		
North and Middle Andaman	Rangat	1 to 4	9	1	11.11	-	-	0.820	0.664
		5	7	2	28.57	3.20	0.23-45.19		
		>6	6	1	16.66	1.60	0.08-31.77		
		Total	22	4	18.18	-	-		

Table 3. Breed wise seroprevalence of IBR in South Andaman, A&N Islands.

Breed	Total tested	Total Positive	Percent Positivity
HF CB	345	83	24.06
Jersey	72	24	33.33
Tharparkar CB	1	0	0.00
Total*	418	107	25.60

*Significance: χ^2=5.55; *p*=0.062.

Table 4. Gender wise details of seroprevalence of IBR in South Andaman, A&N Islands.

Sex	Total tested	Total positive	Total negative	Percent Positivity
Female	415	106	309	25.54
Male	3	1	2	33.33
Total*	418	107	311	25.60

*Significance χ^2=0.949; *p*=0.58.

calculated.

Out of 418 cattle serum samples screened 107 samples were positive for IBR antibodies with an apparent prevalence of 25.60% [23.68 at 95% CI: 19.14-28.73] (Fig. 1).

In the district of South Andaman, within the block of Ferrargunj, village Elephant Point showed the highest seropositivity [31.67% (19/60)] followed by Wimberlygunj [21.15% (11/52)] village. In the block of Port Blair, village Nimbu Bagicha showed the highest seropositivity of 66.67% (2/3) and Rangachang village showed the lowest seropositivity of 12.50% (5/40). In the district of North and Middle Andaman district, village Rangat showed seropositivity of 18.18% (4/22) (Table 1). Statistical analysis of IBR seropositivity between villages of Ferrargunj showed Chi-square test values of 1.95 with *p*=0.586, whereas Chi-square test values of 8.58 with p=0.199 which was significant amongst the villages of Port Blair (Table 1). The variations in the seropositivity of IBR within the same blocks of district may be attributed to the use of frozen semen straws from different sources (contaminated with IBR virus?) or animals must have moved from different places.

In the South Andaman district, cattle of 6 years of age were showing higher seropositivity of 32.43% (12/45) in Ferrargunj block, whereas cattle of 5 years of age showed 38.98% (23/59) seropositivity in the Port Blair block. Rangat block presented 28.57% (2/7) seropositivity of IBR in cattle of 5 years of age in the North and Middle Andaman district. Overall, it is to be noted that cattle in the higher age group (may be higher lactation period) of 5-6years were showing higher seropositivity of IBR antibodies wherein animals might have experienced the infection due to reactivation of the virus in one of the stages of their age (Table 2). It can be seen that the higher age group is positively associated with seropositivity of IBR in the villages of Ferrargunj with Chi-square test values of 4.05 with *p*=0.256 but not significant, whereas, in Port Blair, increasing age is significantly associated with IBR seropositivity having Chi-square test values of 9.01 with *p*=0.011 (Table 2).

In Rangat block, age distribution has not shown any significant association with IBR seropositivity. It is to mention that the apparently healthy animals which are positive for IBR antibodies might have shed the virus whenever there were abortions. This might be one of the reasons how the virus keeps circulating within the herd. The seroprevalence ranged born 10% in Diglipur tehsil to 41.93% in Rangat tehsil with an overall average of 17.68%. Interestingly, in Rangat Tehsil the seroprevalence of IBR (45.16%) (Sunder *et al.* 2005) and Shome *et al.* (1998) showed 86% prevalence, which is considered to be very high as compared to the present findings. The reasons attributed were that of modernization and improvement of the animal husbandry practices and introduction of foreign germplasm, tourist intervention, lack of proper quarantine practices the island ecosystem is in danger facing threat to many of the emergence diseases like Infectious Bovine Rhinotracheitis (IBR) (Sunder *et al.*, 2005). The suggested control measures like proper quarantine/isolation practices at the point of arrival of animals and use of IBR free certified semen straws can reduce the incidence of IBR in the Islands.

The serum samples were collected from HF crossbred (n=345), Jersey (n=72) and, one from Tharparkar cross bred of cattle. HF crossbred cattle showed seropositivity of IBR as 24.06% (n=345), Jersey cattle showed 33.33% (n=72) seropositivity and Tharparkar cross bred cattle was found negative (n=1) for IBR antibodies. There was a positive correlation between breeds of cattle with Chi-square test value of 5.55 and *p*=0.062 (Table 3).

The higher percentage of IBR antibodies in these two breeds of cattle may be attributed to either the animals must have experienced the infection earlier or the virus is circulating in the particular area where the animals are being reared (hypothesized). Only 3 male cattle were included in the study and one bull showed IBR seropositivity (Table 4).

The two districts of A&N Islands, though located far from the mainland, showed the IBR seropositivity of 25.60%. This shows that BoHV-1 is circulating in the cattle population of the region. A&N Islands procure frozen semen straws from the mainland viz., Tamil Nadu and West Bengal for artificial insemination which need to be tested for the IBR virus regularly. Animals showing IBR antibodies are latently infected and need to be tested at regular intervals since they shed the virus whenever there is a stress wherein the virus gets reactivated (Oirschot 1995). Abortions in cattle in villages should be given special attention for testing of IBR. The movement of animals should be strictly monitored and sick animals should be isolated and observed for 21 days for their clinical signs. Quarantine/Isolation units at the point of arrival of animals and procurement of IBR free certified semen for artificial insemination programmes combined with proper biosecurity and management practices would prevent the spread and control of IBR in the Islands.

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