RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 6: 1011-1016 (June 2024)

Seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in Indian Buffaloes

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10.18805/IJAR.B-5039

ABSTRACT

Background: Infectious bovine rhinotracheitis (IBR) is an infectious disease of bovines causing respiratory problem, abortions and reduced milk yield leading to huge economic losses. IBR is endemic in India. India ranks first in buffalo population. Reports on seroprevalence of IBR in cattle in India are available but not in buffaloes. The study was aimed at conducting a seroprevalence of IBR in Indian buffaloes.

Methods: A total of 1000 buffalo serum samples collected randomly from top 10 milk producing states of India and were tested for IBR antibodies using Avidin-Biotin (AB) ELISA.

Result: Cumulative seropositivity was found to be 43.30%. Punjab and Andhra Pradesh states showed highest (58%) and lowest (27%) seropositivity respectively and were part of north and south zone of the country. India has highest number of buffalo population. Presently, India do not vaccinate bovines against IBR and should take call on vaccination of dairy buffalo after having witnessed a high seroprevalence of IBR. This is the first report of such kind of number of buffalo samples tested for IBR antibodies.

Key words: BoHV-1, Buffalo, IBR, Prevalence.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) caused by the Bovine alphaherpesvirus 1 (BoHV-1), a contagious disease of bovines, belongs to the genus *Varicellovirus*, subfamily *Alphaherpesvirinae* family *Herpesviridae* (Mc Lachlan, 2011). Latency and subclinical infections are common in IBR (Ranganatha *et al*., 2013) which can be identified through the detection of antibodies against BoHV-1 in serum (Lotfi *et al*., 2016). Bovine alphaherpesvirus 1 infection was first reported in India in 1976 (Mehrotra *et al*., 1976). Reduced milk production, repeat breeding and abortions are some of the consequences of IBR infection leading to huge economic losses. 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).

IBR is endemic in India and there is no systematic study on the seroprevalence of IBR in buffaloes though many have reported IBR antibody prevalence in cattle either restricted to districts/states/zones (Patil *et al*., 2012 and 2017; Krishnamoorthy *et al*., 2015; Kathiriya *et al*., 2018).

An epidemiological study to estimate the frequency of infectious bovine rhinotracheitis (IBR), zone and species-specific based on serosurveillance of IBR to achieve the goals within the defined populations and inform the researchers and policymakers about the disease (IBR) burden, thereby supporting the process of identification of priorities in Veterinary healthcare, prevention and policy. The study aimed to screen the buffalo serum samples for antibodies against BoHV-1 selected randomly to understand the prevalence.

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How to cite this article: Patil, S.S., Suresh, K.P., Velankar, A., Nayakvadi, S., Hiremath, J., Jacob, S.S., Sridevi, R., Gowda, C.S.S., Gejendiran, N. and Shome, B. (2024). Seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in Indian Buffaloes. Indian Journal of Animal Research. 58(6): 1011-1016. doi: 10.18805/IJAR.B-5039.

Submitted: 20-10-2022 **Accepted:** 21-03-2023 **Online:** 03-05-2023

MATERIALS AND METHODS

Sample collection

Backyard dairy farming is most common in India and husbandry practices remain the same in most of the households having bovines. Two stage random sampling methodology was followed wherein number of random and representative villages and the number of animals in each village were selected using a survey tool box (Sergeant *et al*., 2018).

Enzyme immunoassay for detection of IBR antibodies

Serum samples were subjected to enzyme immunoassay for detection of antibodies against IBR using indigenously developed Avidin-Biotin ELISA (AB ELISA) by Patil *et al*. (2021). Results were interpreted as follows.

Interpretation and analysis

'X'= Average OD of strong positive × 0.64

A test sample is positive if its OD values are greater than 'X'. A test sample is negative if its OD values are less than 'X'.

RESULTS AND DISCUSSION

Sample collection

A total of 1000 buffalo serum samples from 242 villages in 63 districts from top 10 milk producing states were collected (Table 1).

Enzyme immunoassay for detection of IBR antibodies

A total of 1000 buffalo serum samples were collected during 2021 and tested for IBR antibodies which showed a per cent positivity of 43.30 [44.02 (95% CI: 40.53-47.58%)] (Fig 1). Punjab showed the highest positivity of 58% [60.92 (95% CI: 49.66-71.5%)] which is a geographically a small state and known for dairy farming, whereas Andhra Pradesh showed the lowest positivity of 27% [(25.29 (95% CI: 16.4-36.13%)] (Table 1). Following four zones of India had a varied seroprevalence of IBR (Table 2).

South zone

Samples analyzed from this zone were from 3 states (Andhra Pradesh, Karnataka and Tamil Nadu). A total of 300 buffalo serum samples were tested and this zone showed seropositivity of 37% [36.78 (95% CI: 30.73-43.21%)] from 58 villages covering 23 districts. In the present study, Karnataka showed the highest seropositivity of 54% [56.32 (95% CI: 45.13-67.17%)] in buffalo samples collected from 13 villages of 7 districts. Whereas, Renukaradhya *et al*. (1996) have reported 52.5% (530/1010) seropositivity in buffaloes from one slaughter house alone situated in Karnataka that seems to biased. In another study, 72.60% (106/146) seropositivity in buffaloes of two organized farms in Karnataka was recorded (Krishnamoorthy *et al*., 2015). Tamil Nadu has showed 30% seropositivity [28.74 (95% CI: 19.42-39.75%)] in buffalo samples collected from 24 villages belonging to 7 districts in this study. Whereas, Selvaraj *et al*. (2008) showed the seropositivity of 8.56% (16/187) in buffaloes of Tamil Nadu.



Fig 1: Map depicting state wise seropositivity of IBR in India.

West zone

Gujarat, Maharashtra and Rajasthan were a part of this zone. A total of 300 buffalo serum samples were tested and showed a positivity of 39.67% [39.85 (95% CI: 33.67-46.32%)] from 114 villages across 14 districts. Maharashtra showed highest seropositivity of 47% [48.28 (95% CI: 37.37-59.44%)] and Rajasthan showed lowest seropositivity of 33% [32.18 (95% CI: 22.49-43.33%)]. The three states in this zone are the highest milk producers having high yielding dairy animals. Maharashtra has recorded a highest seropositivity of 47% being geographically large state and known for dairy farming. Rajasthan being the second highest milk producing state of India has showed the lowest seropositivity of 33%. Gujarat has showed a seropositivity of 39% in buffaloes which has a good network of milk producer's cooperative units. The buffalo samples of Gujarat were collected from 81 villages of 3 districts. 24 out of 32 (75%) buffalo samples were found positive for IBR antibodies in Gujarat (Patel *et al*., 1983). Pandita and Srivastava, (1993) showed an incidence of IBR as 50.50% in buffaloes in Gujarat. Jain *et al*. (2009) have tested buffalo serum samples for IBR antibodies that showed 34.61% seropositivity in Gujarat which shares borders with Rajasthan. A seropositivity of 33.69% (97/288) of IBR antibodies were recorded in buffaloes of Gujarat during 2018 (Kathiriya *et al*., 2018).

North zone

This zone consisted of Haryana, Punjab and Uttar Pradesh. Cumulative per cent positivity was found to be 52.33 [54.41 (95% CI: 47.92-60.83%)]. Punjab showed the highest seropositivity of 58% [60.92 (95% CI: 49.66-71.5%)]. Punjab is a small state and is a 6th highest milk producing state of India. According to 20th livestock census, Punjab has increased its milk production by 50.14% between 2012 and 2019 (DAHD, 2019). In the present study, a seropositivity of 58% was recorded in Punjab buffalo samples collected from 11 villages belonging to 9 districts. Previous studies have reported seropositivity of IBR antibodies in buffaloes as varying percentage. Aradhana *et al*. (2004) have reported 33.3% of seropositivity of IBR in buffaloes of Punjab. In another study, Dhand *et al*. (2002) have recorded 47.25% (43/91) and 17.47% (43/246) of seropositivity of IBR antibodies in organized and unorganized buffalo farms respectively in Punjab. A small number buffalo serum samples from Ludhiana (Punjab) were tested for IBR antibodies which showed the positivity of 54.50% (12/22)

(Dhami *et al*., 2008). The lowest seropositivity of 48% [49.43 (95% CI: 38.47-60.56%)] was recorded by Haryana. Uttar Pradesh showed 51% seropositivity [52.87 (95% CI: 41.78-63.88%)].

Uttar Pradesh is the largest state of India and is the number one in milk production. The state has recorded a seropositivity of 51% of IBR antibodies in buffaloes of 8 villages from one district only. The sample number should have been more which was not accomplished due to administrative reasons. A small number of buffalo serum samples tested in Uttar Pradesh revealed 85% (17/20) positivity by Nandi *et al*. (2011). Verma *et al*. (2014) have recorded 25.27% (23/91) seropositivity of IBR in buffaloes of 8 districts of Uttar Pradesh.

Central zone

Madhya Pradesh is the only state in this region which is geographically large in nature. The state has showed a seropositivity of 46% [47.13 (95% CI: 36.28-58.32%)] in buffaloes from 5 villages of 2 districts. The Chhattisgarh state which was a part of Madhya Pradesh previously, have reported a seroprevalence of 7.15% (3/42) in buffaloes (Samrath *et al*., 2016). Madhya Pradesh shares a border with Maharashtra (seropositivity: 47%), Gujarat (seropositivity: 39%), Rajasthan (seropositivity: 33%), Uttar Pradesh (seropositivity: 51%) and Chhattisgarh (seropositivity: 7.1% by Samrath *et al*., 2016).

Breed wise seropositivity

Murrah is the most important breed of buffalo in India. Murrah's home is Haryana and Punjab states and the average milk yield per lactation is 1500-2500 kilograms. A seropositivity of 44.08% of IBR antibodies were reported in Murrah breed. Mehasana's home is Gujarat and Maharashtra states and is evolved out of cross-breeding between Murrah and Surti. Murrah and Mehsana were two important breeds of buffaloes along with non-descript buffaloes were tested for IBR antibodies (Table 3). 44.08% (320/726) [44.92% (95% CI: 40.81-49.09%)] seropositivity was recorded in Murrah breeds and Mehsana breed recorded 40.48% (34/84) [40.78% (95% CI: 29.45-53.06%)] seropositivity. Non-descript (not assigned any breed characteristics) showed 41.24% (73/177) seropositivity [41.66 (95% CI: 33.62-50.12%)]. Mehasana buffaloes showed a IBR seropositivity of 40.48%. There were some buffaloes which were non descript in nature showed 41.66% seropositivity. Krishnamoorthy *et al*. (2015) have shown an incidence of 69.15% (92/133) and 66.67% (4/6) in Murrah and Mehasana buffaloes respectively in south India. Kathiriya *et al*. (2018) have reported a seroprevalence of 33.99% in Jafrabadi breed in Gujarat. Very limited reports are there on seropositivity of IBR breed wise in India.

India is the world's largest milk producer, with 22 per cent of global production. Top 10 milk producing states of India contribute to 80% of the national milk production. Additionally, India stands first in the world of buffalo population contributing significantly to the milk production of the nation. India has 109.9 million buffalo (adult female buffaloes: 55 million). Therefore, an attempt was made to screen one hundred buffalo serum samples for IBR antibodies, collected randomly from top 10 milk producing states. Many authors in India and elsewhere have reported

Table 1: Details of samples used in the study from different states of India.

State	Region in India	No. of samples tested	Per cent positivity	True prevalence with 95% CI	No. of district tested	No. of village tested
Andhra Pradesh	South	100	27	25.29 (16.4-36.13%)	9	21
Karnataka	South	100	54	56.32 (45.13-67.17%)	7	13
Tamil Nadu	South	100	30	28.74 (19.42-39.75%)	7	24
Gujarat	West	100	39	39.08 (28.76-50.34%)	3	81
Maharashtra	West	100	47	48.28 (37.37- 59.44%)	6	13
Rajasthan	West	100	33	32.18 (22.49-43.33%)	5	20
Haryana	North	100	48	49.43 (38.47-60.56%)	14	46
Punjab	North	100	58	60.92 (49.66-71.5%)	9	11
Uttar Pradesh	North	100	51	52.87 (41.78-63.88%)	1	8
Madhya Pradesh	Central	100	46	47.13 (36.28-58.32%)	2	5
	Total	1000	43.30	44.02 (40.53-47.58%)	63	242

Table 2: Region wise buffalo serum samples tested for IBR during 2021-22.

Region in India	No. of district tested	No. of village tested	No. of samples tested	No. of samples positive	Per cent positivity	True prevalence at 95% CI
South	23	58	300	111	37.00	36.78 (30.7343.21%)
West	14	114	300	119	39.67	39.85 (33.67-46.32%)
North	24	65	300	157	52.33	54.41 (47.92-60.83%)
Central	2	5	100	46	46.00	47.13 (36.28-58.32%)
Total	63	242	1000	433	43.30	44.02 (40.53-47.58%)

Table 3: Breed wise buffalo serum samples tested for IBR.

Breed	No. of samples tested	No. of samples positive	Per cent positivity	True prevalence at 95% CI
Murrah	726	320	44.08	44.92% (40.81-49.09%)
Mehsana	84	34	40.48	40.78% (29.45-53.06%)
Nili Ravi	8	3	37.50	37.36 (9.98-74.05%)
Non-descript	177	73	41.24	41.66 (33.62-50.12%)
Total	1000	433	43.30	44.02 (40.53-47.58%)

Table 4: Age wise buffalo serum samples tested for IBR.

Age in years	No. of samples tested	No. of samples positive	Per cent positivity	True prevalence at 95% CI
2-5	282	110	39.00	46.83 (40.26-53.54%)
5-11	688	308	44.76	42.54 (38.36-46.81%)
11-15	30	15	50.00	51.72 (32.36-71.09%)
Total	1000	433	43.30	44.02 (40.53-47.58%)

seropositivity of IBR antibodies in buffaloes taking limited number of samples. The present study has revealed seropositivity of IBR antibodies in buffaloes in 100 samples from each top 10 milk producing states of India. Overall seropositivity of IBR antibodies in 1000 buffaloes was found to be 43.30% in India. Buffaloes are domesticated in limited number of countries and many of them have reported seroprevalence of IBR antibodies in buffaloes. A 16.7% (6/30) and 51.50% (103/200) prevalence of IBR in buffaloes was recorded in Pakistan (Akhtar and Asif, 1996; Batool *et al*., 2022), wherein the number of buffaloes screened in both the studies appeared limited. Mahmoud *et al*. (2009) have demonstrated 38.46% (5/13) seropositivity of IBR antibodies in buffaloes in Egypt. A seroprevalence of 82.40% (155/188) of IBR antibodies was recorded in Murrah and Mediterranean buffaloes in Brazil (Ferreira *et al*., 2010). The two studies in Iran have showed a seroprevalence of 5.5% (28/513) and 4.13% (5/121) of antibodies against IBR in buffaloes (Lotfi *et al*., 2016; Kargar Moakhar *et al*., 2002). In Iraq, Ahmed *et al*. (2015) have reported 73.9% (17/23) seropositivity of IBR antibodies in buffaloes.

Trangadia *et al*. (2010) have reported seroprevalence of IBR antibodies in organized buffalo farms located in three zones of India *viz*., Western region reported 40.82% (20/49), Central region has revealed 97.96% (48/49) and Northern region has recorded 26.32% (5/19) and overall seroprevalence was found to be 62.39% (73/117).

Age wise seropositivity

In the present study, the buffaloes in age group between 11-15 years showed the highest seropositivity of 50% (15/30). 688 buffaloes in the range of 5-11 years of age were tested and 42.01% (308/688) of animals showed seropositivity (Table 4). Animals with 3-6 years of age were more prone to infection than were younger animals and the seropositivity of IBR increased with age of animals (Nandi *et al*., 2009). The highest prevalence of IBR (42.07%) was observed in animals aged more than 7 years (Kathiriya *et al*., 2018).

Mahmoud *et al*. (2009) have showed seropositivity of IBR antibodies in different age groups as buffaloes of 3-6 m having 40%, 3-16 m having 33% and adult buffaloes having 40% in different parts of Egypt. Animals above 9 years of age showed the highest seropositivity (45.9%) whereas young animals between 0 to 2 years of age showed the minimum seropositivity (6.89%) (Samrath *et al*., 2016). Increased age has been considered a risk factor for higher prevalence as a result of greater chances of virus exposure to susceptible animals with age and a study noted that BoHV-1 is more prevalent in animals greater than 4 years (Batool *et al*., 2022). Lowest seroprevalence of IBR was found in buffaloes below 2 years of age and highest in animals of above 8 years of age (Dhami *et al*., 2008). Kathiriya *et al*. (2018) showed maximum seropositivity of IBR in animals of more 7 years of age. The incidence of IBR was recorded more as the age of animals increased *i.e*., 6 years and above (Krishnamoorthy *et al*., 2015) might be due to increased susceptibility and stress.

The possible reason for higher seropositivity may be due to increased susceptibility of animals with age or repeated subclinical infection with the virus that boost to keep the antibody titer higher enough to be detected positive or decrease in immunity and increase in stress, which may lead to reactivation of latent virus (Singh and Sinha, 2006; Kathiriya, 2018; Sheza *et al*., 2021).

CONCLUSION

India stands number one in buffalo population in the world. There are health concerns in buffaloes with respect to the productivity and IBR is one of the most important abortifacient which is neglected most. Scanty reports on IBR seropositivity using limited number of samples have revealed that the buffaloes have experienced IBR and is present in the population. Our study is the first report of such kind using significant number of samples revealing the seropositivity in buffaloes. Further study is required using a systematic random sampling methodology with significant numbers which might reveal some more information on its endemicity.

Conflict of interest

All authors declare that they have no conflict of interest.

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