

Seroprevalence of infectious bovine rhinotracheitis (IBR) in India: a 5-year study

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Summary

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease of bovines causing respiratory symptoms, abortions, and reduced milk yield, leading to huge economic losses. Reports on seroprevalence in bovines in India are available and restricted to districts/states. In the present study, a nationwide seroprevalence of IBR in bovines was conducted to provide a national IBR seroprevalence to the Chief Veterinarian who in turn can design the control strategies. A total of 15,592 cattle and buffalo serum samples from 25 states and 3 Union Territories viz., Jammu and Kashmir, Puducherry, and Andaman and Nicobar Islands were tested for IBR antibodies using Avidin-Biotin (AB) ELISA. Cumulative seropositivity was found to be 31.37%. Maharashtra and Rajasthan states, part of the west zone of the country, showed the highest and lowest seroprevalence, respectively. A total of 11,423 cattle and 4,169 buffalo serum samples were tested, which showed 33.91% and 24.39% seropositivity, respectively. India has the highest buffalo population. Presently, no IBR vaccination programs are implemented in India. Considering the high seroprevalence, the authorities should plan control strategies for vaccinating dairy cows and buffaloes in India.

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease of cattle caused by the bovine alphaherpesvirus 1 (BoHV-1) which belongs to the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae* (McLachlan 2011). Four subtypes of the virus are known: 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) (Biswas *et al.* 2013). These serotypes cannot be differentiated by common serological tests, so most of the studies describe them as IBR viruses. Latent 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 (Lemaire *et al.* 2000). BoHV-1 infection 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's 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 though many

have reported IBR antibody prevalence either restricted to districts/states/zones (Choudhury *et al.* 2016, Farooq *et al.* 2019, Goswami *et al.* 2017, Kathiriya *et al.* 2018, Katoch *et al.* 2017, Kollannur *et al.* 2014, Krishnamoorthy *et al.* 2015, Saravanajayam *et al.* 2015, Patil *et al.* 2012, Patil *et al.* 2017, Trangadia *et al.* 2012, Tresamol *et al.* 2019, Verma *et al.* 2014). There are no reports on the seroprevalence of IBR in the bovine population covering the vast majority of states of the country which is reared under a smallholder production system.

In India, dairy farming is not organized and farmers do hold a small number of cattle and buffaloes. Similar husbandry practices are followed at the village level. The animals are grazed in pastures in the daytime and kept in their barns during the night. Therefore, there is a strong possibility that each animal has an equal opportunity of getting infected.

An epidemiological study was conducted to estimate the frequency of zone-, and species-specific IBR. Information, based on IBR serosurveillance about the disease burden within the defined populations is very useful to researchers and policymakers, thereby

supporting the process of identification of priorities in Veterinary healthcare, prevention, and policy. The study aimed to screen the bovine serum samples for antibodies against BoHV-1 selected randomly to understand the prevalence.

A total of 25 states (out of 28 states) and three Union Territories (out of 8 Union Territories) of the country with cattle and buffalo populations were included in the study.

Backyard dairy farming is most common in India and husbandry practices remain the same in most of the households having bovines. 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 (Seargent *et al.* 2018). Villages having a minimum of 100 bovines were selected. A total of 15,592 bovine serum samples from 1,828 villages in 369 districts from 25 states and 3 Union Territories viz., Jammu and Kashmir, Puducherry, and Andaman and Nicobar Islands were collected (Table I).

Bovine serum samples were obtained from the National Livestock Serum Repository (NLSR) maintained at ICAR-NIVEDI, Bengaluru, which were stored at - 20 °C.

Enzyme immunoassay for detection of IBR antibodies

Serum samples were subjected to an enzyme immunoassay for the detection of antibodies against IBR using developed home made Avidin-Biotin ELISA (AB ELISA). Positive and negative serum controls were selected from the repository and were subjected to a serum neutralization test using known BoHV-1. Each serum sample showing a > 1.5 neutralization index (as per WOA) was selected as a positive control and the serum showing the lowest neutralization index (< 1.5) was selected as a negative control. All the controls (positive, negative, and conjugate controls), test samples, and other reagents were used and dissolved in a blocking buffer (1% bovine gelatin 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 °C for 1 hr. Afterward, the plates were washed three 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 °C for 1 hr. Again, plates were washed as described earlier. Then, horseradish peroxidase (HRPO) conjugated Avidin (1:10,000) was added to all wells, incubated at 37 °C for 20 min and followed by the

washing of the plate as described above. Later on, 100 µl of TMB (3,3', 5,5'-tetramethylbenzidine) was added to all wells, incubated at 37 °C for 6-8 min, and kept observed for color development. In the final step, 50 µl of 1M stop solution (H₂SO₄) were added to all wells and 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 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;

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'.

MS Excel v2016 has been used for the entry, storing, and management of surveillance data and SPSS v22 has been used for statistical analysis. The chi-square test has been used to determine the significant difference in the distribution of positive results with the total sample size in a different zone. The significance was assessed at a 5% level. True prevalence (TP) has been computed to adjust for the imperfectness of the test used for screening the samples.

TP was calculated with Blaker's confidence limit using values obtained from apparent prevalence (AP) with Wilson's confidence limit at 95% confidence interval (CI) in the EpiTool: <https://epitools.ausvet.com.au/trueprevalence> (Seargent *et al.* 2018) in which sample size, sensitivity (0.92), and specificity (0.95) of the diagnostic test were considered. The significance of the difference was calculated by

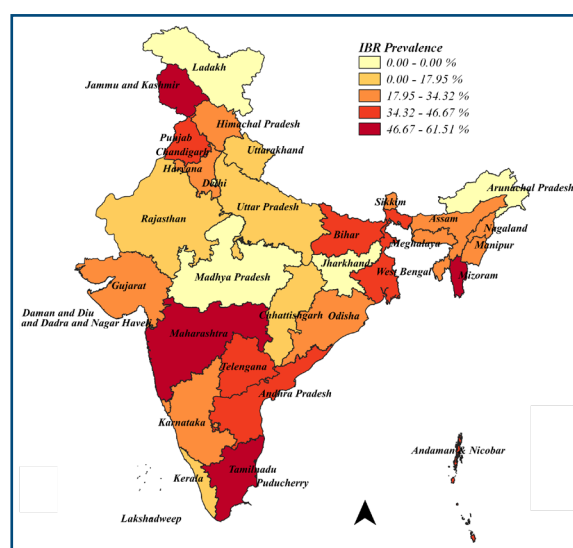


Figure 1. Percent positivity of Infectious bovine rhinotracheitis (IBR) in India.

the chi-square test and $p < 0.001$ was considered statistically significant.

A total of 15,592 bovine serum samples were collected during 2015-2019 and tested for IBR antibodies which showed a percent positivity of 31.37 [30.31 (95% CI: 29.48-31.15)] (Figure 1). Maharashtra showed the highest percent positivity. Maharashtra is a geographically large state and is also known for dairy farming, whereas Rajasthan showed the lowest percent positivity (Table I). European countries like Ireland and Italy have reported seroprevalence of

20-80% in all age groups of dairy animals and 55.49% of national herd prevalence, respectively (Brock *et al.* 2020, Maresca *et al.* 2018). Kipyego and colleagues (Kipyego *et al.* 2020) reported 17.4% animal-level seroprevalence in dairy cattle of Meru County, Kenya. Noaman and colleagues (Noaman *et al.* 2020) have reported a seroprevalence of 72.20% in crossbred dairy cattle in the Isfahan province of Iran. The overall seroprevalence study conducted on 176 serum samples obtained from cattle imported from Sudan to Egypt showed 99.75% BoHV-1 antibodies

Table I. Infectious bovine rhinotracheitis seroprevalence in Indian cattle according to states of origin between 2015-2019.

| SI No | Zone | State/Union Territory | No Districts | No Villages | No tested | No Positive | % Positivity | True Prevalence at 95% CI* |
|-------|--------------------|-----------------------|--------------|-------------|--------------|-------------|--------------|----------------------------|
| 1 | North | Himachal Pradesh | 11 | 116 | 935 | 204 | 21.82 | 19.33 (16.42-22.50) |
| | | Haryana | 23 | 74 | 715 | 176 | 24.62 | 22.55 (19.08-26.33) |
| | | Jammu and Kashmir | 23 | 78 | 519 | 289 | 55.68 | 58.26 (53.31-63.10) |
| | | Punjab | 18 | 60 | 974 | 372 | 38.19 | 38.15 (34.71-41.71) |
| | | Uttar Pradesh | 16 | 22 | 204 | 23 | 11.27 | 7.21 (3.02-13.05) |
| | | | | 13 | 50 | 290 | 50 | 17.24 |
| | Total | | 104 | 400 | 3637 | 1114 | 30.63 | 29.46 (27.76-31.20) |
| 2 | East | Andaman & Nicobar | 2 | 59 | 570 | 266 | 46.67 | 47.89 (43.23-52.61) |
| | | Bihar | 14 | 26 | 217 | 95 | 43.8 | 44.57 (37.18-52.22) |
| | | Odisha | 33 | 80 | 898 | 242 | 26.95 | 25.23 (22.01-28.67) |
| | | | 3 | 11 | 217 | 88 | 40.55 | 40.87 (33.61-48.50) |
| | Total | | 52 | 176 | 1902 | 691 | 36.33 | 36.01 (33.56-38.53) |
| 3 | North East | Assam | 27 | 77 | 657 | 158 | 24.05 | 21.90 (18.32-25.82) |
| | | Manipur | 8 | 55 | 874 | 300 | 34.32 | 33.71 (30.17-37.40) |
| | | Meghalaya | 7 | 52 | 346 | 94 | 27.17 | 25.48 (20.40-31.13) |
| | | Mizoram | 7 | 45 | 111 | 59 | 53.15 | 55.35 (44.74-65.72) |
| | | Nagaland | 11 | 122 | 530 | 171 | 32.26 | 31.34 (26.92-36.05) |
| | | Sikkim | 4 | 101 | 683 | 221 | 32.36 | 31.45 (27.54-35.58) |
| | | | 4 | 110 | 604 | 202 | 33.44 | 32.69 (28.50-37.13) |
| | Total | | 68 | 562 | 3805 | 1205 | 31.67 | 30.65 (28.98-32.37) |
| 4 | Central | Madhya Pradesh | 29 | 89 | 798 | 143 | 17.92 | 14.85 (11.97-18.08) |
| | Total | | 29 | 89 | 798 | 143 | 17.92 | 14.85 (11.97-18.08) |
| 5 | South | Andhra Pradesh | 13 | 59 | 301 | 135 | 44.85 | 45.81 (39.46-52.30) |
| | | Karnataka | 20 | 63 | 519 | 135 | 26.01 | 24.15 (20.03-28.68) |
| | | Kerala | 14 | 92 | 925 | 166 | 17.95 | 14.88 (12.19-17.87) |
| | | Puducherry | 2 | 64 | 387 | 166 | 42.89 | 43.56 (38.00-49.28) |
| | | Tamil Nadu | 2 | 58 | 279 | 159 | 56.99 | 59.76 (53.02-66.28) |
| | | | 8 | 70 | 333 | 155 | 46.55 | 47.75 (41.68-53.92) |
| | Total | | 59 | 406 | 2744 | 916 | 33.38 | 32.62 (30.62-34.68) |
| 6 | West | Goa | 2 | 14 | 251 | 56 | 22.31 | 19.90 (14.48-26.27) |
| | | Gujarat | 26 | 104 | 1650 | 410 | 24.85 | 22.81 (20.49-25.28) |
| | | Maharashtra | 25 | 55 | 530 | 326 | 61.51 | 64.95 (60.11-69.60) |
| | | | 4 | 22 | 275 | 30 | 10.91 | 6.79 (3.16-11.66) |
| | Total | | 57 | 195 | 2706 | 822 | 30.38 | 29.17 (27.21-31.19) |
| | Grand Total | | 369 | 1828 | 15592 | 4891 | 31.37 | 30.31 (29.48-31.15) |

Zone wise = $\chi^2=96.3$, $p < 0.001$, significant; *Diagnostic sensitivity = 92% and Diagnostic specificity = 95%.

(Hekal *et al.* 2019).

The following six zones of India had a varied seroprevalence of the IBR (Table I).

North zone. Six states viz., Himachal Pradesh, Haryana, Punjab, Uttar Pradesh, Uttarakhand, and Jammu and Kashmir Union Territory formed this zone. Cumulative percent positivity was found to be 30.63 [29.46% (95% CI: 27.76-31.20%)]. The samples collected from 78 villages of 23 districts of Jammu and Kashmir, which shares an international border with Pakistan, showed the highest percent positivity of 55.68 [58.26 (95% CI: 53.31-63.10%)]. Pakistan has shown an IBR seropositivity of 69% in dairy cattle of Lahore (Rehman *et al.* 2020). There are illegal movements of men and materials alongside the border though it is under strict vigilance. Uttar Pradesh (UP) showed the lowest seropositivity of 11.27% [7.21 (95% CI: 3.02-13.05)] and it shares a border with Nepal. The overall seroprevalence of IBR was 18.47% in Nepal (Tiwari *et al.* 2020). UP is a very large state and is required to test more samples for the IBR antibodies. Haryana and Punjab are prosperous states having more high-yielding cattle and buffalo and have recorded 24.62% and 38.19% positivity, respectively. Earlier reports have recorded the seroprevalence of IBR in Haryana as 48.78% (Farooq *et al.* 2020) and in Punjab as 29.78% (Gill *et al.* 1984), 42.85% (Aradhana *et al.* 2004), 34.16% (Dhand *et al.* 2002), 36.51% (Kollannur *et al.* 2014), 38.50 (Goswami *et al.* 2017), and 84.50% (Farooq *et al.* 2019).

East zone. This is comprised of West Bengal, Odisha, Bihar, and the Union Territory of Andaman and Nicobar Islands. A total of 1902 samples from 117 villages of 52 districts were tested for IBR antibodies. This zone showed 36.33% positivity [36.01 (95% CI: 33.56-38.53)]. Andaman and Nicobar Islands located 1,350 km from the Indian mainland showed the highest seropositivity of 46.67% [47.89 (95% CI: 43.23-52.61)]. These islands are located at the remotest which procure dairy animals from Tamil Nadu and West Bengal whose IBR seropositivity is also high. The overall seroprevalence of the IBR in the Andaman and Nicobar Islands was recorded as 17.68% in 2005 and 20.58% in 2014 (Sunder *et al.* 2005, Sunder, 2014). Odisha showed the lowest of 26.95% seropositivity against 12.22% seropositivity reported earlier (Das *et al.* 2014).

North East zone. Samples collected were from 7 states of this region viz., Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Sikkim. A total of 3805 samples were tested and 31.67% positivity was recorded. Mizoram showed the highest seropositivity of 53.15%. Meghalaya showed the lowest positivity of 27.17%. Rajkhowa and colleagues (Rajkhowa *et al.* 2004) found a seroprevalence of 52% and 8% in Mizoram and

Meghalaya, respectively. Sikkim showed a percent positivity of 32.35, which shares a border with China and there is a restricted movement of men and materials along the border. Overall, nationwide seroprevalence was found to be 35.8% (481/1,344) in dairy cattle in China (Yan *et al.* 2008).

Central zone. A total of 798 samples from 89 villages of 29 districts of Madhya Pradesh were tested, of which 143 were found positive (17.92%) [14.85 (95% CI: 11.97-18.08)]. A seroprevalence of 68.9% was recorded in Madhya Pradesh in 2011 (Nandi *et al.* 2011).

South zone. Samples analyzed from this zone were from 5 states (Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, Telangana) and one Union Territory of Puducherry. A total of 2,744 bovine serum samples were tested and this zone showed a seropositivity of 33.38%. Patil and colleagues (Patil *et al.* 2017) showed a seroprevalence of 56.20% in bovines from the southern region of India comprising Tamil Nadu, Andhra Pradesh, and Karnataka. In the present study, Tamil Nadu showed the highest seropositivity of 56.99% [56.99 (95% CI: 53.02-66.28)], while the lowest positivity of 17.95% [14.88 (95% CI: 12.19-17.87)] was recorded in Kerala. During 2016-2017, Kerala showed a seroprevalence of 12% (Tresamol *et al.* 2019). Earlier, seropositivity of 65.88% was recorded in Tamil Nadu (Saravanajayam *et al.* 2015). Puducherry showed 42.89% positivity.

West zone. Goa, Maharashtra, Rajasthan, and Gujarat were a part of this zone. A total of 2,706 bovine serum samples were tested and showed positivity of 30.38% [29.17 (95% CI: 27.21-31.19)]. Maharashtra showed the highest seropositivity of 61.51% [64.95 (95% CI: 60.11-69.60)] and Rajasthan showed the lowest seropositivity of 10.91% [6.79 (95% CI: 3.16-11.66)]. Geographically, Maharashtra is a very large state that has a greater number of dairy animals, including organized dairy farms. Patil and colleagues (Patil *et al.* 2017) showed a seroprevalence of 76.5% in Maharashtra, whereas a seropositivity of 0% was recorded in Rajasthan earlier (Tanwar *et al.* 2009).

The chi-square test ($\chi^2 = 96.3$; $p < 0.001$) analysis of zone-wise seroprevalence of IBR antibodies was found significant (Table I).

Seroprevalence of IBR, species-wise

A total of 11,423 cattle and 4,169 buffalo serum samples from 25 states and 3 Union Territories were tested for IBR antibodies. 33.91% [33.23 (95% CI: 32.24-34.24)] positivity was recorded in cattle samples, whereas buffalo samples showed 24.39% [22.29 (95% CI: 20.82-23.82)] seropositivity. India is the highest buffalo-populated country and more

Table II. Seroprevalence of IBR in India, species wise.

| Species | Total Tested | No positive | % Positivity | True Prevalence at 95% CI |
|---------|--------------|-------------|--------------|---------------------------|
| Cattle | 11423 | 3874 | 33.91 | 33.23 (32.24-34.24) |
| Buffalo | 4169 | 1017 | 24.39 | 22.29 (20.82-23.82) |
| Total | 15592 | 4891 | 31.37 | 30.31 (29.48-31.15) |

$\chi^2 = 129$, $p < 0.001$, significant.

samples need to be tested against IBR antibodies to obtain a more detailed picture of IBR prevalence.

Seroprevalence of IBR antibodies between cattle and buffalo was significant, as evidenced by the chi-square test ($\chi^2 = 129$, $p < 0.001$) (Table II).

Year-wise seropositivity of IBR

Bovine serum samples were collected from 2015-2019 (five years) and tested for IBR antibodies. The highest seropositivity of 42.88% [43.55 (95% CI: 41.63-45.49)] during 2019 was recorded. The lowest seropositivity was 18.04% [15 (95% CI: 13.46-16.63)] (Table III). The positivity varied between years though the total samples tested were almost the same and was not able to attribute the reason since the samples did not have sufficient data available.

Presently, the bovine population in the country is not vaccinated against IBR. The seroprevalence of IBR in the bovine population appears to be very high as the bovines must have experienced the infection and are latently infected, such animals are a continuous source of infection whenever there is a reactivation of the virus. Therefore, the authorities should plan control strategies for vaccinating dairy

Table III. Details of year wise collection of bovine serum samples.

| SI No | Year | No tested | No positive | % Positivity | True Prevalence at 95% CI |
|-------|-------|-----------|-------------|--------------|---------------------------|
| 1 | 2015 | 3003 | 542 | 18.04 | 15 (13.46-16.63) |
| 2 | 2016 | 3130 | 1002 | 32.01 | 31.03 (29.20-32.95) |
| 3 | 2017 | 3117 | 861 | 27.62 | 26 (24.23-27.84) |
| 4 | 2018 | 3003 | 1054 | 35.09 | 34.60 (32.66-36.58) |
| 5 | 2019 | 3339 | 1432 | 42.88 | 43.55 (41.63-45.49) |
| | Total | 15592 | 4891 | 31.37 | 30.31 (29.48-31.15) |

cows and buffalo, if not bulls. Bulls should be tested for IBR antibodies periodically and each semen ejaculate must be tested for virus by polymerase chain reaction.

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