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Analysis of Bovine Alphaherpesvirus-1 (BoAHV-1) Antibody Prevalence in Indian Buffalo Populations

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ABSTRACT

Background: Infectious bovine rhinotracheitis (IBR) is a highly contagious disease caused by the important pathogen Bovine Alphaherpesvirus-1 (BoAHV-1), resulting in acute inflammation of the upper respiratory tract, abortion and reduced milk yield in bovines.

Methods: Analyzing the prevalence of Bovine Alphaherpesvirus-1 antibodies in buffaloes throughout five Indian states is the aim of this study. Fifty-two districts and 177 villages provided a total of 4,253 samples, on which Avidin-biotin (AB) ELISA was performed.

Result: The state-wise variations ranged from 11.35% in Andhra Pradesh to 44.43% in Punjab, with an overall per cent positive of 20.20%. The age group belonging to 5 to 10 years had the highest percent positivity (22.56%) and other age groups were 18.56% and 20.93% in less than 5 years and more than 10 years, respectively. Sex-wise, females showed the highest percent positivity, which was found to be 20.85% compared to males being 16.28%. The Nili-Ravi breed was found to have the highest per cent positivity (57.39%) among others. This provides a comprehensive understanding of the distribution of BoAHV-1 antibodies in five states in Indian buffaloes.

Key words: Avidin-biotin (AB) ELISA, BoAHV-1, Epidemiology, IBR, Indian buffaloes, Prevalence.

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is a well-known pathogen belonging to the family *Herpesviridae*, genus *Varicellovirus* and species Bovine Alphaherpesvirus-1 (Mac Lachlan *et al.,* 2011). Bovine Alpha herpesvirus is also responsible for causing Infectious Pustular Vulvovaginitis (IPV), Infectious Balanoposthitis (IBP), abortion and conjunctivitis (Jones *et al.,* 2007). The first ever recorded illness case of BoAHV-1 in Germany during 1841 was venereal transmitted disease among bulls and cows in contact (Rychner *et al.* 1841). Bovine Alpha herpesvirus is differentiated into subtypes of 1.1, 1.2a, 1.2b and 5 (Metzler *et al.,* 1985). In 1976, India reported the first report of IBR-keratoconjunctivitis in calves (Mehrotra *et al.,* 1976). The epidemiology of BoAHV-1 is worldwide except in Austria, Denmark, Finland, Switzerland, a few European countries and parts of Italy and the Czech Republic (Iscaro *et al.,* 2021). Viral strains are associated with different clinical symptoms, type 1 is associated with IBR and abortion is commonly found in North America, South America and Europe. Subtype 2a found in Brazil is associated with IBR, IPV/IBP and abortion. Subtype 2b found in Australia and Europe is linked with IBR, IPV and IBP, excluding abortion (Righi *et al.,* 2023). Latency is a significant concern with BoAHV-1 in bovines, leading to recurring infections (Velankar *et al.,* 2020). Seroprevalence studies have demonstrated the widespread presence of BoAHV-1 and BVDV in ruminant populations (Tamer *et al.,* 2018). A systematic approach is needed to monitoring and controlling BoAHV-1 and the implementation of vaccination to mitigate the spread of BoAHV-1 may ensure the health and productivity of bovines (Patil *et al.,* 2023a). Medical history such as abortion, metritis, repeat breeding and retention of placenta was likely to be highly seropositive for BoAHV-1 (Patil *et al.,* 2017). A review suggests, that glycoprotein D is significantly immunogenic and a subunit vaccine candidate that confers protection against BoVH-1 and BoVH-5 (Rashmi *et al.,* 2024a). Molecular tools such as PCR and restriction endonuclease analysis have proven effectiove in characterizing BoAHV-1 isolates in both cattle and buffaloes (Ranganatha *et al.,* 2013). Recent molecular studies on BoAHV-1 have focused on the US6 (gD) and

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US8 (gE) glycoproteins genes, with the high genetic identity of the gE gene among subtypes indicating its potential for developing sensitive immunoassays for sero-epidemiological surveillance (Rashmi *et al.,* 2024b). Implementation of a vaccine and eradication policy in India may lead to protection against a significant pathogen (BoAHV-1) affecting livestock, leading to substantial economic losses. Moreover, understanding the prevalence of antibodies is crucial for effective management and disease control strategies in both cattle and buffalo population in India.

MATERIALS AND METHODS

Research design and sampling strategy

Using a survey toolbox, the number of random and representative villages and the number of buffaloes in each village were chosen as part of a two-stage random sample procedure (Sergeant, 2018). Serum samples were collected from buffaloes during the period 2023-2024 and were maintained and processed at the National Livestock Serum Repository (NLSR), ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru, Karnataka, India. Detection of antibodies against BoAVH-1 using indigenously developed Avidin-biotin (AB) ELISA by Patil *et al.* (2021).

Statistical analysis

Appropriate statistical analyses, such as true prevalence and chi-square tests, were calculated using Epitools (Sergeant, 2018). In order to account for the limitations of the test, the true prevalence has been calculated using Epitools. In the epitools, true prevalence (https://epitools.ausvet.com.au/trueprevalence) was computed using the Blaker confidence limit by using values derived from apparent prevalence with the Wilson confidence limit at a 95% confidence interval. The Chi-square test (https://epitools.ausvet.com.au/chisq) was utilized to identify significant differences in the distribution of positive outcomes. Risk factor analysis involves statistical models such as prevalence estimation, confidence intervals and p-values to determine the precision of estimates and the statistical significance of findings. A p-value of 0.05 or less was considered significant. (Ferrara *et al.,* 2024).

RESULTS AND DISCUSSION

A total of 4,253 samples from 52 districts and 177 villages were used in this study from five Indian states: Andhra Pradesh, Karnataka, Goa, Punjab and Rajasthan (Fig 1). Cumulative seropositivity was found to be 20.20% (859/4253). [17.47(16.11-18.89)] was found to be on the higher side and was significant ($\chi 2$ = 488.5, p =<0.0001) (Table 1).

State wise prevalence

Andhra Pradesh is part of the southern zone of India. 2070 buffaloes were tested and showed seropositivity of 11.35% [7.3 (95% CI: 5.81- 8.96%)] from 84 villages covering 20 districts. Aruna and Babu (1992) have reported 21.1% (236/1121) seropositivity in buffaloes from Andhra Pradesh. A study involving both cattle and buffalo tested for IBR showed 27.4% (423/1541) seropositivity in Andhra Pradesh (Sarumathi *et al.,* 2002). In 2012, serological investigation for IBR by ELISA in cattle and buffaloes showed 16.26% (237/1458) seropositivity, reported in Andhra Pradesh by Trangadia *et al.* (2012). In 2023, randomly collected milk-producing buffalo serum samples showed 27% (27/100)

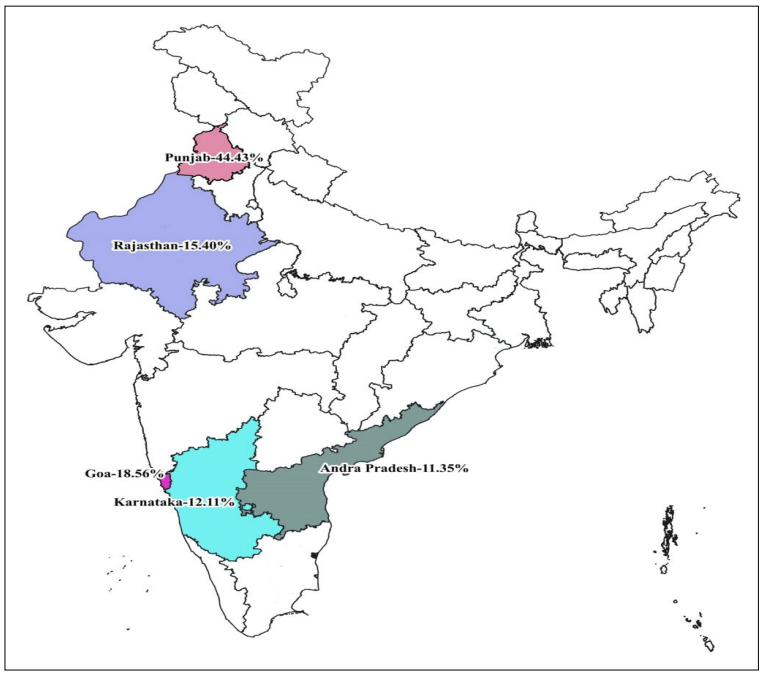

Fig 1: Map depicting the state-wise prevalence of bovine alphaherpesvirus-1in the respective states of India.

seropositivity in Andhra Pradesh (Patil *et al.* 2023b). In another study, 41.01% (89/217) seropositivity was reported in buffaloes, particularly those belonging to the Palnadu district of Andhra Pradesh (Kalavathi *et al.,* 2024).

Karnataka is also in the southern zone. Two hundred and twenty-three buffaloes were tested and showed seropositivity of 12.11% [8.17 (95% CI: 3.97-13.84%)] from 12 villages covering five districts. In 1996, buffalo samples collected from a slaughterhouse in Bangalore, Karnataka, showed 52.5% (530/1010) seropositivity in buffaloes (Renukaradhya *et al.,* 1996). In 2015, samples collected from buffaloes on dairy farms in Karnataka showed 51.22% (42/82) seropositivity (Krishnamoorthy *et al.,* 2015). In 2023, randomly collected milk-producing buffalo serum samples showed 54% (54/100) seropositivity in Andhra Pradesh (Patil *et al.,* 2023b).

Goa is also a part of the southern zone; 97 buffaloes were tested and showed seropositivity of 18.56% [15.58 (95% CI: 8.13-25.79%)] from 6 villages covering 2 districts. Not much data is available on the Goa region tested for IBR except for Patil *et al.* (2022), who reported 22.31% (56/251) seropositivity in bovine serum samples.

Table 1: Geographic distribution of total sample collection in Indian States.

State	Districts surveyed	Villages surveyed	Samples collected
Total	52	177	4253
Andhra Pradesh	20	84	2070
Karnataka	5	12	223
Goa	2	6	97
Punjab	13	40	1006
Rajasthan	12	35	857

Punjab is a part of the northern zone in India. One thousand and six buffaloes were tested and showed seropositivity of 44.43% [45.33 (95% CI: 41.83-48.87%)] from 40 villages covering 13 districts. In 2002, a study conducted in organized and unorganized dairy farms in the state of Punjab showed 17.48% seropositivity in buffaloes (Dhand *et al.,* 2002). In 2004, a study conducted in two phases showed 33.3% seropositivity in buffaloes (Aradhana *et al.*, 2004). A study involving the history of buffalo abortions from 2 dairy farms in Punjab showed 54.50% seropositivity (Dhami *et al.,* 2008). In 2023, randomly collected milk-producing buffalo serum samples showed 58% (58/100) seropositivity in Punjab (Patil *et al.* 2023b).

Rajasthan is a part of the western zone in India. 857 buffaloes were tested and showed seropositivity of 15.40% [11.96 (95% CI: 9.36-14.91%)] from 35 villages covering 12 districts. In 2023, randomly collected milk-producing buffalo serum samples showed 33% (33/100) seropositivity in Rajasthan (Patil *et al.,* 2023b).

Age wise prevalence

In the present study, buffaloes belonging to the age group of 5 to 10 years showed a higher seropositivity of 22.56% [20.19 (17.94-22.58)]. Buffaloes belonging to <5 years and >10 years showed 18.56% [15.58(13.87-17.41)] and 20.93% [18.31(12.09-25.99)] respectively (Table 2). Statistical analysis showed that the p-value is less than 0.05 and is significant (χ^2 = 9.81, p = 0.0074). In 2021, Buffaloes belonging to age groups (years) of <2.5, 2.5–4.5, 4.5-6.5 and ≥ 6.5 showed seropositivity for IBR 41.38% (12/29), 69.64% (39/56), 58.51% (55/94) and 75.61% (31/41) respectively from Haryana state (Farooq *et al.,* 2021). In a study focusing on ≤ 3-year-old cattle belonging to the districts Tutak and Doğubeyazıt, IBR seropositivity was 3.39% and

Table 2: Distribution of BoAHV-1 prevalence by State, age, sex and breed in Indian buffaloes.

Factor	n	Positive	%	95% CI	χ^2	p-value
Total	4253	859	20.2	17.47 (16.11-18.89)		
State						
Andhra Pradesh	2070	235	11.35	7.3 (5.81-8.96)	488.5	<0.0001
Karnataka	223	27	12.11	8.17 (3.97-13.84)		
Goa	97	18	18.56	15.58 (8.13-25.79)		
Punjab	1006	447	44.43	45.33 (41.83-48.87)		
Rajasthan	857	132	15.4	11.96 (9.36-14.91)		
Age						
<5 years	2441	453	18.56	15.58 (13.87-17.41)	9.81	0.0074
5 to 10 years	1640	370	22.56	20.19 (17.94-22.58)		
>10 years	172	36	20.93	18.31 (12.09-25.99)		
Sex						
Male	608	99	16.28	12.97 (9.84-16.58)	6.46	0.011
Female	3645	760	20.85	18.22 (16.74-19.77)		
Breed						
Murrah	2446	510	20.85	18.22 (16.42-20.12)	238.59	<0.0001
Nili-ravi	230	132	57.39	60.22 (52.79-67.37)		
Non-descript	1577	217	13.76	10.07 (8.22-12.13)		

1.70%, respectively, in turkey (Aktaþ *et al.,* 2021). In 2022, a study in turkey cattle revealed that the 0-3 year old group had a seropositivity of 12.38% (14/113), the 4-6 year old group had a seropositivity of 28.12% (9/32) and the 7-9 year old group had a seropositivity of 53.3% (8/15) (Alçay *et al.,* 2022). Hamdy *et al.* (2022) studied the sero-surveillance of IBR in cattle and buffalo belonging to the age groups <2, 2-4 and >4 and reported 19.81% (21/106), 21.90% (30/137) and 71.9% (41/57), respectively, in Egypt. Ortiz-gonzález *et al.* (2022) accessed dairy herds in Columbia for IBR seroprevalence and reported that age groups belonging to < 1 year had 55.9% (100/179), 1-2 years had 48.8% (102/209), 2-4 years had 42.9% (48/112) and > 4 years had 65% (325/500). Trinidad *et al.* (2024) focused their study on IBR/IPV in the regions of Peru and reported seropositivity in age groups of 2-5 years with 87.5% (70-80) and >5 years with 56.58% (43/76). Kalavathi *et al.* (2024) reported IBR seropositivity of 17.86% (15/84), 41.76% (76/182) and 56.70 (55/97), which belongs to the age groups of <3 years, 3-7 years and >7 years in cattle and buffalo. Across different studies in the world, age groups that are typically 4-5 years old show higher seropositivity. The variation may be because the older animals are exposed to BoAHV-1 many times, leading to higher seropositivity rates.

Sex wise prevalence

In the present study, female buffaloes showed a higher IBR seropositivity of 20.85% [18.22 (16.74-19.77)] and male buffaloes showed an IBR seropositivity of 16.28% [12.97 (9.84-16.58)]. The statistical analysis was found to be significant (χ^2 = 6.46, p = 0.011) (Table 2). In 2017, a study in Baghdad, Iraq, showed not much of a difference between IBR seropositivity, with males being 38.09% (8/21) and females being 41.34% (43/104). (Nezzal *et al.,* 2017). Kalavathi *et al.* (2024) reported the seropositivity of males at 14.29% (12/84) and females at 48.03% (134/279) in buffaloes and cattle. In 2016, reports of male buffaloes showed seropositivity of 11.11% (1/9) and female buffaloes showed seropositivity of 6% (2/33) in Chhattisgarh (Samrath *et al.,* 2016). In 2015, samples collected from cattle and buffaloes on dairy farms in Karnataka showed seropositivity of males being 57.89% (11/19) and females being 61.67% (333/540) (Krishnamoorthy *et al.,* 2015). In 2017, selected farms of Uttarakhand buffaloes showed seropositivity, with males being 25% (2/8) and females being 39.32% (35/89) (Thakur *et al.,* 2017). Saravanajayam *et al.* (2015) reported seropositivity of males at 33.33% (5/15) and seropositivity of females at 67.92% (163/240). Lotfi *et al.* 2016 reported the seropositivity of females at 5.5% (28/513). Across different studies in the world, females have higher seropositivity compared to male buffaloes. This is because females have been exposed more to artificial insemination and gynecological examinations. Other than that, physiological and environmental stress, such as during pregnancy and lactation, Stress can compromise the immune system, making them more susceptible to infection.

Breed wise prevalence

In the present study, Nili-ravi breed buffaloes showed higher seropositivity of 57.39% [60.22(52.79-67.37)], Murrah breed buffaloes showed seropositivity of 20.85% [18.22(16.42-20.12)] and non-descript buffaloes showed 13.76% [10.07(8.22-12.13)] seropositivity, the statistical analysis found to be significant (χ^2= 238.59, p=<0.0001) (Table 2). Romero-Salas *et al.* (2018) reported 58.93% (122/207) seropositivity in Murrah, which belongs to Veracruz, Mexico. In 2015, a study showed 54.34% (18/34) and 51.85% (14/27) seropositivity in Murrah breed and non-descript, respectively (Saravanajayam *et al.,* 2015). In 2015, a study on an organized dairy farm reported 69.18% (92/133) seropositivity in Murrah (Krishnamoorthy *et al.* 2015). Patil *et al.* (2023b) reported 44.08% (320/726), 37.50 (3/8) and 41.24% (73/177) seropositivity in Murrah, Nili-ravi and non-descript, respectively. Saravanajayam *et al.* (2017) reported seropositivity of 22.22% and 17.28% in Murrah and non-descript, respectively. Selvaraj *et al.* (2008) reported 8.56% (16/187) seropositivity belongs to 179 nondescript and 8 Murrah-graded buffaloes. Batool *et al.* (2022) reported a seropositivity of 51.5% (103/200) in the Nili-Ravi bred, which belongs to Pakistan.

CONCLUSION

Globally, India is No. 1 in the population of buffaloes and most of them are used in the dairy industry. Although health concerns arise constantly because of losses in productivity due to causative agents such as BoAHV-1, Previous reports are limited in the sense of tested sample numbers, yet they prove findings of BoAHV-1 prevalence in buffaloes. For deeper insights on the prevalence of BoAHV-1, a much bigger study with a similar study plan might reveal its endemicity. Apart from regular monitoring and surveillance, the government of India should implement a control program and an eradication program through vaccination for all bovines.

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Disclaimers

The authors' views are their own and do not reflect their institutions. They ensure accuracy but are not liable for any losses from this content.

Informed consent

All procedures related to serum collection adhere to ethical guidelines and have been reviewed and approved by the appropriate regulatory committees. The collection process follows established protocols to ensure animal welfare and scientific integrity.

Conflict of Interest

No conflicts of interest or external funding influenced this study or its publication.

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