ISSN: 2456-2912
VET 2024; SP-9(1): 476-479

www.veterinarypaper.com
Received: 20-10-2023
Accepted: 22-12-2023

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Sero-prevalence of infectious bovine rhinotracheitis in bovines of Palnadu district, Andhra Pradesh

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Abstract
The study was taken up to note the prevalence of antibodies in the sera of bovines against Infectious Bovine Rhinotracheitis (IBR). The survey for the study of seroprevalence was carried out to detect the extent of IBR infection prevailed in the area. Infectious bovine rhinotracheitis infection, clinically exhibits rhinotracheitis, conjunctivitis, pustular vulvovaginitis and balanoposthitis, abortion, infertility, and encephalitis. Three hundred and sixty three bovine serum samples were procured from the suspected clinical cases of IBR in different mandals of Palnadu district of Andhra Pradesh. The sera were processed for the detection of antibodies against IBR using indirect enzyme linked immunosorbent assay (Indirect ELISA). The results showed that IBR antibodies were profoundly prevalent in many mandals of the Palnadu district with an overall prevalence rate of 40.22%. Of the 363 serum samples tested, 146 samples were tested positive for the Infectious bovine rhinotracheitis in bovines. The prevalence in cattle was 39.04% and buffaloes was 41.01% which was statistically not significant. On sex wise basis, it was found that females (48.03%) had higher level of antibodies than males (14.29%). Age-wise seroprevalence differed significantly with higher seroprevalence (56.70%) among adults (over 7 years of age) with least seroprevalence in below three years of age group (17.86%) indicating animals of older age were more susceptible. The present paper indicated the prevalence and current status of IBR infection in Palnadu district of Andhra Pradesh.

Keywords: ELISA, IBR, seroprevalence, seropositivity

Introduction
Infectious Bovine Rhinotracheitis (also known as IBR, BoHV-1, or “Red nose”) is a viral disease predominantly affecting the upper air ways and trachea. The etiological agent is, Bovine Herpes Virus -1, DNA virus of *Herpesviridae* family. Its infection is seen both in domestic and wild cattle. In cattle, the disease is characterised in many forms like inflammation of the upper respiratory tract, mucopurulent nasal discharges, hyperaemia of the muzzle (red nose disease), conjunctivitis, infectious pustular vulvovaginitis affecting the reproductive tract and causing endemic abortions, infectious balanoposthitis (external genitalia of male). The neonatal septicaemic form of disease is characterized by encephalitis (Simon *et al.* 2018) [14]. Transmission of the disease to healthy animals is facilitated by the inhalation of nasal exudates, respiratory droplets, genital secretions, semen, foetal fluids and tissues. They aid for the spread of the disease. (Nandi *et al.* 2009) [8]. Infectious bovine rhinotracheitis infection though has a low mortality and morbidity but poses a drastic decline in means of its productivity and milk production.
BoHV-1 has a large host range, short multiplication cycle to produce its virions and the ability of inducing latent infection. IBR causes significant losses in the dairy industry due to its disease and trading restrictions. (Chandranaik *et al.* 2016) [1]. BoHV-1 infections can be diagnosed by detection of virus antigen or virus protein components and antibody by serological tests or by detection of genomic DNA using Polymerase chain reaction (PCR), nucleic acid hybridization and sequencing. The screening, surveillance and monitoring are important criteria that should be done regularly for proper sustaining of the herd health status and to minimize the financial losses caused by IBR disease (Raizman *et al.*, 2011) [11].

Materials and Methods

In the present study, a total of 363sera samples were procured from 146 cattle and 217 buffaloes. Among the 146 cattle, 65 were from males including bulls, bull calves and remaining 81 samples were collected from females. Among the 217 buffaloes, 19 were from males including bulls, bull calves and remaining198 were females. The collected serum samples had an history of any one or two clinical symptoms of the IBR disease and were collected from Palnadu district of Andhra Pradesh during the period from April 2020 to March 2023 (Table 1). Blood for serological analysis was collected aseptically in vacutainers and made stand still to clot for the production of serum. The collected sera were aliquoted in 2ml containers and stored at -20 °C until further use.

ELISA procedure

The detection of antibody levels to Infectious bovine rhinotracheitis infection in the test serum samples was estimated by indirect ELISA test kit (IDVET IBR total Ab kit, France). In brief, microwells coated with Infectious bovine rhinotracheitis virus specific antigen were added with 50 µL test sera and 50 µL dilution buffer. Positive and negative controls of 50 µLwere also placed that were provided in the kit. The ELISA plate was covered and incubated at 21 °C (± 5 °C) for overnight (16 -20 hrs). The wells were emptied and the microwells were washed thrice with 300 µL wash solution. Then100 µL of conjugate was added and incubated at 21 °C (± 5 °C) for 30 min. The wells were emptied and washed thrice with 300 µl of wash solution. Substrate solution of 100 µL was added to each well and incubated for 15 min ± 2min at 21 °C (± 5 °C) in dark. Finally, the reaction was stopped by adding 100 µL of stopping solution to each well and the results were read with optical density at a wavelength 450nm in an ELISA reader (Thermo, 51119000, Germany).
Interpretation for each sample was done by calculating the S/N percentage (S/N%)

$$S/N\ \% = \frac{\text{OD of the sample}}{\text{OD of the Negative control}} X100$$

- Less than or equal to 60% are considered positive
- Greater than 60% are considered negative

Statistical analysis

The various parameters of seroprevalence of bovines (cattle and buffaloes) statistically was carried out by Chi-square (χ^2) test using the software WEB AGRI STAT PACKAGE, ICAR research complex, Goa.

Results

Out of 363serum samples tested for Infectious bovine rhinotracheitis antibodies by the Indirect ELISA, the test revealed 40.22 % (146/363) for the IBR infection in bovines and produced a S/N percentage of less than or equal to 60%. The highest number of positives were noted in, Macherla (100%), Piduguralla (100%), Nuzendla (100%) followed by Amaravathi (77.5%), Nadendla (71.43%), Savalyapuram (57.14%), Chilakaluripeta (50%), Machavaram (50%), Rajupalem (50%), Narasaraopet (40.54%), Muppalla (36.36%) and Rompicherla (33.33%). (Table 1).
The prevalence of BHV-1 antibodies in white cattle and black cattle was noted to be 39.04% (57/146) and 41.01% (89/217), respectively which was statistically not significant ($p<0.05$). In this study, the female cattle showed significantly ($p<0.05$) higher prevalence (48.03%) as compared to males (14.29%). The seroprevalence of BoHV-1 based on age differed significantly ($p<0.05$) with the highest prevalence (56.7%, 55/97) in older animals (over 7 years of age) followed by young animals (41.8%, 76/182) among animals between 3 to 7 years of age and lowest (17.9%, 15/84) in animals below 3 years of age. (Table 1).

Table 1: Seroprevalence of IBR considering different parameters

Species	No. of samples tested	No. of samples tested positive	Percentage of Positivity
Species			
White Cattle	146	57	39.04
Black cattle	217	89	41.01
Total	363	146	40.22
$\chi 2 = 0.141$ (Not Significant)			
Sex-wise			
Males	84	12	14.29
Females	279	134	48.03
Total	363	146	40.22
$\chi 2 = 30.573$ (Significant at 5% Level of significance)			
Age wise			
Below 3 yrs	84	15	17.86
3 to 7 yrs	182	76	41.76
Above 7 yrs	97	55	56.70
Total	363	146	40.22
$\chi 2 = 28.609$ (Significant at 5% Level of significance)			

Discussion

India has a huge livestock population with a wide variety of cattle and buffalo breeds supporting the socio- economics of the people living in India. Of all diseases, important silent diseases of Cross bred bovines is the Infectious bovine rhinotracheitis, a highly severe disease of bovines occurs throughout the world, including India. The first report of the disease was in Uttar Pradesh (Mehrotra *et al*., 1976) [7] and thus prevalent in most parts of the state like Kerala, Karnataka, Bihar, Tamil Nadu, Nagaland, Gujarat and Odissa (Nandi *et al*., 2011; Kollannur *et al*., 2014) [9, 5].
Serological diagnostic methods play a major role in detecting the occurrence of antibodies against the IBR infection. Indirect ELISA is a simple technique to perform and does not need well skilled persons. Since the infection manifests a variety of symptoms, it is challenging to distinguish it from an IBR infection in clinical conditions. Hence, indirect ELISA is the best approach because of its high specificity less cumbersome (Gokce *et al*., 2007) [2].
IBR seropositivity have been reported by several investigators from different parts of India. Patel *et al*. (2023) [10] reported 29.73% positivity in South Gujarat. Similarly, Thakur *et al*., (2017) [15] reported 29.03% prevalence in Uttarakhand, while Verma *et al*. (2014) [17] reported 32.84% seropositivity in Uttar Pradesh, Goswami *et al*., (2017) [3] recorded 38.5% in Punjab while Kathiriya *et al*., (2018) [4] observed 35.19% sero prevalence in Saurashtra of Gujarat. Higher rates of prevalence was noticed by Renukaradhya *et al*., (1996) [12], in her survey three states of the southern region had an overall prevalence of 50.9%, and 52.5%, respectively. The infected semen was responsible for the rapid spread of the infection of the BoHV-1 in southern states. Trangadia *et al*. (2010) [16] studied the seroprevalence of IBR infections in organised dairy farms of India and the overall prevalence rate of antibodies was found to be 60.84%. Krishnamoorthy *et al*. (2015) [6] made an effort for the sero-surveillance of IBR in 11 farms situated in 4 different regions of India. The results posed an overall prevalence of 61.6%ranging from 36.5% in

Central region to 84.5% in Northern region. Saravanajayam *et al*. (2015) [13] conducted a study in unvaccinated cattle present in Northern part of Tamil Nadu which revealed 65.88% seroprevalence.

The results obtained for species wise prevalence of IBR were not statistically significant. An approximately comparable percentage of seroprevalence in cattle and buffaloes was reported by Goswami *et al*. (2017) [3] as 41.46% and 36.19% respectively, in Punjab. Renukaradhya *et al.* (1996) [12] reported seropositivity as 50.9% and 52.5% in cattle and buffaloes respectively. Kathiriya *et al.* (2018) [4] showed 36.31% in cows and 33.99% in buffaloes were seropositive for IBR in Saurashtra region of Gujarat. Higher prevalence in cattle (38.16%) was reported by Patel *et al.* (2023) [10] than buffaloes (15.78%) in Gujarat. In Uttar Pradesh, Verma *et al*. (2014) [17] reported higher positivity in cattle (48.84%) than that of buffaloes (35.28%). Thakur *et al.* (2017) [15] reported more prevalence in buffaloes (38.14%) than in cattle (26.78%).

In this study, the female cattle showed a significantly higher prevalence as compared to males. Our findings were in accordance with Thakur *et al*. (2017) [15] who found that higher number of females (30.08%) were harbouring antibodies to IBR virus than the males (16.21%). The study conducted by Saravanajayam *et al*., (2015) [13] also showed higher prevalence in females (67.92%) than males (33.33%). Patel *et al.* (2023) [10] showed that the females had an unsignificant higher prevalence (30.04%) in comparison to their counterparts. On the contrary, Verma *et al*. (2014) [17] identified higher positivity in males (48%) than females (29.35%).

The percentage positivity of IBR infection increased with the age of the animal which was in accordance with the studies of Krishnamoorthy *et al.* (2015) [6], Verma *et al.* (2014) [17], Goswami *et al.* (2017) [3], Kathiriya *et al.* (2018) [4], Saravanajayam *et al.* (2015) [13] and Patel *et al.* (2023) [10]. There was a positive corelationship between the age and IBR infection which might be due to reduced immunity levels as the age advances. Patel *et al.* (2023) [10] opined that higher prevalence in cattle above 7 years of age might be due to having more chances of exposure to infection, lower immunity, work taken from them and nutritional status of animals.

Conclusion

The study significantly stated that the bovine population in the area studied has been exposed to BoHV-1 infection. Development and implementation of relevant control measures should be taken up at state and national level in regarding to management practices in purchase of animals which affects the disease prevalence in the respected area.

Conflict of Interest: Authors have no conflict of interest in this study.

Author's Contributions: SK and KKSL were involved in the design of the research. Collection of samples and processing was carried out by SK and KKSL. The laboratory tests and interpretation of the tests were carried out by BVD and NTB. SK, KKSL and GDK drafted and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors are thankful to the Director, Animal Husbandry Department, Andhra Pradesh, Joint Director (AH), VBRI, Vijayawada and District Animal Husbandry Officer, Palnadu District for providing necessary facilities and infrastructure tocarry out the work.

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