Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 5 Issue 2 February 2023

Research Article

Sero-Epidemiological Study of Infectious Bovine Rhinotracheitis (IBR) in Bovine of South Gujarat

Dharmeshkumar Rameshbhai Patel[1]*, IH Kalyani[2] and PM Makwana[1]

[1]Assistant Professor Department of Veterinary Microbiology, Vanbandhu College of Veterinary Science and Animal Husbandry, NAU campus, Kamdhenu University (KU), Navsari, Gujarat State, India

[2]Professor and Head, Department of Veterinary Microbiology, Vanbandhu College of Veterinary Science and Animal Husbandry, NAU campus, Kamdhenu University (KU), Navsari, Gujarat State

***Corresponding Author:** Dharmeshkumar Rameshbhai Patel, Assistant Professor Department of Veterinary Microbiology, Vanbandhu College of Veterinary Science and Animal Husbandry, NAU campus, Kamdhenu University (KU), Navsari, Gujarat State, India.

DOI: 10.31080/ASVS.2022.05.0603

Received: December 19, 2022

Published: January 27, 2023

Abstract

A study conducted at Vanbandhu College of Veterinary Science, Kamdhenu University, Navsari, Gujarat State, India on the sero-epidemiology of infectious bovine rhinotracheitis (IBR) in 1396 bovine (870 cattle and 526 buffaloes) of Southern Gujarat revealed 29.73% overall BoHV-1 antibody in 415 animals. The seroprevalence among cattle (38.16%) was significantly ($P \leq 0.05$) higher in comparison to buffaloes (15.78%). Significant variation in prevalence rate was noted across the districts with Navsari (35.70%) top on the list followed by Valsad, Tapi and Surat (23.45%). Females of these species showed a non-significant higher seroprevalence (30.04%) rate in comparison to their male counterparts. Age-wise seroprevalence differed highly significantly. The higher seroprevalence (58.09%) was recorded among very adult animals (over 7 years of age) with least seroprevalence in below one year of age group (10.75%) indicating animals of increasing age to be more susceptible.

While in ELISA, out of 870 cattle serum samples, 332 (38.16%) samples were found to be positive for IBR antibodies with non-significantly maximum seroprevalence recorded in Valsad district (42.45%) followed by Tapi, Navsari and least in Surat (35.71%). The rate of seroprevalence was highly significant and recorded highest in pure exotic breed, HF (44.45%) followed by HF cross (40.47%), Jersey (40%), Jersey cross (33.33%), nondescript (28%) and the least in pure indigenous breed, Gir (18.75%). Seroprevalence was seen to be non-significantly higher in females (38.28%) than male (34.48%). Cattle above seven years of age showed 59.40% prevalence followed by other younger age groups with least in below one year of age (23.47%).

In buffaloes, out of 526 sera screened, 15.78% cases were found to be positive for IBR antibodies. It was noted significantly to be highest in Valsad (27.45%) followed by Navsari, Surat and Tapi districts. Among different breeds, Surti showed 50.00% seropositivity followed by Mehsani (13.19%) and non-descript breed (9.09%) with least recorded in Jafarabadi (8.33%). Females showed non-significantly higher (15.90%) prevalence than males (13.79%). Like cattle, animals of increasing age (below one to more than 7 years age) appeared more susceptible with highest in the oldest group (34.26%) and the least (8.45%) recorded in the youngest group.

In cattle, the prevalence of IBR was statistically higher in clinically ailing (30.40%, 173/569) than apparently healthy (14.29%, 43/30) animals. Among clinically ailing cattle, history of genital tract infection on the top (48.61%, 70/144) followed by respiratory disorder (30.66%), eye infection (25.26%), pyrexia and/anorexia (22.35%), repeat breeder (17.50%) and abortion (14.29%,4/28) were noticed in different combinations. Among clinically ailed cattle, the highest in older group having 37% seroprevalence and the lowest (4.76%) in the youngest age group (1-4 years). Female showed non-significantly higher (31.07%) than males (16%).

Keywords: IBR; BoHV-1; Bovine; Indirect ELISA; Seroprevalence; Significance

Introduction

Bovine herpesvirus-1 (BHV-1) is the causative agent of infectious bovine rhinotracheitis (IBR), belonging to the genus *Varicellovirus* (subfamily *Alphaherpesvirinae*) of the *Herpesviridae* family, which impacts on the dairy sector by affecting both milk production and health of animals. The virus is responsible for a variety of clinical conditions in cattle and buffaloes, including rhinotracheitis, pustular vulvovaginitis, abortion, mastitis, balanoposthitis, infertility, tracheitis, conjunctivitis-keratoconjunctivitis, encephalitis and fatal disease in newborn calves, and thus causes great economic losses to the livestock industry [4,14]. It is considered to be one of the most common pathogens present in the semen of breeding bulls [3]. BoHV-1 transmission through semen may cause serious reproductive complications in inseminated cows, such as abortion, infertility, endometritis and embryonic absorption [40].

IBR was originally recognized as a respiratory disease of feeder cattle in the western United States during the early 1950s. The first published report on respiratory IBR came from Schroeder and Moys in 1954. The first case of IBR in Europe was found in 1960. BoHV-1 infection was first reported in India by Mehrotra., *et al*. [32] and various workers have since reported the widespread prevalence of the disease in various parts of the country [21,26,45,46]. The infection has serious economic implications for India, which is an emerging as the world's biggest milk producer and has the world's largest cattle and buffalo population.

Mucous membrane of either upper respiratory or genital tract is the most common route of entry of BoHV-1. Conjunctival route transmission has also been reported. Direct nose to nose contact is the preferential way of transmission of BoHV-1. However, airborne transmissions by the aerosol route were demonstrated on short distances [27]. Genital infection requires direct contact at mating. Genital transmission also occurs through virus contaminated semen [30]. After genital infection and seroconversion, BoHV-1 localizes and persists, latently, in sacral ganglia [1]. The virus is excreted through secretions (nasal and ocular) and is present in the placenta of aborted animals and semen. BoHV-1 can spread through artificial insemination (AI), causing a variety of genital tract disorders such as endometritis, infertility and abortion.

Due to the latent nature of most of BoHV-1 infections and intermittent shedding of the virus in secretion and excretion, the situation is complex since the various farm operations is distributed for the dairy animals, which further results into dissemination of the viral agent to the virus-free herds. Such a situation causes huge economic losses to the dairy industry. Keeping above points in view, the present study was designed to detect the presence of BoHV-1 antibody in sera of apparently healthy and or clinically ailing bovine (cattle and buffaloes) of Southern Gujarat by an indirect Enzyme-Linked Immunosorbent Assay (ELISA) test by using commercially available kit.

Materials and Methods

Location of study area

The study was carried out at Vanbandhu College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Gujarat State. It is located south region of Gujarat. An Average annual rainfall is 1000 to 1700 mm. The mean temperature varies from 23 to 270C with a high humidity of 65 to 90%. The state Gujarat is dense populated plain tract (more than 750 persons/sq km), situated on the western coast of the Indian Peninsula. The state is bound by the Arabian Sea on the west, Pakistan and Rajasthan in the north and northeast, Madhya Pradesh in the Southeast and Maharashtra in the south

Source of animals and collection of serum

Materials comprising serum and other clinical samples (vaginal, nasal and conjunctival swabs as well as placental cotyledons) were collected randomly from cattle and buffaloes of different age groups, breeds and sex living in different districts (Valsad, Navsari, Tapi and Surat) of South Gujarat. The samples were mainly collected from Livestock Research Station and clinic of our college, Panjarapol Navsari/Valsad, clinical camps organized by Department of Animal Husbandry, Gujarat State at different villages of aforesaid four districts. At the time of sample collection, anamnesis details (like species, breeds, age, sex etc) and clinical signs (genital tract infection, respiratory disorder, eye infection, pyrexia or anorexia, repeat breeder, abortion etc were recorded. None of the animals has been vaccinated against BoHV-1. All the animals under study were reared under different managemental practices. About 9ml of blood was collected aseptically from the jugular vein of each animal in a vacuette with serum clot activator (Greiner bio-one, Austria). The vacuettes were kept in upright position at room temperature for about two hours. The separated serum was collected in a screw capped plastic vials and transported to the laboratory. The serum

samples were heat inactivated at 56ºC for 30 min and merthiolate (1:10,000) was added in all vials as a preservative. The sera were stored at -20ºC/-800C temperature till further use. All the serum samples were collected from 1396 animals (870 cattle and 526 buffaloes) under the present study were subjected to indirect ELISA. Samples were tested in due period of time after collection.

Serological test: Indirect ELISA

The serum samples from bovine cattle and buffaloes were subjected to indirect ELISA kit obtained from ID.vet, rue Louis Pastuer-Grabels, France and supplied by Artec Diagnostic Systems, Navi-Mumbai , Maharashtra to determine the presence serum antibodies to BHV 1 (Figure 1). The test was performed as per the protocol outlined in the user manual supplied with the kit. We complied and followed the test protocol of short incubation period. Add 90 ul of Dilution Buffer 2 to each microwell then add 10 ul of the Negative Control to wells A1 toB1. Add 10 ul of Positive Control to wells C1 and D1 then add 10 ul of each sample to be tested in the remaining wells and Incubate 45 minutes + 4 minutes at 370C (+ 30C). Empty the wells and wash each well three times with approximately 300 ul of the Wash Solution (1X). Avoid drying of the wells between washings. Prepare the Conjugate 1X by diluting the Concentrated conjugate 10X to 1/10 (short incubation) and 1/15 (overnight incubation) in Dilution Buffer 3. Add 100 ul of the Conjugate 1X to each well and Incubate 30 minutes + 3 minutes at 370C (+ 30C). This washing cycle was repeated atleast 3 times as per earlier steps. Add 100 ul of the Substrate Solution to each well and Incubate 15 minutes + 2 minutes at 210C (+ 50C) in the dark then add 100 ul of the Stop Solution to each well in order to stop the reaction. Finally, the plate was read at 492 nm and optical density (OD) value (absorbance) was recorded with the help of ELISA reader with installed programme. The per cent positivity (PP) value was calculated using the following formula as per the instruction manufacturer. A sample was considered positive when the P/P% was ≥ 60%, negative when the ratio was < 50% and the sample was retested when between 50% and 60%.

$$P/P\% = \frac{OD_{sample} - OD_{negative\ control}}{OD_{positive\ control} - OD_{negative\ control}} \times 100$$

The test is validated if the mean value of the Positive Control O.D. (OD_{PC}) is greater than 0.350 and the ratio of the mean O.D. values of the Positive and Negative Control is greater than 3.

Figure 1: An Indirect ELISA kit for detection of IBR antibodies

Statistical analysis

The statistical analysis of data generated on various parameters of seroprevalence of bovine (cattle and buffaloes) was carried out by Standard Statistical procedures [48]. Chi-square (X^2) test was used according to WEB AGRI STAT PACKAGE software developed by Ashok Kumar, ICAR research complex, Goa and Statistical Packages for social Science (SPSS) software (version 17).

Results and Discussion

A total of 1396 serum samples (cattle - 870, buffalo -526) from bovine were screened by IBR indirect ELISA. Of these, 415 serum samples were found positive for IBR antibodies with an overall seroprevalence of 29.73%. Thus, it shows the prevalence of IBR antibodies in bovine of Southern Gujarat. The details on species-wise, district-wise, sex-wise and age-wise seroprevalence are presented in table 1.

In this study, the overall prevalence of IBR was found to be 29.73% (415/1396) in bovine as a whole. However, in cattle and buffaloes, seropositivity was noted to be 38.16% (332/872) and 15.78% (83/526), respectively by Indirect-ELISA (Figure 2). The species-wise difference was statistically highly significant ($P < 0.01$) and sex-wise non-significant ($P < 0.05$). Deka., *et al.* [10] revealed 45.01% prevalence of BoHV-1 antibodies in cattle bulls of Punjab by A-B ELISA while Khan [25] reported 21.30% seropositivity of IBR by using i-ELISA in cattle and buffaloes of Gujarat.

Attributes	No. of Tested	No. of Positive	Percent Positive
Region			
South Gujarat	1396	415	29.73
Districts			
Valsad	263	76	28.90
Navsari	493	176	35.70
Tapi	333	91	27.32
Surat	307	72	23.45
Total	1396	415	29.73
$\chi^2 = 15.21^{**}(P \leq 0.01)$			
Species			
Cattle	870	332	38.16
Buffalo	526	83	15.78
Total	1396	415	29.73
$\chi^2 = 78.61^{**}(P \leq 0.01)$			
Sex-wise			
Male	58	13	22.41
Female	1338	402	30.04
Total	1396	415	29.73
$\chi^2 = 1.55^{NS}(P > 0.05)$			
Age-wise			
<1 year	186	20	10.75
1-4 years	436	53	12.16
5-7 years	533	202	37.90
>7 years	241	140	58.09
Total	1396	415	29.73
$\chi^2 = 206.34^{**}(P \leq 0.01)$			

Table 1: Overall seroprevalence of IBR in bovine using Indirect ELISA.

Note: NS - Nonsignificant at P > 0.05; ** - Highly significant at P ≤ 0.01.

More or less similar results were obtained in different states of India by different researchers [9,11] with a seropositivity of 38.01% and 32.26%, respectively.

The higher seropositivity ranging from 40 to 80% were recorded using ELISA by different workers [28,34,38]. The higher seropositivity of the animals against IBR is probably due to the lack of control measures towards infection. While Jai., *et al.* [19] Jain., *et al.* [21] and Rajesh., *et al.* [44] observed a lower seropositivity of 14.88%, 17.68% and 10.39%, respectively, employing ELISA.

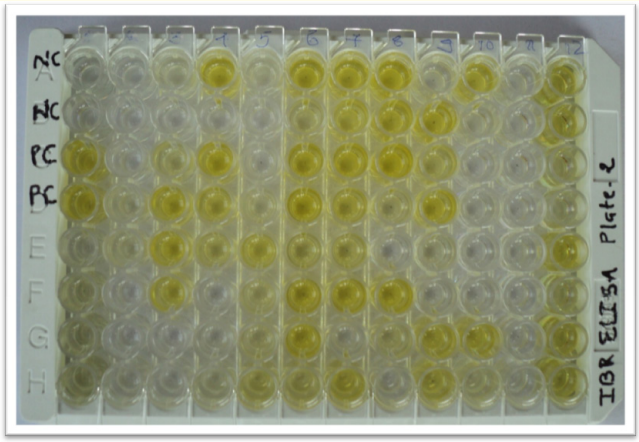

Figure 2: An ELISA module showing positive and negative reactions for IBR antibodies by Indirect ELISA.
Well A1 and B1 : Negative control serum
Well C1 and D1 : Positive control serum
Rest all wells : Field serum samples

In the seroprevalence study applying serological tests other than ELISA, supporting results were obtained by different workers. Aruna and Suri Babu [6], Ionescu [18] and Kargar Moakhar., *et al.* [24] found the overall seroprevalence of 33.97%, 31.9% and 29.11%, respectively.

A higher seroprevalence of 73%, 64.72% and 52.31% were observed, respectively, by Mehrotra and Rajya [31], Pandey., *et al.* [37] and Suri Babu., *et al.* [49] by tests other than ELISA. While a lower seroprevalence of 14.77% was reported by Mohan., *et al.* [33].

In present study, out of 870 sera from cattle 332 were found positive. Similarly 83 sera of buffaloes were found positive out of 526. Thus the higher seroprevalence (38.16%) was found in cattle than (15.78%) in buffaloes. Jain [20] yielded overall seroprevalence of 29.21% with the higher seroprevalence in cattle (34.21%) than in buffalo bulls (25.94%). Similarly Khan [25] in Gujarat reported species-wise higher seroprevalence of 23.40% in cattle in comparison to 18.97% in buffaloes by i-ELISA. The higher seroprevalence in cattle than in buffaloes were reported by different

workers in India. In Haryana, Pandita and Srivastava [39] reported seropositivity of 50.16% and 38.78% in cattle and buffaloes, respectively. Ganguly and Mukhopadhayay [13] have reported 48.15% BoHV-1 seroprevalence in breeding animals of West Bengal state. While in Punjab, Dhand., *et al.* [11] found 34.16% and 17.8% seropositivity in cattle and buffaloes, respectively.

More or less equal percentage of seroprevalence both in cattle and buffaloes were also reported in India. Suri Babu., *et al.* [49] observed seroprevalence of 65.78% and 62.30% in cattle and buffaloes, respectively from Andhra Pradesh. While Renukaradhya., *et al.* [45] also reported 50.9% and 52.5% seropositivity in cattle and buffaloes, respectively. Chinchkar., *et al.* [9] found 32.26% and 31.00% seroprevalence in cattle and buffaloes, respectively.

On a global basis our results of 29.72% overall seroprevalence was comparable with the seroprevalence 33.97% [24] in Iran in Asian continent; 25.9% [15] in Tunisia of African continent; 37.8% [12] in Canada, 37% [16] in Uruguay of American continent; 35.9% [7] in Belgium, 34.99% [8] in Italy, 38% [2] in Poland of European continent.

District-wise difference was highly significantly ($P \leq 0.01$) marked. Higher prevalence of IBR was found in Navsari (35.70%) followed by Valsad (28.90%), Tapi (27.32%) and Surat (23.45%). Jain., *et al*. [22] recorded 75% in Anand and 21.43% sero-prevalence in Surat districts. The disease occurs to be more prevalent in areas of intensive animal husbandry practices such as organized farms.

Similarly, Dhand., *et al.* [11] reported seroprevalence of 28.76 per cent in cattle and buffaloes in Punjab state. However, contrary to the present findings, Khan [25] reported the slightly lower rate of seroprevalence of 21.30 per cent in the cattle and buffalo population of Gujarat state. Rajesh., *et al.* [44] reported very low rate of seroprevalence of 14.88 percent in cattle from Kerala state. While a higher rate of seroprevalence 49.97 percent was reported by Pandita and Srivastava [39]. The variation in this may be due to the sample size, location of the samples collected, inclusion of samples from the sexes, seasons, etc. Nandi., *et al*. [35] conducted seroprevalence in other states of India like Bihar, West Bengal, Gujarat, Assam, Madhya Pradesh etc. The per cent positivity was higher in cattle than that of buffaloes. The possible reason for higher seropositivity in cattle might be due to the inclusion of crossbred animals and the involvement of artificial insemination in cattle breeding. During the present investigation, the rate of seroprevalence recorded in buffaloes was 15.78 per cent, which corroborates the finding of Aruna and Suri Babu [6] who reported 21.05 per cent seroprevalence of IBRV antibodies in buffaloes of Andhra Pradesh. However, Manickam and Mohan [29] failed to detect IBRV antibodies in buffaloes in Tamil Nadu.

Age-wise seroprevalence differed highly significantly ($P \leq 0.01$). The highest seroprevalence (58.09%, 140/241) was recorded among the adult animals (over seven years of age) followed by younger groups (37.90%, 202/533 between 5 to 7 years; 12.16%, 53/436 between 1 to 4 years) having lowest in age group of below one year (10.75%, 20/186). The possible reason may be increased susceptibility of animals with age or repeated subclinical infection with virus that boost to keep the antibody titre higher enough to be detected positive or decrease in immunity and increase in stress, which may lead to reactivation of latent virus [47].

OIE [36] advocates removal of animal only if positive by both antigen detection and antibody detection tests. Considering the nature of herpes virus pathogenesis (latency and reactivation) and the fact that there is no BoHV-1 vaccination practiced in India, the present study recommends removing seropositive animals from breeding herd, because it is speculated that these seropositive animals will excrete the virus under stress conditions and will be source of infection to other susceptible cattle.

In the present study, a total of 870 cattle sera were screened for IBR seroprevalence study. District-wise, breed-wise, sex-wise and age-wise seroprevalence details are presented in table 2.

Out of 870 sera screened 332 were found to be positive for IBR antibodies. The sera were tested from four different districts viz., Valsad, Navsari, Tapi and Surat of South Gujarat. The highest Prevalence was recorded in Valsad (42.45%) followed by Tapi (37.33%), Navsari (37.07%) and Surat (35.71%). The district-wise difference was statistically non-significant ($P > 0.05$).

The serum samples were collected from different breeds of cattle viz., Holstein Friesian (HF), Jersey, Gir, HF cross, Jersey Cross and Non descript. The rate of prevalence was highest in HF (44.55%), HF cross (40.47%), Jersey (40%), Jersey cross (33.33%), Non-descript (28.00%) and Gir (18.75%). The breed-wise seroprevalence

in cattle differed highly significantly (P ≤ 0.01). Our findings are in agreement with previous worker [17] who showed higher prevalence in Gir and Deoni (15%), while Sahiwal and nondescript cattle showed least rate of prevalence (5%).

Female cattle (cows) showed higher seroprevalence (38.29%) as compared to males (34.48%) and could be due to difference in sample size in these two cases. Sex-wise seroprevalence did not differ significantly (P > 0.05). Our observations are in accordance with earlier worker [43].

In respect of age, maximum seroprevalence (59.40%) was found in cattle of more than seven years of age followed by animals of age group of 5 to7 years (44.80%), 1 to 4 years (25.72%) and below 1 year of age (23.47%). Statistically age was not found to influence the prevalence rate. However, 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.

Attributes	No. of Tested	No. of Positive	Percent Positive
Region			
South Gujarat	870	332	38.16
Districts			
Valsad	212	90	42.45
Navsari	259	96	37.07
Tapi	217	81	37.33
Surat	182	65	35.71
Total	870	332	38.16
$\chi^2 = 2.31^{NS}$ (P > 0.05)			
Breed wise			
Holstein Friesian	101	45	44.55
Jersey	65	26	40.00
Gir	80	15	18.75
HF Cross	551	223	40.47
Jersey Cross	48	16	33.33
Nondescript	25	07	28.00
Total	870	332	38.16
$\chi^2 = 17.43^{**}$(P ≤ 0.01)			
Sex wise			
Male	29	10	34.48
Female	841	322	38.29
Total	870	332	38.16
$\chi^2 = 0.17^{NS}$ (P > 0.05)			
Age wise			
<1 year	115	27	23.47
1-4 years	276	71	25.72
5-7 years	346	155	44.80
>7 years	133	79	59.40
Total	870	332	38.16
$\chi^2 = 60.47^{NS}$ (P > 0.05)			

Table 2: Seroprevalence of IBR in cattle using indirect ELISA.

Note: NS - Nonsignificant at P > 0.05;
** - Highly significant at P ≤ 0.01.

Higher seroprevalence rate in cattle (38.16%) concurred with the findings of earlier workers [9,11,42]. Seroprevalence of IBR in cattle has been reported from other Indian states too [19,23,25,28,39,44]. The prevalence rate reported by these workers varied between less than five per cent to more than 80%.

The higher prevalence of IBR reported in cattle in South Gujarat might be due to more number of organized farms, large no. of crossbred animals, intensive farming, adopting cross breeding programme, non-inclusion of preventive and control measures especially vaccination schedule against IBR.

The highest Prevalence was recorded in Valsad (42.45%) followed by Tapi (37.33%), Navsari (37.07%) and Surat (35.71%). The district-wise difference was statistically non-significant (P > 0.05). The variation in prevalence in different districts might be due to different rearing practices adopted, following different managemental practices and general awareness of livestock farmers regarding disease prevention measures. Our observation supported the findings of Jain., *et al*. [22] who also reported higher seroprevalence in Surat district.

A total of 526 buffaloes sera were screened. Details of breed-wise, sex-wise and age-wise seroprevalence in buffaloes are presented in table 3.

Out of 526 sera screened 83 (15.78%) were found to be positive for IBR antibodies. These samples were collected from Valsad,

Attributes	No. of Tested	No. of Positive	Percent Positive
Region			
South Gujarat	526	83	15.78
Districts			
Valsad	51	14	27.45
Navsari	234	42	17.95
Tapi	116	12	10.34
Surat	125	15	12.00
Total	526	83	15.78
$\chi^2 = 9.98^{*}(P \leq 0.05)$			
Breed wise			
Surti	347	61	17.58
Mehsani	144	19	13.19
Jafarabadi	24	02	08.33
Nonde-script	11	01	09.09
Total	526	83	15.78
$\chi^2 = 2.94^{NS}(P > 0.05)$			
Sex wise			
Male	29	04	13.79
Female	497	79	15.90
Total	526	83	15.78
$\chi^2 = 0.09^{NS}(P > 0.05)$			
Age wise			
<1 year	71	06	8.45
1-4 years	160	17	10.63
5-7 years	187	23	12.30
>7years	108	37	34.26
Total	526	83	15.78
$\chi^2 = 35.53^{**}(P \leq 0.01)$			

Table 3: Seroprevalence of IBR in buffaloes using indirect ELISA.

Note: NS - Nonsignificant at P > 0.05;

** - Highly significant at P ≤ 0.01; * - Significant at P ≤ 0.05.

Navsari, Tapi and Surat districts of Southern Gujarat. The highest seroprevalence was recorded in Valsad district (27.45%), followed by Navsari (17.95%), Surat (12%) and Tapi (10.34%) districts in descending order. The district-wise distribution of seroprevalence in buffaloes differed significantly (P ≤ 0.05). Our observations are in accordance with Khan (2004) who noted 18.97% seropositivity in buffaloes.

The serum samples were collected from different breeds of buffaloes viz., Surti, Mehsani, Jafarabadi, and non-descript. The lowest rate of seroprevalence was recorded in Jafarabadi (08.33%). The exact reason for lower positivity is not known but might be due to species resistance character. The highest seroprevalence was recorded in Surti (17.58%) followed by Mehsani (13.19%) and non-descript breed (09.09%). The breed-wise seroprevalence in buffaloes did not differ significantly (P > 0.05). Our findings are in accordance with Jain., *et al*. [22] who reported 42.86% and 30% in Surti and Mehsani while they noted significantly higher level of seroprevalence in Jafarabadi breed (20.59%) which is contrary to our current study (8.33%).

The male buffaloes showed lower seroprevalence (13.79%) in comparison to females (15.90%). The sex-wise seroprevalence did not differ significantly (P > 0.05) in the present study.

The sera were tested from four age groups i.e., below 1 year, 1to 4 years, 5 to 7 years and more than 7 years. Maximum seroprevalence (34.26%) was found in animals above 7 years of age followed by 5-7 years (12.30%) and 1-4 years (10.63%). The venereal transmission of infection during breeding age (above 7 years of age) might be responsible for higher prevalence among buffaloes. The least seroprevalence was noted in age group of below 1 year (8.45%). The age-wise difference was highly significant statistically (P ≤ 0.01) but Aradhana., *et al*. [5] summarized that no difference in the disease prevalence between age group of up to 3 years and more than 3 years.

Summary and Conclusion

An Indirect ELISA based seroepidemiological survey on IBR was carried out in cattle and buffaloes. For overall seroprevalence out of 1396 (870- cattle and 526-buffalo) serum samples, BoHV-1

antibody could be detected in 415 (29.73%) animals. The seroprevalence among cattle 38.16% (332/870) was significant higher in comparison to buffaloes 15.78% (83/526). Significant variation in prevalence rate was noted across the districts and Navsari topped the list (35.70%, 176/493) followed by Valsad (28.90%, 76/263), Tapi (27.32%, 91/333) and Surat (23.45%, 72/307). Females of these species showed a non-significant higher seroprevalence (30.04%, 402/1338) rate in comparison to their male counterparts (22.41%, 13/58). Age wise seroprevalence differed highly significant ($P \leq 0.01$). The higher seroprevalence (58.09%, 140/241) was recorded among very adult animals (over 7 years of age) followed by younger groups (37.90%, 202/533, between 5 to 7 years) with least seroprevalence in below one year of age group (10.75%, 20/186).

While in ELISA, out of 870 cattle serum samples 332 (38.16%) samples were found to be positive for IBR antibodies. In cattle, maximum seroprevalence was non-significantly recorded in Valsad district (42.45%, 90/212) followed by Tapi (37.33%, 81/217), Navsari (37.07%, 96/259) and Surat (35.71%, 65/182). The rate of seroprevalence was highly significant and recorded highest in pure exotic breed, HF (44.45%, 45/101) followed by pure exotic breed/cross bred i.e., Holstein Friesian cross (40.47%, 223/551), Jersey (40%, 26/65), Jersey cross (33.33%, 16/48), nondescript (28%, 7/55) and least in pure indigenous breed, Gir (18.75%, 15/80). Seroprevalence was seen to be non-significant higher in females (38.28%, 322/841) than male (34.48%, 10/29). More number of seropositivity was found in cattle above seven years of age (59.40%, 79/133) followed by age between 5-7 years (40.80%, 155/346), 1-4 years age group (25.72%, 71/276) while least seropositivity was found below 1 year of age (23.47%, 27/115).

In buffaloes, 526 sera screened 83 (15.78%) were found to be positive for IBR antibodies. The district-wise seroprevalence of IBR was noted significantly to be highest in Valsad (27.45%) followed by Navsari (17.95%), Surat (12%) and Tapi (10.34%) districts. Among different breeds, Surti showed 50.00% seropositivity followed by Mehsani (13.19%) and non-descript breed (09.09%) with least recorded in Jafarabadi (08.33%, 2/24). In female buffaloes, the seropositivity was noted non-significantly higher having 15.90% cases. However, males exhibited a bit of lower (13.79%, 04/20) seropositivity. The highest rate of seroprevalence (34.26%) was observed significantly in the age group of more than 7 years followed by age between 5-7 years (12.30%), 1-4 years (10.63%) while the least (8.45%) recorded in below 1 year age group.

Acknowledgements

Authors are thankful to Dr. N.H. Kelawala, Honorable Vice-Chancellor and former Dean and Principal, Navsari Veterinary College, KU, Navsari for providing all essential facilities to conduct this study. Authors are also thankful to supplier of indirect ELISA kit for timely supply and quality product. We are also grateful to the in charges/Veterinarians at Veterinary Dispensary or College clinic or Panjarapol or Clinical camp of Surat, Navsari and Valsad districts of South Gujarat for extending their help for collection of clinical samples for this study.

Bibliography

1. Ackermann M and Wyler R. "The DNA of an IPV strain of bovid herpesvirus 1 in sacral ganglia during latency after intravaginal infection". *Veterinary Microbiology* 9 (1984): 53-63.

2. Ackermann M., *et al*. "Pro and contra IBR-eradication". *Veterinary Microbiology* 113 (2006): 293-302.

3. Afshar A and Eaglesome MD. "Viruses associated with bovine semen". *Veterinary Bulletin* 60 (1990): 93-109.

4. Afshar A and Bannister GL. "Viral infections of the bovine mammary gland". *Veterinary Bulletin* 40 (1970): 681-686.

5. Aradhana Sharma DR., *et al*. "Status of infectious bovine rhinotracheitis (IBR) in Punjab state". *Indian Journal of Animal Sciences* 74 (2004): 264-266.

6. Aruna D and Suri Babu T. "Prevalence of infectious bovine rhinotracheitis (IBR) virus antibodies in buffaloes of Andhra Pradesh". *Indian Journal of Animal Sciences* 62 (1992): 540-541.

7. Boelaert FP., *et al*. "Prevalence of Bovine herpesvirus-1 in the Belgian cattle population". *Preventive Veterinary Medicine* 45 (2000): 285-295.

8. Castrucci G., *et al*. "A serological survey of bovine herpesvirus-1 infection in selected dairy herds in Northern and Central Italy". *Comparative Immunology, Microbiology and Infectious Diseases* 20 (1997): 315-317.

9. Chinchkar SR., *et al*. "Seroprevalence of infectious bovine rhinotracheitis in Maharashtra state". *The Indian Veterinary Journal 79* (2002): 68-69.

10. Deka D., *et al*. "Detection of bovine herpesvirus-1 infection in breeding bull semen by virus isolation and polymerase chain reaction". *Revue scientifique et technique (International Office of Epizootics)* 24 (2005): 1085-1094.

11. Dhand NK., *et al*. "Seroprevalence of infectious bovine rhinotracheitis in Punjab". *The Indian Veterinary Journal* 72 (2002): 850-852.

12. Durham PJK and Hassard LE. "Prevalence of antibodies to infectious bovine rhinotracheitis, Parainfluenza-3, bovine respiratory syncytial and bovine viral diarrhoea viruses in cattle in Saskachewan and Alberta". *Canadian Veterinary Journal* 31 (1990): 815-820.

13. Ganguly S and Mukhopadhayay SK. "Serological survey of infectious bovine rhinotracheitis". *The Indian Veterinary Journal* 87 (2010): 711-712.

14. Gibbs and Rweyemamu. "Bovine herpesviruses. Part I, Commonwealth Bureau of Animal Health". *Veterinary Bulletin* 47 (1977): 317-343.

15. Graham A and Minocha HC. "Neutralizing antibodies to bovine herpes virus-1 (BHV-1) and bovine parainfluenza-3 (PI-3) viruses in cattle in Tunisia". *Archives de l'Institut Pasteur de Tunis* 67 (1990): 25-31.

16. Guarino H., *et al*. "Prevalence of Bovine Herpesvirus-1, Bovine Viral Diarrohea, Parainfluenza-3, and Bovine Respiratory Syncytial antibodies in dairy farms of Uruguay". XXI Word Buiatrics Congress 4-8 December 2000, Punta del Este, Uruguay (2000).

17. Gupta AK., *et al*. "Seroprevalence of infectious bovine rhinotracheitis in cattle". *Veterinary Practitioner* 11 (2010): 169-170.

18. Ionescu A. "Studies on the incidence of IBR-IPV in some cattle farms for bull semen production and dairy cows". Lucrai Stiinifice Medicina Veterinara, Universitatea de Stiinte Agricole si Medicina Veterinara Ion Ionescu de la Brad 43 (2000): 218-220.

19. Jai S., *et al*. "Incidence and prevalence of livestock diseases of Andaman and Nicobar Islands". *Indian Journal of Animal Sciences* 75 (2005): 1041-1043.

20. Jain L. "Detection of Bovine herpesvirus 1 (BHV-1) infection in breeding bulls by serological and molecular methods and its characterization by sequencing of PCR products. M. V. Sc. thesis submitted to A.A.U, Anand (2006).

21. Jain L., *et al*. "Seroprevalence of bovine herpesvirus 1 (bhv-1) in indian breeding bulls of Gujarat". *Buffalo Bulletin* 27 (2008): 165-169.

22. Jain L., *et al*. "Detection of Bovine Herpesvirus 1 (BoHV-1) Infection in Semen of Indian Breeding bulls by polymerase chain reaction and its characterization by DNA sequencing". *Buffalo Bulletin* 28 (2009): 2-6.

23. Jain V., *et al*. "Seroprevalance of IBR among bovines of Garwal region". *The Indian Veterinary Journal* 83 (2006): 340-342.

24. Kargar Moakhar R., *et al*. "Seroepidemiological survey for antibodies against infectious bovine rhinotracheitis and bovine herpes 4 viruses among cattle in different provinces of Iran". *Archives of Razi Institute* 52 (2001): 93-100.

25. Khan OA. "Seroprevalence of Infectious Bovine Rhinotracheitis in Gujarat State". M.V.Sc. thesis submitted to G.A.U, Sardarkrushinagar (2004).

26. Krishnamoorthy P., *et al*. "Sero-epidemiology of infectious bovine rhinotracheitis and brucellosis in organized dairy farms in southern India". *Indian Journal of Animal Sciences* 85 (2015): 695-700.

27. Leuzinger H., *et al*. "Herpes simplex virus 1 envelopment follows two diverse pathways". *Journal of Virology* 79 (2005): 13047-13059.

28. Lyaku JRS., *et al*. "Prevalence of antibody to bovine herpesvirus-1 (BHV-1) in Tanzanian Cattle". *Tropical Animal Health and Production* 23 (1991): 106-107.

29. Manickam R and Mohan M. "Seroepidemiological studies on Infectious bovine rhinotracheitis (IBR) viral abortions in cows". *The Indian Journal of Animal Sciences* 57 (1987): 959-962.

30. Mars MH., *et al*. "Airborne Transmission of bovine herpesvirus 1 infections in calves under field conditions". *Veterinary Microbiology* 76 (2000): 1-13.

31. Mehrotra ML and Rajya BS. "Note on seroepidemiology of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus in India". *The Indian Journal of Animal Sciences* 51 (1981): 561-562.

32. Mehrotra ML., *et al*. "Infectious bovine rhinotracheitis (IBR) keratoconjunctivities in calves". *Indian Journal of Veterinary Pathology* 1 (1976): 70-73.

33. Mohan M., *et al*. "Seroepidemiological studies on infectious bovine rhinotracheitis (IBR) in bulls". *Indian Veterinary Journal* 66 (1998): 914-916.

34. Motha MXJ and Hansen MF. "Prevalence of IBR (infectious bovine rhinotracheitis) PI-3 (Parainfluenza type 3), BRS (Bovine respiratory syncytial) and BCV (bovine corona virus) infections in the dairy cattle population of New Zealand". *New Zealand Veterinary Journal* 46 (1998): 239-240.

35. Nandi S., *et al*. "Serological evidences of bovine herpesvirus-1 infection in bovines of organized farms in India". *Transboundary and Emerging Diseases* 58 (2011): 105-109.

36. Office Internationale Des Epizooties. "Infectious bovine hinotracheitis/pustular vulva infectious vaginitis. *In*: OIE terrestrial manual (2010): 752-767.

37. Pandey AB., *et al*. "Investigation of an outbreak of infectious pustular balanoposthitis in breeding bulls". *Indian Journal of Veterinary Research* 9 (2000): 27-37.

38. Pandita N and Srivastava RN. "A study on seroepizootiology of BHV-1 in Haryana". *Indian Journal of Virology* 9 (1993): 31.

39. Pandita N and Srivastava RN. " Dot-immunobinding assay for detection of bovine herpes virus-1 (BHV-1) antibodies". *Indian Journal of Virology* 11 (1995): 27-29.

40. Parsonson IM and Snowdon WA. "The effect of natural and artificial breeding using bulls infected with or semen contaminated with IBR virus". *Australian Veterinary Journal* 51 (1975): 365-369.

41. Pharande RR., *et al*. "Seroprevalence and characterization studies of infectious bovine rhinotracheitis virus. Animal health: a breakpoint in economic development. The 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress, August 23-27, 2004, Petaling Jaya, Malaysia (2004): 300-301.

42. Raghuvanshi S and Kumar M. "Sero-epidemiological investigation of infectious bovine rhinotracheitis in cattle and buffaloes using Avidin-Biotin ELISA. "Xth Annual Conference of IAAVR and National Symposium on "Challenges and strategies for Sustainable Animal Production in Mountains" and Indian Veterinary Congress-"4th Vetex 2003" held at Veterinary College, CSK HPKVV, Palampur (2003): 14-15.

43. Raghuvanshi S., *et al*. "Avidin biotin ELISA for sero-monitoring of infectious bovine rhinotracheitis in cattle and buffaloes". *Indian Journal of Veterinary Medicine* 26 (2006): 49-51.

44. Rajesh JB., *et al*. "Seroprevalence of Infectious Bovine Rhinotracheitis in cattle population of Kerala". *The Indian Veterinary Journal* 80 (2003): 393-396.

45. Renukaradhya GJ., *et al*. "Prevalence of infectious bovine rhinotracheitis in Southern India". *Revue Scientifique Et Technique - Office International Des Épizooties* 15 (1996): 1021-1028.

46. Samal SK., *et al*. "Note on the incidence of IBR virus infection among cattle in India". *The Indian Journal of Animal Sciences* 51 (1981): 895-897.

47. Singh A and Sinha BK. "Seroprevalence of infectious bovine rhinotracheitis (IBR) in cattle in Bihar". *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* 27 (2006): 107-108.

48. Snedecor GW and Cochran WG. "Statistical Methods. 6th Edition". Oxford and IBH Publishing Company, Calcutta.

49. Suri Babu T., *et al*. "Prevalence of infectious bovine rhinotracheitis virus (BHV-1) antibodies in bovines". *The Indian Veterinary Journal* 61 (1984): 195-200.