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Seroprevalence of Infectious Bovine Rhinotracheitis (BHV-1) in Dairy Animals with Reproductive Disorders in Saurashtra of Gujarat, India

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A B S T R A C T

Respiratory and reproductive disorders in dairy animals due to various etiological agents have led to significant economic losses to dairy industry. These losses are due to abortions, metritis, retention of placenta, repeat breeding, death of animals, loss of production and trade restrictions etc. The objectives of this cross-sectional study were to detect the seroprevalence of infectious bovine rhinotracheitis (IBR, BHV-1) in dairy animals of Junagadh, Rajkot and Bhavnagar Districts of Gujarat, India. Anti BHV-1 antibodies were measured using a commercial ELISA kit. Blood samples were collected from a total of 598 animals of different age, gender from the different places of the districts. Overall individual seroprevalence was 35.19%. The study revealed that BHV-1 is comparatively more widespread in cattle (36.31%) than buffalo (33.99%). The seropositivity of IBR increased with age of animals. The highest prevalence of IBR (42.07%) was observed in animals aged more than 7 years. As vaccination against IBR is not practiced in the region and higher percent positivity (>20%) in all age group of animals indicated the natural circulation of BHV-1 virus in the population. Because of less awareness on the vaccination of animals against this virus, the disease may spread rapidly. The results of present study also indicate that strict monitoring and surveillance of IBR is need of today to protect the animals from infection and further spread.

Introduction

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease of cattle caused by the bovine herpes virus-1 (BoHV-1) belongs to the genus Varicellovirus, subfamily Alphaherpesvirinae family Herpesviridae. Four subtypes of virus are known: 1.1 and 1.2a (associated with infectious bovine rhinotracheitis), 1.2b (associated with infectious pustular vulvovaginitis and infectious balanopothitis (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 virus. Latent and the 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). It is one of the most widespread respiratory/ reproductive viral diseases of bovines in India (Kiran *et al.,* 2005). IBR has been known to exist in India since 1976 (Mehrotra *et al.,* 1976) and since then sporadic studies on seroprevalence have been conducted in different parts of India (Nandi *et al.,* 2011; Kollannur *et al.,* 2014).

The reported seroprevalence in Cattle and buffaloes varied from 2.75 to 81.0 per cent (Sinha *et al.,* 2003; Malmurugan *et al.,* 2004). It is an economically important disease of bovine and causes abortions, infertility, meningoencephalitis, keratoconjunctivitis, respiratory and genital tract infections.

The present study records the current status on seroprevalence of infectious bovine rhinotracheitis based on avidin-biotin ELISA in bovine as ELISA is more sensitive (Payment *et al.,* 1979) of all the serological techniques available for the assay of antibodies to BHV-1 infection.

Materials and Methods

Study area

A cross-sectional study was conducted during 2016-17 to 2017-18 in Twelve (12) organised & unorganised dairy farms (Gir, Holstein Friesians, Jersey and Indigenous breeds) of cattle and Jafrabaddi breed of buffalo located in Junagadh, Rajkot and Bhavnagar districts of Gujarat state of India. A total 310 cattle and 288 buffaloes were included in the study of which 499 were female with age ranging from 3 to 12 years (pubertal heifers and cows), 99 bulls. A medical historyfrom each animal was collected. The information included age of the animals, occurrence of abortions, post abortion complications, infertility within the last three years.

Samples

Blood sample was collected from each of the 598 dairy animals of Gujarat state *via* jugular venipuncture into sterile vacutainer tubes with no anticoagulant. Blood samples were collected from unvaccinated normal as well as from reproductive disordered animals. Sera samples were inactivated at 56°C for 30 minutes and preserved at –20°C until use.

Detection of antibodies against BoHV-1

The sera samples were analysed using avidin-biotin ELISA kit developed at National Institute of Veterinary Epidemiology and Disease Information (NIVEDI), Bangaluru. The AB-ELISA technique previously described (Suresh *et al.,* 1999) was employed with modifications. BHV-1 antigen coated (100 ng, 100 µl per well, 1:100 dilution in carbonate bicarbonate buffer pH 9.6) flat-bottomed polysorp plates (Nunc-immunoplates) were incubated at 37°C for one hour over a rotary shaker and then washed three times with washing buffer (PBST-20, pH 7.4). Gelatin (1%) and Tween-20 (0.05%) in phosphate buffer saline were used as diluting as well as blocking buffer. The wells were filled with 100 µl each of test and reference control sera (1:100 dilution) in duplicate and incubated at 37°C for one hour. After washing the plates, biotinilated anti-bovine IgG (1:30,000 dilution, Pierce, 100µl) was added to each well and incubated for 1 hour. The plates were washed again and avidin-HRPO (1:15,000 dilution, Sigma, 100 µl) was added to each well. The plates were incubated at 37°C in a shaker for 20 minutes. Then the plates were washed and treated with 50 µl of freshly prepared substrate (3.7 mM OPD and 3.5 mM H_2O_2) for 7 minutes at room temperature. The enzyme substrate reaction was stopped by adding 1M H_2SO_4 (50 µl) and the absorbance values (OD) were recorded at 492 nm. The test results were expressed as

percent positivity (PP) values calculated as follows. Serum samples showing PP 45% considered as positive. PP (%) = Mean OD of the sample / Mean OD of the strong positive serum X 100. All the positive sera samples in duplicate were again retested by serial dilution of the sera starting from 1:100 to 1:800.

Results and Discussion

In this study, AB ELISA has been employed to screen the serum samples since the test is well suited for screening of viral infections and for analysis of a large number of samples (Salas *et al.,* 2013). Internationally serum neutralization test (SNT) is the accepted test for screening of animals for trade purpose, but it suffers from interference by non-antibody neutralizing factors in some sera, time consuming and require cell culture facilities. However, contrasting reports exist that show a good positive correlation found between micro SNT and ELISA in detecting BoHV-1 antibodies (Das *et al.,* 2014).

AB ELISA screening of serum samples showed that 36.31% cows and 33.99% buffaloes were seropositive for IBR. The district wise seroprevalence varied from highest in Bhavnagar (39.33%) followed by Junagadh (35.38%) and Rajkot (30.85%) districts of Saurashtra region. In this study, the overall seroprevalence of IBR antibodies was observed 35.19% (Table 1).

These results were similar to previous findings of some scientists (Nandi *et al.,* 2011; Chandranaik *et al.,* 2014; Samrath *et al.,* 2016) who recorded 39%, 34.90% and 34.69% seropositivity of IBR respectively in Indian cattle. These finding are lower than scientist (Trangadia *et al.,* 2009), who reported IBR seroprevalence of 55.26 % in Western region and 70.48 % in Southern region of the country in the organized dairy farms. Seropositivity of 61.6% suggests alarmingly wide spread prevalence of IBR in Indian cattle, considering the fact that India does not practice IBR vaccinations. Since none of the farms included in the study were vaccinated against BoHV-1, the seroprevalence obtained indicated that the animals in the farms had been exposed to the virus, assuming that the presence of antibodies can only be caused by exposure to the pathogen (Kampa *et al.,* 2004).

However, the rate of prevalence still suggests the importance of the disease in this area. Previous studies showed that IBR prevalence in different parts of India was highly variable ranging from 0% to 71.1% (Nandi *et al.,* 2011). The variation in prevalence rate of IBR antibodies in different farms may be attributed to differences in management and/or geographical differences. Based on medical history it was concluded that non- vaccination, intensive rearing, purchase and mixing of animals without IBR screening in all the farms and natural insemination from unscreened bulls in one farm are thought to be the main cause of the high prevalence.

High density of dairy cows and intensive management promotes viral spread and increases the chances that healthy susceptible animals will come into contact with infected animals (Chandranaik *et al.,* 2014). In larger farms, there is usually more movement of animals for the purchase and replacement of animals increasing the risk of infection and higher seroprevalence. A high number of seropositive animals may also be due to virus latency, which was inherent characteristic of the BoHV-1 infection (Chandranaik *et al.,* 2014).

Based upon the medical history for the heard, it was found that 79.69% Abortion cases, 76.32% Repeat breeding cases, 76.09% Retention of placenta cases and 58.33% Metritis cases were seropositive for IBR respectively (Table 2).

Table.1 Details of samples collected and seroprevalance of IBR antibodies in bovines

Districts	Cattle		Buffalo		Total	
	ST(SP)	% Seropositive	ST(SP)	% Seropositive	ST(SP)	% Seropositive
Junagadh	130 (48)	36.92	130(44)	33.85	260(92)	35.38
Rajkot	100(32)	32.00	88(26)	29.55	188(58)	30.85
Bhavnagar	80(32)	40.00	70(27)	38.57	150(59)	39.33
Total	**310(112)**	**36.31 (Average)**	**288(97)**	**33.99 (Average)**	**598(209)**	**35.19 (Average)**

ST-Serum Tested, SP-Seropositive

Table.2 Seroprevalance of IBR in cattle showing abortion, metritis, repeat breeding and ROP

Districts	Abortion (SP)	Metritis (SP)	Repeat Breeding (SP)	ROP (SP)
Junagadh	23 (19)	5 (3)	33 (25)	22 (18)
Rajkot	19 (14)	4 (3)	25 (18)	16 (12)
Bhavnagar	22 (18)	3 (1)	18 (15)	8 (5)
Total	**64 (51)**	**12 (7)**	**76 (58)**	**46 (35)**
Seroprevalance	**79.69**	**58.33**	**76.32**	**76.09**

SP-Sero positive, ROP-Retension of placenta

Table.3 Seroprevalance of IBR in Cattle according to age and lactation

Age group wise			Lactation wise		
Age group (yrs.)	No. of Tested Animals (SP)	PS (%)	Animal with lactation No.	No. of Tested Animals (SP)	PS (%)
3 to 4	22 (06)	27.27	1	36(08)	22.22
4 to 5	48 (15)	31.25	2	94(28)	29.79
5 to 6	68 (25)	36.76	3	108 (33)	30.56
6 to 7	145 (61)	42.07	4	148 (52)	35.14
Above 7	315 (152)	48.25	Above 4	212 (88)	41.51

SP-Sero positive, PS-Percent Sero prevalance

One of the scientists (Nandi *et al.,* 2009) similarly reported higher seroprevalence of IBR in cows having increased rate of abortion, stillbirth and repeat breeding. Seroprevalence of 79.69% in abortion cases is much higher than previous finding (Renukaradhya *et al.,* 1996), who recorded 55.4% seroprevalence of IBR in crossbred of southern India. Maximum seropositivity of IBR was found in animals above 7 years of age (Table 3) which was in agreement with (Verma *et al.,* 2014) who reported that animals more than 2 years of age are more prone to IBR virus infection than younger animals. The possible reason might be production stress in this age group might lead

to activation of latent infection and there were more chances of such animals get exposed to a natural infection.

In conclusion it was found that IBR prevalence was higher in some farms which might be due to use of contaminated semen for insemination, intensive management practices and introduction of unscreened new cows/bulls, since vaccination against IBR is not practiced in India and higher percentage positivity in all age group indicate natural circulation of virus in the population. This study also suggested need of an intensive control and surveillance program for reducing BoHV-1 infection rates in cattle in India.

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