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Seroprevalence of infectious bovine rhinotracheitis in organized dairy farms of India

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ABSTRACT

In this study, a systematic sero-surveillance of IBR was undertaken from 11 dairy farms located in 4 different regions of India. A total of 1000 cattle serum samples were tested for the presence of antibodies against IBR using Avidin Biotin ELISA. The results revealed that IBR antibodies were widely prevalent in all regions of the country ranging from 36.5% in Central region to 84.5% in Northern region with an overall prevalence of 61.6%. The prevalence of IBR antibodies was different between various age groups being 22.3%, 62.1%, 59.3%, 76.1% and 66.78 % in the age groups less than 1 year, 1-2 years, 2-3 years, 3-4 years and more than 4 years old respectively. Based upon the medical history of the herd, it was found that 83% abortion cases, 76% metritis cases, 83% repeat breeding cases and 65% retention of placenta cases were seropositive for IBR.

Key words: AB ELISA, BoHV-1, Cattle, IBR, Seroprevalence.

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).

The disease was first time reported in India from Uttar Pradesh (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). It causes huge economic losses due to drop in milk production, repeat breeding and abortions. Screening, surveillance and monitoring is important to maintain the herd health status and to decrease the economic losses caused by this disease (Raizman *et al.,* 2011).

The present communication deals with sero-prevalence of IBR in eleven dairy farms located in different parts of the country.

MATERIALS AND METHODS

Study Area: A cross-sectional study was conducted during 2013-14 in eleven (11) intensive dairy farms (Holstein Friesians, Jersey and Indigenous breeds) of cattle located in four regions of India. Dairy farms of Uttarakhand and Punjab in Northern region, Madhya Pradesh and Chhattisgarh in Central region, Maharashtra in Western region and Andhra Pradesh, Karnataka and Tamilnadu in Southern region were selected for the study. A total 1000 cattle were included in the study of which 893 were female with age ranging from 1 to 12 years (pubertal heifers, first-calf heifers and cows), 103 calves (1-12 month) and 4 bulls. A medical history from 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 1000 animals *via* jugular venipuncture into sterile vacutainer tubes with no anticoagulant. Samples were transported on ice to the laboratories, where they were centrifuged at 1000 g for 15 min to separate the serum, which was stored at -20°C until further use.

Detection of antibodies against BoHV-1: The serum based NIVEDI Avidin Biotin-ELISA was used for detection of antibodies against BoHV-1. The wells of the immunoassay plate were coated with BoHV-1 antigen (100 ng/well) in the volume of 100 µl diluted in 0.05M carbonate-bicarbonate buffer (pH 9.6) and incubated at 37^0C for 1 hr . The plate was washed three times with washing buffer (0.05% Tween 20 in 0.1M PBS pH 7.2). Test serum samples, along with strong and moderate positive and negative reference samples, were diluted (1:100) in blocking buffer (1% bovine gelatin in washing buffer). 100 µl of test sera samples and all the three controls were added in duplicates and incubated at 37^0C

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for 1hr and washed. Subsequently 100 ìl of biotinylated anti-bovine IgG at 1:30,000 dilutions in blocking buffer was added to each well and incubated at 37^{0}C for 1hr. after washing 100 ìl of Avidin-HRPO conjugate diluted to 1:15,000 in blocking buffer was added and incubated at 37^{0}C for 20 min. After washing, 100 µl of chromogen/ substrate solution (3.7 mM OPD and 3.5% hydrogen peroxide dissolved in distilled water) was added and observed for colour development at room temperature after 10 min. The enzyme-substrate reaction was stopped by adding 50 µl of 0.5M sulphuric acid to each well. The absorbance was 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.

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 Das *et al*. (2014) found a good positive correlation found between micro SNT and ELISA in detecting BoHV-1 antibodies.

AB ELISA screening of serum samples showed that 65.9% cows and 22.3% calves were (true prevalence 70.88% and 24% respectively) seropositive for IBR. Seroprevalence varied from 36.5 % in Central region to 84.5% in Northern region with over all seroprevalence of 61.6% ± 0.59 with confidence interval at 95 % level was 60.44 % to 62.76 % (Table 1). The overall true prevalence was 66.26% and 90.88%, 39.27%, 82.28% and 56.20% respectively in north, central, west and south region respectively (Table 1). These results were higher than previous findings of Nandi *et al.* (2011), Chandranaik *et al*. (2014a) and Annual Reports NIVEDI (2013-14) recorded 39%, 34.90% and 52% seropositivity of IBR respectively in Indian cattle. These finding are consistent with 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).

State wise analysis showed that seroprevalence of IBR varies from 7% in Madhya Pradesh to 85% in Uttarakhand (Table1) which were located distantly. The farms in Madhya Pradesh (7%) and Tamilnadu (34%) revealed comparatively low prevalence. These farms might be using high quality certificated semen which was thought to be the main causes of the lower prevalence rate. 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.,* 2014b). 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.,* 2014b).

Seroprevalence of 61.6% of IBR in Asia is comparable with the 67% of IBR seroprevalence in Thailand (Kampa *et al.,* 2004) and 61.17 % in Turkey (Okur *et al.,*

Table 1: Region and state wise sero-prevalence of IBR in cattle

Region	Farm location	Tested animals (Positive animals)	Percent Seroprevalence	Percent Seropositive cows	Percent Seropositive calves
North	Uttarakhand	100(85)	84.5	90	50
	Punjab	100(84)		95	40
Central	Madhya Pradesh	100(7)	36.5	7.7	0
	Chhattisgarh	100(66)		72	10
West	Maharashtra	200(153)	76.5	82.2	25
South	Andhra Pradesh	200(117)	55.25	60.6	15.4
	Tamilnadu	100(34)		37.7	0
	Karnataka	100(70)		75.5	20
	Total	616/1000	61.6	65.9	22.3

2007). All bulls from Andhra Pradesh in this study were found positive for IBR antibodies which might be potential source of spread of IBR in farm as virus can be transmitted by semen. Based upon medical history of farms it was found that 83% abortion, 76% metritis, 83% repeat breeding and 65 % retention of placenta cases were seropositive for IBR (true seroprevalence 89.41%, 81.65%, 89.41% and 69.74% respectively) (Table 2). Nandi *et al.* (2009) similarly reported higher seroprevalence of IBR in cows having increased rate of abortion, stillbirth and repeat breeding. Seroprevalence of 83% in abortion cases is much higher than previous finding of Renukharadhya *et al.* (1996) who recorded 55.4% seroprevalence of IBR in crossbred of southern India.

Table 2: Sero-prevalence of IBR in cattle showing abortion, metritis, repeat breeding and retention of placenta

Regions	Abortion (Seropositive)	Metritis (Seropositive)	Repeat breeding (Seropositive)	Retention of Placenta (Seropositive)
North	23(21)	0	34(33)	43(28)
Central	7(2)	8(6)	12(12)	16(6)
West	43(39)	61(51)	69(55)	68(48)
South	10(7)	10(3)	46(33)	1(1)
Total	83(69)	79(60)	161(133)	128(83)
Seroprevalence	83%	76%	83%	65%

Maximum seropositivity of IBR was found in animals between 3-4 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 this study, 21.8% animals of less than 6 months old were positive for IBR. This might indicate that maternal transfer of antibodies in the calf (Ezzi *et al.*, 2011). During the study, 3 seropositive calves were from naive dams for BoHV1. This situation may suggest a postnatal infection in these animals within 6 months of birth following decline in maternal immunity.

Table 3: Sero-prevalence of IBR in cattle according to age and lactation

Age group (Years)	Tested animals (Seropositive)	Percent Seroprevalence	Animals with lactation No.	Tested animals (Seropositive)	Percent Seroprevalence
0-1	103(23)	22.3	1	221(159)	71.9
1-2	82(51)	62.1	2	247(162)	65.5
2-3	165(98)	59.3	3	190(138)	72.6
3-4	105(80)	76.1	above 4	202(134)	66.3
above 4	545(364)	66.78			

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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