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Research Article

SEROPREVALENCE STUDIES ON BOVINE VIRAL DIARRHEA VIRUS IN HARYANA, INDIA

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ABSTRACT

Bovine viral diarrhea virus (BVDV) has a worldwide distribution and it tends to be endemic among cattle populations. The BVDV infection produces a wide range of disease symptoms including diarrhea, respiratory distress, abortion, congenital defects, acute and chronic mucosal disease. Considering the known presence of this disease in India, limited research has been carried out on the seroprevalence of this disease in India. Therefore, the study was undertaken to know the prevalence of bovine viral diarrhea virus (BVDV) antibodies in bovine sera of Haryana. The 440 sera samples which were received from two different sources, RRC-FMD and CCL, LUVAS, were tested to detect the BVDV specific antibodies by a commercially available ELISA kit. Out of 220 cattle sera samples tested, 85 (38.64%) were found to be positive while out of 220 buffaloes' sera samples, 84 (38.18%) were found to be positive. Overall 169 sera samples out of 440 were found to be positive for BVDV antibodies by ELISA. The seroprevalence of BVDV in bovines of Haryana was found to be 38.41%. The presence of a high number of seropositive animals was an indirect indication of widespread presence of BVDV in bovines of Haryana.

Keywords: Antibodies, Bovine viral diarrhea virus, ELISA, Seroprevalence

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Bovine viral diarrhea (BVD) is considered one of the most significant infectious diseases in the livestock industry due to its high prevalence, persistence and clinical consequences (Khezri, 2015). The disease has a global distribution and impacts animal health and reproductive performance resulting in significant economic losses in the cattle industry. It is a listed notifiable disease by the World Organization for Animal Health. The worldwide seroprevalence of BVD has been reported between 40 and 80% while persistently infected (PI) animals' prevalence is between 0.5 and 4% (Barbosa *et al.*, 2019). Bovine viral diarrhea virus (BVDV), belonging to the genus Pestivirus of the family Flaviviridae, is the causative agent of BVD. Although cattle are the primary host for BVDV, but most even-toed ungulates are also susceptible (Singh *et al.*, 2017). Direct or sexual contact with PI cattle is the major mode of BVDV transmission. All the species of BVDV i.e. BVDV-1, BVDV-2 and BVDV-3 have been detected in cattle in India (Mishra *et al.*, 2014). The serological and virological studies indicate widespread BVDV infections in most parts of India but there is scarcity of serosurveillence data from Haryana. Therefore, the present study was undertakento know the seroprevalence of BVDV antibodies in Haryana state.

MATERIALS AND METHODS

A total of 440 sera samples, 220 each of cattle and buffaloes from eighteen districts of Haryana, were received from two different sources:100 samples from Regional Research Centre on foot-and-mouth disease (RRC-FMD) and 340 samples from College Central Laboratory (CCL), LUVAS, Hisar. These samples were screened to detect the BVDV specific antibodies using a commercial ELISA Kit (PrioCHECK®BVDV Ab Kit, Prionics) according to the manufacturer's recommendations. The sera samples were tested to detect antibodies against the highly conserved NS3 (p80) protein of BVDV. The test was based on the principle of competition between BVDV specific antibodies present in the test serum sample and a peroxidase conjugated monoclonal (mAb) anti-p80-antibody.The detection of antibodies against highly conserved p80 protein of BVDV by competitive ELISA provides reliable results in determining seroconversion after field virus infection and has been used in several countries where control and eradication programmes are in progress. After completion of ELISA kit steps, the corrected OD450 of all the samples was expressed as percent inhibition (PI) which was calculated as follows:

$$\text{Percent inhibition} = 100 - \frac{\text{Corrected OD 450 test sample}}{\text{Corrected OD 450 max}} \times 100$$

The test was considered valid when the mean OD450 blank was <0.15; the corrected mean OD450 max was ≥1.00; the percent inhibition of reference serum 2 was >50; and the percent inhibition of reference serum 3 was <50. Sera samples showing percent inhibition of <50 and ≥50 were considered negative and positive, respectively.

RESULTS AND DISCUSSION

Out of the 100 sera samples received from RRC-FMD, LUVAS, 30% (30/100) were found positive for

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Table 1

Seroprevalence of BVDV antibodies in bovine sera samples received from RRC-FMD and CCL, LUVAS, Hisar

Source of sera samples	Species	No. of sera samples tested	No. of positive samples	Percent positive for BVDV antibodies
RRC-FMD, LUVAS	Cattle	50	16	32.00%
	Buffaloes	50	14	28.00%
	Total	100	30	30.00%
CCL, LUVAS	Cattle	170	69	40.58%
	Buffaloes	170	70	41.17%
	Total	340	139	40.88%
	Total cattle	220	85	38.64%
	Total buffaloes	220	84	38.18%
	Total cattle + buffaloes	440	169	38.41%

BVDV antibodies in ELISA in which 32% (16/50) samples were of cattle and 28% (14/50) were of buffaloes while from 340 sera samples of CCL, LUVAS, 40.58% (69/170) samples of cattle and 41.17% (70/170) samples of buffaloes were found positive for BVDV antibodies (Table 1). In aggregate, out of 440 samples, 38.64% (85/220) samples of cattle and 38.18% (84/220) of buffaloes were found positive with the antibody detection ELISA kit. The seropositivity was highest in district Palwal i.e. 100% (3/3) followed by 66.67% in Ambala (2/3), 52.38% in Faridabad (11/21), 50% in Fatehabad (9/18) and Panipat (2/4), 44.74% in Karnal (17/38), 44.44% in Sirsa (32/72), 41.67% in Jhajjar (5/12) and Mahendergarh (10/24) as shown in Table 2. There was low seropositivity in districts of Yamunanagar (10%) and Sonipat (28.57%) while no seropositivity was detected in Panchkula (0%). Several epidemiological factors like cattle density, different cattle housing practices, breeding methods, use of common pastures and water sources, overstocking of animals, animal trade practices, variation in temperature and humidity, immunosuppression and stress condition, vaccination practices, poor biosecurity measures and implementation of different control or eradication programs can account for variation in the seroprevalence of infection (Brodersen, 2010). The influence of farm management practices on regional variation in pestivirus antibody prevalence has been reported earlier (Graham *et al.*, 2001). In the present study, the wide variation in seroprevalence rate between the different districts can be attributed to such factors.

The prevalence rate for BVDV antibodies in cattle and buffaloes (38.41%) found in this study approximated well with the observation of Bhatia *et al.* (2008) who reported 37.6% of Indian cattle positive for BVDV antibodies. But it is high compared to the 17.31% (76/439) in 17 states of India (Sudarashana *et al.*, 1999), 30% in 14 states of India (Sood *et al.*, 2007), 27.4% (46/168) at Chennai (Selvaraj *et al.*, 2007), 17.1% in cattle from 13 states of India (Behera *et al.*, 2011), 13.2% (66/500) in Tamil Nadu (Kumar *et al.*, 2018) and 12.34% (113/916) in the north east India (Chakaraborty *et al.*, 2018). Seroprevalence rate observed in this study is less as compared to that reported in Gujarat in buffaloes which was 65.9% showing gastrointestinal and reproductive disorders (Mukherjee *et al.*, 1989). The increase in seroprevalence of BVD in the

Table 2

Seropositivity of BVDV antibodies in different districts of Haryana

S. No.	District Names	No. of sera samples tested	No. of positive samples
1.	Ambala	3	2
2.	Bhiwani	39	13
3.	Faridabad	21	11
4.	Fatehabad	18	9
5.	Gurugram	26	9
6.	Hisar	79	23
7.	Jhajjar	12	5
8.	Jind	50	21
9.	Kaithal	18	6
10.	Karnal	38	17
11.	Mahendragarh	24	10
12.	Palwal	3	3
13.	Panchkula	1	0
14.	Panipat	4	2
15.	Rohtak	5	2
16.	Sirsa	72	32
17.	Sonipat	7	2
18.	Yamunanagar	20	2
	Total Samples	440	169

country can be attributed to import of exotic germplasm from Europe and America for crossbreeding in India for the last few decades, transmission of the virus between animals through needles during mass vaccination programs, uncontrolled movement of cattle between herds and spread of the virus through contaminated semen by artificial insemination. Through detailed genetic analysis of 13 BVDV isolates from India, Mishra *et al.* (2004) has indicated the introduction of present BVDV infection in the country from Europe and America. Different surveys, based on serological assays, across the world show considerable variation in seroprevalence ranging from 40 to 90% in individual cattle and 28–66% in cattle herds (Scharnbock *et al.*, 2018). The seroprevalence of BVDV in cattle was 16.85% in Pakistan (Gohar *et al.*, 2013), 33.2% in Malaysia (Daves *et al.*, 2016), 51.1% in Bangladesh (Uddin *et al.*, 2017), 58.09% in China (Deng *et al.*, 2015) and 80% in both Australia and New Zealand (Reichal *et al.*, 2018). Though the sample size in some of the districts was limited and may not be a true representative of the target population, this is the first epidemiological study in determining the seroprevalence of BVDV antibodies in the bovines of Haryana. However, large scale serosurveillance is required in the country to have a thorough picture of BVDV seroprevalence so that the appropriate steps could be taken up by policy makers to control this disease. The present results form an important basis for initiating a step towards the control of spread of BVDV infection in the country.

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