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Prevalence of Bovine Viral Diarrhoea infection in cattle and buffaloes from Andhra Pradesh, India

B. Vimala Devi[1*], G. Sireesha[2], D. Neeraja[1], L. Ratna Kumari[3] and R. Amarendrakumar[4]

Department of Animal Husbandry Veterinary Biological & Research Institute
Labbipet, Vijayawada-520010
Andhra Pradesh, India

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Abstract

The present study was directed at assessing the prevalence of Bovine Viral Diarrhoea (BVD) in various districts of Andhra Pradesh. Samples from organized cattle and buffalo farms and also from clinical cases were tested from January 2022 to December 2022. A total of 673 serum samples from five organised cattle and buffalo farms and 86 clinical samples from animals suffering from severe diarrhoea, respiratory problems and reproductive disorders were screened for the presence of antibodies against BVD. Serum samples were tested for antibodies against BVD virus p80-125 protein (NSP2-3) using a commercially available competitive ELISA kit (ID Screen). The results revealed that a total of 442 out of 759 animals (58.23 per cent) were positive for antibodies against Bovine viral diarrhoea virus. Statistically significant association was observed between BVD seropositivity and different risk factors such as district, farm, species and breed. Sample size and Geographical location may have contributed to this result. In two organized farms where seropositivity was high (more than 70%), all the seronegative animals (55) were screened for presence of BVD virus using a commercially available BVD p80 antigen ELISA kit (ID screen) to detect persistently infected animals. As no animal was found positive, all the remaining seropositive animals (414) in these two farms were also tested for BVD antigen on the suspicion of a current transient infection or presence of a seropositive PI due to exposure to a BVDV strain that is antigenically different to the persisting strain. All the animals tested were negative for BVD antigen. Out of all the clinical samples tested, one animal that was antibody positive was found positive for BVD virus also, indicating a transient infection. The high seropositivity in the organized cattle farms without a PI animal might either be due to an earlier transient infection in the herd or transfer of a PI animal away from the herd before testing. The high seropositivity in the state and presence of BVD antigen in the field animal indicates that a circulating BVD virus was present in most parts of the state.

Keywords: *Bovine Viral Diarrhoea Virus, BVD p 80 ELISA, indigenous breeds*

1. *Veterinary Assistant Surgeon*
2. *Assistant Director (A.H)*
3. *Joint Director (A.H), VBRI AP, Vijayawada*
4. *Director of Animal Husbandry, Andhra Pradesh*

**Corresponding author: bvimala96@gmail.com, Ph. 8978544133*

Bovine viral diarrhoea virus (BVDV) is a single-stranded RNA virus that belongs to the genus Pestivirus of the family *Flaviviridae*. Bovine Viral Diarrhoea is an infectious and highly contagious viral disease of cattle with a cosmopolitan distribution. The World Organisation for Animal Health (OIE) listed it as a notifiable disease (OIE, 2018). The infection causes huge economic loss on account of accompanying abortions, birth of congenitally defective calves, reduced fertility and drop in milk yield (Brodersen *et al*., 2014). Prenatal infection can result in the birth of immune tolerant persistently infected (PI) animals, that remain lifelong virus carriers and are the main source of infection for other non-infected cattle. The worldwide seroprevalence of BVD has been reported to be between 40 to 80 per cent while the prevalence of persistently infected animal is between 0.5 to 4 per cent (Barbosa *et al*., 2019 and Charleston *et al*., 2001). In India, the presence of this infection was first reported in Odisha (Nayak *et al.,* 1982) and later in Gujarat (Mukherjee *et al*., 1989). The overall prevalence of BVD antibodies in Indian cattle and buffaloes was reported as 30 per cent from 14 states (Sood *et al.*, 2007). Existence of BVDV infection was also reported from other states like Kerala (Kulangara *et al.,* 2015), West Bengal (Ghosh *et al*., 2015), Himachal Pradesh (Katoch *et al.,* 2017), Tamil Nadu (Kumar *et al.,* 2018) and Haryana (Khaneja *et al.,* 2021). Although the prevalence of BVD disease is reported from most parts of India, data reported from Andhra Pradesh state is scarce. Hence the present study was taken up to study the prevalence of BVD in the state.

Materials and methods

Sample collection

As part of regular screening of organised cattle and buffalo government farms for various sexually transmitted diseases, 673 serum samples were collected from all the cattle and buffaloes maintained in five organized farms located in three districts of Andhra Pradesh state during the year 2022. Serum samples (86) collected by the field veterinary institutions from animals suffering with severe diarrhoea, respiratory problems and reproductive disorders like abortions and repeat breeding in different villages of the state were also included in the study (Table 1). Animals of various ages and breeds were included in the study.

Competitive ELISA for detection of BVDV antibody in serum

All the 759 serum samples collected were tested for BVDV antibodies using a commercially available competitive ELISA kit (ID Screen BVD p80 Antibody Competition). This kit was designed to detect specific antibodies directed against the p80-125 protein (NSP2-3) of BVD virus by competitive ELISA. As NSP2-3 is highly conserved among Pestiviruses and is one of the most immunogenic proteins of the virus (Chimeno *et al*., 2006 ; Bhatia *et al*., 2008) this kit was selected for the present study. The test was conducted as per the manufacturer's instructions.

For each sample competition percentage (S/N per cent) was calculated as mentioned below.

$$\text{S/N per cent} = \frac{\text{Mean O.D of Test sample}}{\text{Mean OD of Negative control}} \times 100$$

Positive cases were determined as those individuals with S/N percentage ≤ 40 and negative cases as those with S/N percentage >50. In cases of S/N percentage between 40 per cent to 50 per cent, these were considered as doubtful and not included in the present study.

Sandwich ELISA for detection of BVDV antigen

To detect BVD virus, a commercially available p80 antigen capture ELISA (ID screen BVD p80 antigen Capture) was used because of the highly conserved nature of BVDV p80 (nonstructural NS3 protein) among Pestiviruses (Shapouri *et al*., 2022; Sandvik *et al.,*1995). As high seropositivity was detected in two organized cattle and buffalo farms *viz.* CLF, Chintaladevi farm (P.P : 95.26) and NKBC, SPSR Nellore farm (P.P: 83.51), all the sero negative animals (55) in these farms were screened for the presence of BVD virus by antigen capture ELISA to detect the presence of persistently infected animal in the herd. Later remaining

seropositive animals (414) in the same farms were also screened for the presence of BVD virus by antigen capture ELISA in order to rule out both, the presence of seropositive PI animals and also current transient infection in the herds. All the 86 clinical samples were also screened for the presence of BVD virus by p80 antigen capture ELISA. The test was conducted as per the manufacturer's instructions.

For each sample S/P per cent was calculated as mentioned below.

$$\text{S/P per cent} = \frac{\text{Mean O.D of Test sample - Mean O.D of Negative control X 100}}{\text{Mean OD of Positive control -Mean OD of Negative control}}$$

Positive cases were determined as those individuals with a S/P percentage ≥35 and negative cases as those with S/P percentage < 35.

Statistical analysis

Statistical analysis of district wise, breed wise and species wise seroprevalence of BVDV was performed using Chi square test (SPSS software version 20.0).

Results and discussion

The mean prevalence of BVD antibodies in Andhra Pradesh was 58.23 per cent with 63.74 per cent in organised farms and 15.11 per cent in field cases as given in Table 1. The worldwide seroprevalence of BVD has been reported to be between 40 to 80 per cent (Barbosa *et al.,* 2019) where as in India it was reported as 15 to 30 per cent (Sudharsana *et al.,* 1999; Sood *et al.,* 2007). As vaccination against BVD is not practiced in India, a serological response usually indicates natural infection. The high seroprevalence indicates the presence of circulating BVD virus (Sudharsana *et al*.,1999) caused either by the presence of one or more persistently infected animals in the farms or transient infection (Garoussi *et al*., 2019). The high seroprevalence in the farms compared to field cases may be due to the confinement and proximity of the animals with each other enabling spread of the disease from infected to healthy animals. The leersser seroprevalence in the field samples might also be due to the smaller sample size of the field animals tested.

The association between location (district and farm) and seroprevalence of BVD is statistically significant at 0.01% level (P< 0.01) (Table 2). The results are in agreement with Kumar *et al.* (2018) whose findings shows a significant statistical association of districts of Tamil Nadu with BVD seroprevalence. In SPSR Nellore district, the high seropositivity (P.P: 88.27 per cent) can be attributed to the presence of two large farms in close proximity out of the five farms selected for the study.

Species wise analysis for seroprevalence of BVDV revealed a statistically significant association at 0.01 level (Table 3). The seroprevalence in buffaloes (76.81) was much higher than cattle (54.11 per cent). This result is in contrast to that reported by Sood *et al.* (2007) who reported a higher incidence in cattle than in buffaloes.

For breed wise seroprevalence analysis, NKBC cattle farm, SPSR Nellore district was chosen where different indigenous cattle and buffalo breeds are maintained. (Table 4). Statistically significant association ($p < 0.01$) between breed and BVD seroprevalence was

Table 1. Overall seroprevalence of BVDV in different cattle and buffalo farms of Andhra Pradesh state during the year 2022

Source of sample	No of farms/ No of villages (for clinical cases)	Total number of animals tested	Number of animals positive for BVDV antibody	Per cent Prevalence
Organized cattle and buffalo farms	5 farms	673	429	63.74
Clinical cases	20 villages	86	13	15.11
Total samples		759	442	58.23

observed. The results are in agreement with Gonzalez-Bautista *et al.* (2021) whose findings shows a significant statistical association of breed, age, management practices with BVD seropositivity. In Khillari breed, the incidence was less (P.P:11, 2/18) and in Motu breed no incidence was recorded (P.P:0, 0/11). Further study with larger sample size is required to confirm whether these two breeds are resistant to BVD.

As PI animals are generally antibody negative (Hilbe *et al.,* 2007), all the seronegative animals (55) in the farms with high seropositivity (CLF, Chintaladevi and NKBC farm) were tested for presence of BVD p80 antigen. All the animals were found to be negative for BVD antigen. Generally seronegative, viraemic animals are considered as persistently infected. However, a small proportion of persistently viraemic animals may produce antibodies to some of the viral proteins if they are exposed to another strain of BVDV that is antigenically different to the persisting virus (OIE, 2018). As there is evidence of circulation of BVDV-1, BVD-2 and BVD-3 virus strains in India (Mishra *et al*., 2004, 2005, 2014 and Behera *et al.,* 2011) presence of PI animal in seropositive animals was suspected. Absence of PI animals in high seropositive herds can also indicate either a current or past transient infection. To rule out the presence of another BVD strain and current infection, all the remaining animals (414) in the above farms were also tested for BVD antigen and all of them were found negative. This indicates the absence of PI animal and current infection in the two tested farms. The high seropositivity in the absence of PI animal and current infection might be due to earlier transient infection or transfer of PI animal away from the herd before testing.

Out of 86 clinical samples screened for presence of BVD virus by antigen ELISA, one sample collected from an animal in Krishna district suffering with severe diarrhoea was found positive. The animal was also antibody positive indicating that it might not be a PI animal

Table 2. District and farm wise seroprevalence of BVD in different districts of Andhra Pradesh state during 2022

Sl. No	Name of the district	Name the farm/village	No.of samples tested	No. of samples positive	Per cent Positivity	Chi-square	p Value
1	Prakasam	Chadalawada farm	114	2	1.75	483.02	0
2	SPSR Nellore	CLF, Chintaladevi farm	190	181	95.26		
		NKBC farm	279	233	83.51		
3	Kurnool	FSBS, Nandyal farm	76	13	17.11		
		Clinical cases from various villages	13	5	38.46		
4	Guntur	YBRC farm	14	0	0.00		
		Clinical cases from various villages	17	5	29.41		
5	Krishna	Clinical cases from various villages	2	2	100.00		
6	Chittoor	Clinical cases from various villages	54	1	1.85		
	Total		759	442	58.23		

Table 3. Species wise seroprevalence of BVDV

Species	No.of samples Tested	No. of samples Positive	No. of samples Negative	% Positivity	Chi-square	p Value
Cattle	621	336	285	54.11	23.9324	9.980×10^{-7}
Buffaloes	138	106	32	76.81		

Table 4. Breed wise sero-prevalence of BVDV in NKBC cattle farm

Sl. No	Breed	No.of samples tested	No of samples Positive	No of samples Negative	per cent Positivity	Chi-square	p Value
1.	Banni	15	15	0	100	146.041	0
2.	Kankrej	16	16	0	100		
3.	Krishna valley	5	5	0	100		
4.	Mehasana	20	20	0	100		
5.	Murrah	11	11	0	100		
6.	Ongloe	4	4	0	100		
7.	Punganoor	9	9	0	100		
8.	Jaffarbadi	24	23	1	95.83		
9.	Pandharipuri	29	27	2	93.1		
10.	Sahiwal	21	19	2	90.48		
11.	Malnadgidda	9	8	1	88.89		
12.	Gir	16	14	2	87.5		
13.	Tharparkar	16	14	2	87.5		
14.	Red sindhi	14	12	2	85.71		
15.	Deoni	25	21	4	84		
16.	Rathi	11	9	2	81.82		
17.	Kangayam	5	4	1	80		
18.	Khillari	18	2	16	11.11		
19.	Motu	11	0	11	0		

and the infection might have been transient. Both antibody and antigen test results of the present study suggests that there is circulating BVD virus in the state. This study indicates the prevalence of BVD in most districts of the state and clinical form of the disease with severe diarrhoea is also evident.

The present results form an important basis for initiating measures towards control of further spread of BVDV infection in the state. Different measures like regular serosurveillance of animals in the organized farms, identification and removal of PI animals by antigen testing will help in removal of the source of infection. Testing of new entrants into the herd and new born calves will help in stopping the infection from entering the herd. Testing of cattle and buffaloes especially breeding bulls and pregnant animals in organized farms before movement to other farm or herd will help in identification of source of BVD infection there by limiting the spread of infection.

Conclusion

The status of the Bovine Viral Diarrhoea virus in five cattle and buffalo farms of Andhra Pradesh state has been reported. The study indicates the serological evidence of BVD in cattle and buffaloes in the state. The overall prevalence of BVDV antibodies in the state is 58.23 per cent. Statistically significant association observed between BVD seroprevalence and various risk factors like location, species and breed. Sampling method and sample size might have attributed to this. Absence of PI animal in the organized cattle and buffalo farms in spite of high seropositivity might be attributed to the previous transient infection in the herd or movement of one or more *PI* animals away from the farm before testing. This indicates the necessity of screening of animals especially breeding bulls and pregnant animals in organized farms before movement to another farm or herd. The study also reports a case of current BVD infection in a buffalo suffering with severe diarrhoea. This indicates the necessity of screening field cases with history of diarrhoea, respiratory problems and reproductive disorders against BVDV. Hence large scale serosurveillance and antigen testing of BVD suspected animals is necessary to know the prevalence of BVDV in all the districts of the state to plan suitable control measures.

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Conflict of interest

The authors declare that they have no conflict of interest.

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