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Seroprevalence of bovine viral diarrhoea in organized herds in India

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ABSTRACT

Bovine viral diarrhoea (BVD) is an important infectious viral disease affecting cattle populations all over the world. In addition to direct loss caused by the disease, the virus causes immunosuppression thereby predisposing the host to other diseases. A cross-sectional study was undertaken to detect the prevalence of BVD in 14 well-organized herds located in different parts of India. A total of 880 serum samples (646 cattle and 234 buffaloes) were screened by a commercial ELISA kit, detecting antibodies towards the p80 (NS3) region of BVDV. The overall true prevalence was 56.67% (95% CI: 53.26-60.02%) and within herds, it ranged from 0-99.99%. The prevalence rate was higher in cattle (65.42%) than in buffaloes (32.49%) and the difference was statistically significant. Further, a significant difference in prevalence among cattle breed types was recorded, with the lowest in indigenous cattle (16.49%) followed by crossbreeds (16,97% and exotic breeds (87.80%). Higher positivity was detected among females (68.87%) than males (48.83%) but this difference was not significant, as revealed by multivariate regression analysis. Of the 10 semen stations studied, the prevalence varied from 9.72% to 72.68%. However, none of the animals from these semen stations turned positive in the antigen ELISA test, suggesting the antibodies detected in this study were from past infections. On the two dairy farms/bull mother farms showing very high positivity, two (one each) persistently infected cows were detected during whole herd screening by antigen ELISA test. One bull mother farm was free of BVD antibodies suggesting it is possible to maintain BVDV-free herds. The present study indicates the endemicity of BVDV in Indian organized herds, and therefore a suitable testing strategy and management should be adopted in response to testing to control the introduction and further transmission of the disease on farms.

Key words: bovine viral diarrhoea; BVD virus; prevalence; India; persistently infected; antibody; antigen

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Introduction

Bovine viral diarrhoea (BVD) is an economically important disease of cattle and other ruminants. It causes enteric, respiratory and reproductive disorders in animals. In addition, the virus also causes immunosuppression in the host, thereby predisposing the affected animal to other pathogens (WOAH, 2015, YARNALL and THRUSFIELD, 2017). The disease can be manifested in different clinical presentations ranging from a subclinical to a severe fatal mucosal disease. In acute cases, fever, diarrhoea, respiratory disease, haemorrhagic lesions and sudden death may be observed (BAKER, 1995; WOAH, 2015). In breeding females conception failure, abortions, still birth, teratogenic abnormalities and birth of persistently infected (PI) calves are observed, depending on the stage of in-utero infection (BAKER, 1995, WOAH, 2015). Further, it causes reductions in milk yield and weight gain (YARNALL and THRUSFIELD, 2017). PI animals are the major source of infection to other animals as they excrete the virus in various secretions (OIE, 2015, SCHARNBÖCK et al., 2018).

BVD is caused by the BVD virus (BVDV), a RNA virus belonging to the genus *pestivirus* of the *flaviviridae* family. Being an RNA virus, it mutates, resulting in genetic and antigenic variations (WALZ et al., 2010). The BVDV contains two genotypes - BVDV-1 and BVDV-2, which in turn contain many subtypes (BVDV-1a to1u; BVDV-2a to 2d) (MIROSŁAW and POLAK, 2019). Further, recently natural infection has been reported with various subtypes of HoBi like virus (HoBiPev), also putatively known as BVDV-3 (MOORTHY et al., 2019).

A very high prevalence of BVD has been reported from almost all over the world, although some countries have been able to eradicate the disease (MOENNIG et al., 2005; WOAH, 2015). An exhaustive meta-analysis study reviewing 334 publications across the world revealed the mean antibody prevalence to be 49.2% (95% CI: 46.14-52.25). YARNALL and THRUSFIELD (2017) studied the economic impact of BVD by meta-analysis, and found it ranged from £0 to as high as £552 per cow per year, with a mean impact of £46.5.

The first serological evidence of BVD in India was found in 1982 in Odisha (NAYAK et al., 1982). SOOD et al. (2007) screened cattle and buffaloes from 17 states of India from 1999-2004, and reported an overall prevalence of 30%. To date serological evidence of BVD infection has now been reported in buffaloes, sheep, goat, pig and mithun in India (MUKHERJEE et al., 1989; SUDHARSHANA et al., 1999, MISHRA et al., 2009, MISHRA et al., 2011, SINGH et al., 2017, CHAKRABORTY et al., 2018). Although serological evidence of BVDV was reported long ago, very few systematic studies have been undertaken and the serological studies have mostly relied on samples collected from fields of unknown origin or small holding farms. Information on the prevalence of this important disease in organized herds is lacking. Therefore, this study was undertaken to estimate the prevalence of BVD in well-organized herds located in different parts of the country.

Materials and methods

Study design and sampling. A cross-sectional study was undertaken in 14 organized herds (10 semen stations and 4 bull mother farms/ dairy farms) located in different parts of India. The sample size was determined by considering an expected disease prevalence of 30% (SOOD et al., 2007), with desired precision of 5%, confidence level of 95% and considering an imperfect test with 95% test sensitivity and specificity, using epitools software (http://epitools.ausvet.com.au) (HUMPHRY et al., 2004, SERGEANT, 2017). The estimated sample size was 413. The number of animals from each farm to be screened for detection of disease was determined by using the finite population size of the farm, 30% expected prevalence and 99% probability of detection (PUTT et al., 1988). As a required protocol, the animals on these farms are periodically screened for many of the sexually transmitted and/ or abortion causing infectious diseases. The serum samples received from these farms after the required testing were stored at -20°C. In this study, the latest batch of serum samples from the respective farms that were available in the laboratory were used. A simple random sampling method was used to select the

individual animals from each farm to be screened in this study. Random numbers were generated from the list of animals available from each farm by using the epitools software. A total of 880 animals (646 cattle and 234 buffaloes) were selected for detection of antibodies against BVDV.

Sample analysis. The serum samples were tested by a commercially available competitive ELISA test kit (Priocheck BVDV antibody test, Prionics) for detection of antibodies against BVDV. This kit utilizes two monoclonal antibodies recognizing different epitopes located on the NS3 non-structural protein (p80) of BVDV. The sensitivity and specificity of the kit has been reported to be 97.9% and 99% respectively (KRAMPS et al., 1999). The test was performed and the results were interpreted as per the manufacturer's instructions. The percentages of inhibition were calculated as per the formula: PI=100-(corrected OD_{450} test sample/ corrected OD_{450} max)* 100. Test samples with a PI value < 50 were recorded as negative, and samples with PI value ≥50 were recorded as positive.

The presence of BVD antigens in the serum samples was detected using an E^{RNS} based antigen ELISA kit (IDEXX BVDV Ag/serum plus kit; IDEXX). The test method records 100% sensitivity and specificity for detection of BVD antigens when compared with virus isolation and fluorescent activated cell sorting (FACS) methods, according to the manufacturer's report. The test was able to detect infection with various subtypes of BVDV-1, BVDV-2 and HoBiPeV (BAUERMANN et al., 2012, MOORTHY et al., 2019). The test was performed according to the manufacturer's instructions, and the S-N (sample –negative) value was calculated. If the S-N value was >0.3, the sample was declared positive, and others with a value <0.3 were declared negative. The animal was declared persistently infected (PI) if it tested positive twice in the antigen ELISA test when sampled at an interval of at least three weeks.

Statistical analysis. The apparent and true prevalence were determined using epitools software, considering test sensitivity at 0.979, test specificity at 0.99 and a confidence level of 95% (SERGEANT, 2017). The Chi square test or the Fisher exact test was used to compare the prevalence results for species, breed, sex and farms. The difference was considered significant if the P value was <0.05. Logistic regression analysis was performed to detect the effect of other independent variables. The statistical analyses, *viz*. Chi square test and logistic regression analysis, were performed using SPSS software (version 16).

Results and discussion

The overall true seroprevalence was 56.67% (95% CI: 53.26-60.02). As BVD vaccination is not practised in India, the positivity was only considered as originating from infection. The observed prevalence was similar to the world mean antibody prevalence of 49.2% (SCHARNBÖCK et al., 2018). However, previous studies in India have reported a comparatively lower prevalence (15.29%-30%) of BVD (SUDHARSHANA et al., 1999, SOOD et al., 2007, KULANGARA et al., 2015). This could be because the previous studies undertook random sampling from the field (village conditions) as part of various projects. It has been reported that BVD prevalence is influenced by many external factors, such as sampling period, production type, the age of the animals sampled, farm management practices adopted, the sensitivity and specificity of the diagnostic method used etc. (reviewed in SCHARNBÖCK et al., 2018).

Of the 14 herds screened in this study, the presence of BVD antibodies was ascertained in 13 herds, suggesting the between herds prevalence to be 92.86%. The true animal level prevalence within positive herds varied from 9.72% to 99.99% (Table 1). The finding of this study was similar to a herd level seroprevalence study undertaken in Ireland that reported 90% between herd prevalence, and 77.7% within herd prevalence, ranging from 42.8% to 88.3% (BARRETT et al., 2018). A previous study undertaken in Tamil Nadu reported within herd prevalence of 12-65%, which is lower than our study (KUMAR et al., 2018). This discrepancy in prevalence rates could be because in this study we screened medium and large size herds, whereas the others concentrated mostly on small and medium size herds. Increases in herd size have been reported to be an important risk factor for high BVD seropositivity (BARRETT et al., 2018, KUMAR et al., 2018).

Table 1. Prevalence of antibodies to BVDV in different variables

Variable	Description	No of samples tested	No of samples positive	Apparent Prevalence		True Prevalence	
				% positivity	95% CI	% positivity	95% CI
Species	Cattle	646	416	64.40	60.63-67.99	65.42	61.54-69.14
	Buffalo	234	76	32.48	26.80-38.72	32.49	26.63-38.93
Cattle breedtype	Indigenous	106	18	16.98	11.02-25.25	16.49	10.34-25.03
	Cross breed	267	163	61.05	55.08-66.70	61.97	55.81-67.80
	Exotic	273	235	86.08	81.47-89.69	87.8	83.05-91.53
Sex	Male	536	259	48.32	44.12-52.55	48.83	44.50-53.20
	Female	344	233	67.73	62.62-72.45	68.87	63.59-73.74
Farm	Farm 1	89	49	55.06	44.73-64.97	55.79	45.13-66.01
	Farm 2	27	19	70.37	51.52-84.15	71.59	52.14-85.81
	Farm 3	44	22	50.00	35.83-64.17	50.57	35.95-65.19
	Farm 4	53	32	60.38	46.94-72.41	61.28	47.41-73.70
	Farm 5	63	26	41.27	29.96-53.58	41.56	29.88-54.27
	Farm 6	68	65	95.59	87.81-98.49	97.61	89.59-100
	Farm 7	48	5	10.42	04.53-22.17	9.72	03.65-21.84
	Farm 8	56	11	19.64	11.34-31.84	19.24	10.67-31.83
	Farm 9	121	120	99.17	95.47-99.85	99.99	97.49-100
	Farm 10	27	12	44.44	27.59-62.69	44.83	27.44-63.66
	Farm 11	85	52	61.18	50.55-70.84	62.1	51.13-72.07
	Farm 12	84	60	71.43	61.0-79.98	72.68	61.92-81.51
	Farm 13	38	19	50.00	34.85-65.15	50.57	34.93-66.20
	Farm 14	77	0	0.00	0-04.75	0	0-03.87
	Total	880	492	55.91	52.61-59.16	56.67	53.26-60.02

Species-wise sorting of the results revealed much higher positivity among cattle (65.42%; 95%CI: 61.54-69.14) than buffaloes (32.49%; 95%CI: 26.63-38.93), and the difference was statistically significant (P<0.001). Multivariate logistic regression analysis revealed the odds ratio to be 4.212 (95% CI: 2.769-6.408) and the P value <0.001 (Table 2). Perusal of previous studies also revealed a lower prevalence in buffaloes than cattle (SOOD et al., 2007, DENG et al., 2015, EVANS et al., 2019). This indicates that buffaloes are less susceptible to BVDV infection than cattle, but the possibility of false negative results because of the test kit used should not be ignored. The test kit used in this study was validated in cattle and not in buffaloes. Previous studies using a virus neutralization test for detection of BVDV antibodies reported a high prevalence in buffaloes (MUKHERJEE et al., 1989, PAIXÃO et al., 2018).

Sex-wise analysis revealed 48.83% (95%CI: 44.50-53.20) positivity in males and 68.87% (95% CI: 63.59-73.74) in females, and this difference was also found to be statistically significant (P<0.001). However, multivariate logistic regression analysis revealed an odds ratio of 1.008 (95% CI: 0.684-1.485) and a P value of 0.970, indicating that the observed difference was not statistically significant (Table 2). A BVD prevalence study in mithuns revealed a higher prevalence (20.9%) in males than in females (12.1%) (SINGH et al., 2017).

Table 2. Significance of the difference observed in the prevalence of BVDV among different variables

Type	Description	Crude P value	Odds ratio	95% confidence interval	adjusted P value
Species	Cattle	<0.001	4.212	2.769-6.408	<0.001
	Buffalo				
Cattle breed-type	Indigenous-Exotic	<0.001	30.739	6.50-57.268	<0.001
	Indigenous-crossbreed				
Sex	Male	<0.001	1.008	0.684-1.485	0.97
	Female				
Farm-type	Medium Size	<0.001	1.071	0.710-1.614	0.744
	Large size				

Analysis of BVD prevalence among cattle breed types revealed a lower prevalence among indigenous breeds (16.49%; 95%CI: 10.34-25.03) than cross breeds (61.97%; 95%CI: 55.81-67.80) and exotic cattle (87.80%; 95%CI: 83.05-91.53), and the difference was statistically significant (P<0.001). Multivariate logistic regression analysis between indigenous and exotic cattle revealed an odds ratio of 30.739 (95% CI: 16.500-57.268; P value <0.001), and between indigenous and crossbreed cattle the odds ratio was 7.754 (95% CI: 4.395-13.681; P value <0.001) (Table 2). It has been suggested that indigenous breeds are better adapted to the local climatic conditions, and therefore may be less susceptible to infectious agents than cross breeds and exotic breeds.

Farm-wise prevalence ranged from 0.0-99.99% and this difference was found to be statistically significant (P<0.001). For logistic regression analysis, the farms were categorised as medium (<100 herd size) and large (>100 herd size). The analysis revealed an odds ratio of 1.071 (95% CI: 0.710-1.614), and a corrected P value of 0.744. Wide variation in prevalence among herds has been reported world-wide, and various management practices, artificial insemination, sex, herd demographic structure, herd size, frequency of purchase and trading activities have been reported to be important risk factors (SCHARNBÖCK et al., 2018). Among the semen stations, the prevalence varied from 9.72-72.68%. In order to ascertain the presence of PI animals in the farm, whole herd

screening was carried out using a BVD antigen ELISA kit, and none of the animals were found to be positive. These semen stations are open herds as new high genetic merit bull calves are procured regularly through a progeny selection/ pedigree selection programme, followed by adaptation of quarantine measures. The MSP protocol mandates screening of all quarantined bull calves, so they are tested for BVD antigens by ELISA or real-time PCR (below 6 months age), and should be declared negative before induction onto the farm. Since these semen stations strictly adhere to the MSP guidelines and all the animals in the herd were declared negative by an antigen ELISA test, it appears that the BVD antibodies are due to past infections. However, although rare, a persistent testicular infection (PTI) has been detected in some bulls after recovering from acute infections (VOGES et al., 1998, GIVENS et al., 2009). These PTI bulls can excrete the virus in their semen resulting in the spread of infection to naïve cows (GIVENS et al., 2009, WOAH, 2015). Therefore, it is recommended to screen the continuous semen batches from BVD seropositive bulls to detect PTI bulls, and remove those PTI bulls from semen stations.

The screening of animals from the four bull mother farms/ dairy farms revealed interesting results. All these four farms are large, closed herds and therefore the chances of introduction of a new infection to the farm is negligible, but the possibility of within herd transmission is very high due to several management practices (PAIXÃO et al., 2018). One herd housing nearly 400 animals was found to be free of BVD antibodies, suggesting that the virus had never been introduced onto the farm, and also confirming it is possible to maintain BVD free herds. On two farms a very high BVD antibody prevalence (97.6% and 99.99%) was recorded, which led to the suspicion of the possibility of PI animals in the herds. Whole herd screening by antigen ELISA testing confirmed this, and one animal from each of the farms was detected as the PI animal. The farm authorities were advised to remove the PI animal from their farm. In the farm showing 62% prevalence, none of the animals turned positive by the antigen ELISA test. Further, the farm screens all new born calves for BVD PI status at regular interval. Therefore, this high prevalence of BVD on the farm was puzzling and needs further investigation.

Control of BVD infection on farms is important to prevent direct losses due to disease, on animal welfare grounds, as well as to reduce the use of antibiotics and other reactive measures resulting from the immunosuppressive effect of the virus (YARNALL and THRUSFIELD, 2017). Vaccination for control of BVD has not been initiated in India yet, and furthermore the effectiveness of the currently available vaccines in control of HoBiPev infection is questionable (BAUERMANN et al., 2013). Therefore, the current control of BVD depends on the prompt detection and removal of PI animals from herds, and implementation of biosecurity measures to prevent introduction of new infections to the herd. Therefore, all new born calves from BVD infected herds should be screened as early as possible for PI status. Further, all new animals to be introduced onto a farm should undergo quarantine, where they should be tested by both BVD antigen and antibody tests. The animals should be found negative by the BVD antigen test and there should not be any new seroconversion during the quarantine period (WOAH, 2015).

In conclusion, an overall seroprevalence of 56.67% was recorded. The presence of BVD antibodies could be detected in all herds screened but one, suggesting the endemicity of the disease in India. Buffaloes were found to be less susceptible than cattle, and among the cattle, indigenous breeds were found to be less susceptible. Two PI calves were detected on farms reporting very high BVDV prevalence. The study advocates screening of new-born calves and newly purchased animals, detection of PI animals and adaptation of other biosecurity measures for control of this important disease.

Conflict of Interest

The authors declare no conflict of interest related to this article.

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Author Contributions

Laxmi Narayan Sarangi: Conceptualization, Methodology, Investigation, Project administration, Data Curation, Formal Analysis, Writing- Original draft; Kota Sri Naga Leela Surendra: Investigation, Samir Kumar Rana: Conceptualization, Funding acquisition, Project administration, Supervision, Writing-Review and Editing; Naveena Thodangala: Investigation; Amitesh Prasad: Resources, Investigation; Ponnanna Nadikerianda Muthappa: Resources, organising resources, Writing-Review and Editing; Girish Kumar Sharma: Conceptualization, Funding acquisition, Supervision, Writing-Review

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SAŽETAK

Virusni proljev goveda (BVD) važna je zarazna virusna bolest od koje obolijevaju goveda diljem svijeta. Osim izravnoga gubitka uzrokovanog bolešću, virus uzrokuje imunosupresiju zbog čega domaćin postaje podložan drugim bolestima. Provedeno je presječno istraživanje kako bi se otkrila prevalencija BVD-a u 14 organiziranih uzgoja koji su uključivali farme bikovskih majki i stanice za proizvodnju sjemena za UO. Uzgoji su se nalazili u različitim područjima Indije. Ukupno je 880 uzoraka seruma (646 goveda i 234 bivola) analizirano komercijalnim ELISA testom za otkrivanje protutijela na regiju p80 (NS3) BVDV-a. Ukupna je stvarna prevalencija iznosila 56,67 % (95 % CI: 53,26 – 60,02 %), a unutar stada kretala se u rasponu od 0 do 99,99 %. Stopa prevalencije bila je veća u goveda (65,42 %) nego u bivola (32,49 %) i razlika je bila statistički znakovita. Nadalje, zabilježena je znakovita razlika u prevalenciji među pasminama goveda, s tim da je najmanja bila u autohtonih pasmina goveda (16,49 %), slijede zatim križanci (61,97 %) te egzotične pasmine (87,80 %). Veća je pozitivnost zabilježena u ženki (68,87 %) u odnosu na mužjake (48,83 %), ali multivarijantna regresijska analiza nije potvrdila znakovitost te razlike. Među deset istraživanih stanica za proizvodnju sjemena za UO, prevalencija je varirala od 9,72 % do 72,68 %. No ni jedna životinja iz tih stanica nije bila pozitivna na antigenskom ELISA testu, što pokazuje da protutijela pronađena u ovom istraživanju potječu od prijašnjih infekcija. Na dvjema farmama mliječnih krava - bikovskih majki tijekom testiranja cijelog stada antigenskim ELISA testom, utvrđena je visoka pozitivnost pri čemu su dvije krave bile stalno zaražene. Na jednoj farmi bikovskih majki nisu pronađena BVD protutijela što upućuje na to da je moguće održati stada bez ove bolesti. Rezultati istraživanja upućuje na endemičnost BVDV-a u organiziranim uzgojima goveda u Indiji zbog čega postoji potreba za odgovarajućim strategijama testiranja i upravljanja stadom kako bi se kontrolirao unos bolesti i njezino širenje na farmama.

Ključne riječi: virusni proljev goveda; virus BVD; prevalencija; Indija; trajna infekcija; protutijelo; antigen