

- reproductive failure -Tamilnadu

Bovine viral diarrhoea (BVD) is an economically important disease of dairy cattle all over the world with two biotypes viz., cytopathic and non – cytopathic viruses and further classified as various genotypes like BVDV -1, BVDV -2 and Hobi like pestivirus called BVDV-3. Cattle of all ages are susceptible to infection with bovine viral diarrhoea viruses (BVDV). Bovine viral diarrhoea infection results in a wide variety of clinical manifestations, including enteric, respiratory and reproductive disease in susceptible hosts. (Neibergs *et al.*, 2011). Most of the BVDV isolates from dairy animals belong to BVDV -1 and subtype of BVDV-1b strain based on the 5' UTR and Npro region (Mishra *et al.*, 2004; Deng *et al.*, 2012). Mukherjee *et al.* (1989) reported that the prevalence of BVD antibodies in cattle (65.9%) exhibited a wide range of clinical signs such as chronic diarrhoea, buccal ulceration, conjunctivitis and abortion in dairy Serasssanimals. Seroprevalence of the disease among tropical countries has been reported (Mishra *et al.*, 2011), but detailed reports on actual epidemiology of the disease with pertaining to the genotype of BVD on the small dairy holder of Tamil Nadu is lacking. Grooms (2004) observed that interruption of normal fertilization or embryonic death may be the mechanism for decrease in conception rate in BVDV-infected cattle. The decrease in milk yield, high bulk milk somatic cell count and increased risk of clinical mastitis in dairy cows were associated with BVDV infections were also believed by immunosuppressive effect (Waage, 2000; Beaudeau *et al.*, 2005).

Materials and Methods

Source of clinical samples

A total of each 500 sera and whole blood in EDTA tubes, were collected by random sampling method from 62 organized dairy farms located in north western zone of Tamil Nadu for assessing the prevalence and detection of BVDV infection in the study population and transported to the laboratory under cold chain as per the procedure described in OIE (2015). Clinical samples were collected from animals not vaccinated against BVD. IDEXX BVDV Total Ab Test kit (HerdChek* BVDV Ab IDEXX, Lieberfeld- Bern, Switzerland) was used to detect BVD antibodies

from serum samples.. The sensitivity and specificity of commercial indirect ELISA (FarmChek* BVDV Ab IDEXX, Liebefeld- Bern, Switzerland) was 68.18 and 98.18 per cent respectively. One step RT PCR protocol was followed to detect BVDV in whole blood. The cell culture and BVDV strains used for this assay was MDBK cell line, BVDV -1 (Ind S -1449), BVDV -1 (Ind 5 1966) maintained at ICAR -National Institute of High Security Animal Disease, Bhopal

Results

Out of 500 sera samples randomly collected from 62 dairy cattle farms from north western zone of Tamil Nadu. Out of 66 animals were seropositive for BVD by indirect ELISA. Out of 500 samples, 77 sera samples randomly selected and screened for BVD antibodies by virus neutralization test (VNT), 22 sera samples were found to be positive with per cent positivity of 28.57. Among the 22 positive sera samples, 16 samples (72.72%) were positive for BVD-1 and 19 samples (86.36%) were positive for BVD-2. Twelve samples showed both BVD1 and BVD2. Among the samples positive for virus neutralization test, 13 animals (59.09%) were suffering from reproductive disorders, two animals showed enteritis & mastitis, 27.27% (6/22) animals were pregnant and not showed any clinical signs and one animal showed pneumonia One serum sample was positive for BVD by ELISA but negative by VNT and seven sera samples were BVD positive by VNT but negative by ELISA (Table I). Among the BVD positive sera samples, 3 samples were positive for IBR, one sample positive for Brucellosis and six samples shown positive for Tuberculosis with per cent positivity of 4.50, 1.50 and 9.10 respectively by ELISA Among 434 sera samples were negative for BVD antibodies, six samples were shown positive for IBR, each one sample were found to be positive for Brucellosis and Tuberculosis respectively. The sero prevalence of BVD was statistically significant with Brucellosis and Tuberculosis at 0.05 level ($p < 0.05$). However, the seroprevalence of BVD in dairy cattle was statistically not significant ($p > 0.05$) between IBR in dairy cattle of north western zone of Tamil Nadu (Table II).

Discussion

Bovine viral diarrhoea is an economically important viral disease contributes to a variety

Table I. Comparison of serum i-ELISA with VNT

i -ELISA Values	VNT values		Total
	Positive	Negative	
Positive	15	1	16
Negative	7	54	61
Total	22	55	77
Statistic	Formula	Value	95% CI
Sensitivity (%)	a/a+b	68.18%	45.13% to 86.14%
Specificity (%)	d/c+d	98.18 %	90.28% to 99.95%

BVD-1 positive -16, BVD-2 positive-19 and BVD-1 and BVD-2 Positive-12

Table II. Concurrent infection of IBR, Brucellosis and Tuberculosis in BVD seropositive dairy cattle of north western zone of Tamil Nadu

Variable	IBR		Brucellosis		Tuberculosis		Total
	Positive	Negative	Positive	Negative	Positive	Negative	
BVD Positive	03	63	01	65	06	60	66
BVD Negative	06	428	01	433	01	433	434
Total	09	491	02	498	07	493	500
% within BVD sero positive	4.5	95.5	1.5	98.5	9.1	90.9	
P value	3.242		2.373		32.582		13.20
Chi square value	0.072		0.123		0.000^a		

Note: Values with superscripts significantly differs at 0.05 level ($p < 0.05$)

of important clinical symptoms that includes reproductive, respiratory and gastrointestinal systems in dairy cattle, sheep, goat, pigs and wildlife animals' species. The present seroprevalence of BVD in dairy cattle of north western zone of Tamil Nadu was 13.20 per cent during the study period. The seropositivity percentage in i-ELISA was 13.2 and in VNT 28.6. It seems that VNT has more sensitivity than iELISA in this study, seven sera samples were positive in VNT and negative in i-ELISA. This might be due to VNT would detect both IgM and IgG antibodies where as in i-ELISA detects only IgG since antiIgG antibodies were used as secondary antibody and in VNT, Indian BVDV strains (BVD-1 and BVD-2) were used for neutralization of serum antibodies. This is in agreement with the findings of OIE (2015) who reported that diagnostic test developed for

detection of BVD identify one or more biological response for hosts. Chase *et al.* (2003) and Sarrazin *et al.* (2013) stated that both diagnostic tests have been used to classify dairy cattle at different stage of disease in the present study. In the present study only one serum sample was positive in i-ELISA which was negative in VNT. This could be due to higher dilution of serum (1 in 10) in VNT when compared to (1 in 5) i-ELISA. Among 24 sera samples positive by VNT, 15 samples were positive for BVD-1 and 19 were positive for BVD-2 whereas 12 sera samples were positive for both. This shows that both the BVDV genotypes are circulating in the study area. Hence further studies required with monoclonal BVD antibodies to avoid cross reactivity which commonly encountering in serological studies. The concurrent infection of IBR, Brucellosis and Tuberculosis in BVD

seropositive dairy cattle was 4.50, 1.50 and 9.10 per cent respectively. The seroprevalence of BVD was significantly higher at 0.05 level ($p < 0.05$) in dairy cows concurrently infected with TB when compared with IBR and Brucellosis in north western zone of Tamil Nadu during the study period. Higher seroprevalence of BVD with concurrent infection was observed in dairy cattle is in agreement with the findings of Shirvani *et al.* (2012) who observed strong significant association between being antibody positive to BVD with TB and IBR at farm and animal level, because of BVDV immunosuppressive in nature. Similar findings were also reported by Scott *et al.* (2006) who revealed that concurrent infection of BVD and TB was 28.40 and 9.10 respectively. There are no reports available on the concurrent infection of IBR, Brucellosis and TB in dairy cattle of north western zone of Tamil Nadu to substantiate this claim. Further serosurveillance of BVD in dairy cattle is needed. Out of 500 sera samples, 77 sera samples randomly selected and screened for BVD antibodies by virus neutralization test, 22 sera samples were found to be positive with per cent positivity of 28.57. Among the 22 sera samples positive by VNT, 16 samples (72.72%) were positive for BVD-1 and 19 samples (86.36%) were positive for BVD-2. Twelve samples showed both BVD1 and BVD2. Among the virus neutralization positive samples, 13 animals (59.09%) showed reproductive failure, two animals showed enteritis & mastitis, 27.27% (6/22) animals were pregnant and not showed any clinical signs and one animal showed pneumonia. It was concluded that BVD1&2 genotype causes reproductive failure among the dairy cattle of Tamilnadu.

Conclusion

The seroprevalence of bovine viral diarrhoea with genotype was studied in this work. BVD1&2 was detected in the study population with highest percentage of reproduction failure (54.16%) followed by enteritis, mastitis and pneumonia. A sum of 25% of BVD1 & 2 sero positive animals showed no clinical signs with pregnant. By this research work, it was concluded that, BVD 1 and 2 genotype causes reproductive failure than others and VNT was more sensitive than iELISA to detecting BVD antibodies.

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