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MOLECULAR DETECTION OF BOVINE VIRAL DIARRHOEA VIRUS (BVDV) INFECTION AMONG CATTLE IN DAURA ZONE, KATSINA STATE, NORTHWEST, NIGERIA

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ABSTRACT: *Bovine viral diarrheal virus (BVDV) infection causes a diverse range of clinical outcomes from being asymptomatic, or a transient mild disease, to producing severe cases of acute disease that leading to animal death. The infected animals may suffer from mild diarrhoea or respiratory symptoms or else show no signs of infection at all. BVDV is a small, enveloped single-stranded positive-sense RNA virus, measuring about 12.5kb that belongs to the Pestivirus genus and Flaviviridae family. Proper control of BVDV involved the removal of infected animals from the herd, this can be achieved through proper detection of BVDV-infected animals. This study aimed at molecular detection of BVDV infection among rearing cattle in the Daura zone, Katsina State, Northwestern Nigeria. 125 blood samples were analysed for the presence of BVDV using RT-PCR according to manufacturer information. An overall prevalence rate of 10.4% was obtained, and diarrhoea, and nasal and eye discharge remained major signs of the infection. The study suggests the need for improving sanitary politics in the veterinary sector to prevent potential transmission the BVDV infection among the cattle and other domestic animals in the study area.*

KEYWORDS: Bovine viral diarrhoea virus, Cattle, Daura, Polymerase Chain Reaction,

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INTRODUCTION

Bovine viral diarrheal virus (BVDV) infection serves as one of the important viral infections of cattle in developing countries including Nigeria, and many other countries of the world. The infection causes a diverse range of clinical outcomes from being asymptomatic, or a transient mild disease, to producing severe cases of acute disease leading to death (Strong *et al.*, 2015). The BVDV is a small, enveloped single-stranded positive-sense RNA virus, measuring about 12.5kb that belongs to the *Pestivirus* genus and *Flaviviridae* family (Meyers and Thiel, 1996). The infection with this virus causes severe economic losses due to decreased fertility and milk production, slow fetal growth, diarrhoea, respiratory symptoms, and reproductive dysfunctions such as abortion (Khodakaram-Tafti *et al.*, 2017). Due to the broad nature of the disease, transmittance, and lack of treatment have made it a globally enzootic, and one of the most significant cattle diseases (Uzal *et al.*, 2016).

Genetically and antigenically the virus has two genotypes of BVDV have been classified as BVDV-1 and BVDV-2, with at least 21 sub-genotypes in BVDV-1 and 4 sub-genotypes in BVDV-2 (Donoso *et al.*, 2018), the two genotypes are closely associated with two fundamental difference forms of infection in cattle which may be either transient or persistent (Peterhans *et al.*, 2010). The infected animals may suffer from mild diarrhoea or respiratory symptoms or else show no signs of infection at all. But, after seroconversion, the animals are protected from re-infection for life. Persistent infection is initiated in embryos from the second to fourth of the 9.5 months of development (Peterhans *et al.*, 2010).

Cattle are found throughout Nigeria, which is most common in the northern part (two-thirds of the country) (Bourn 1983). Nigeria is one of the great potential markets for million tons of milk production which is valued at around $1.5 million annually (Annatte *et al.*, 2012). The estimated total domestic fluid milk of about 600,000 litres which is worth about $770,000 produced in 2006 entered through formal marketing channels that include, corporate, public, and other private milk collection co-operatives schemes from migrant herdsmen, and the output of the few commercial dairy farms (Annatte *et al.*, 2012). The White Fulani or Bunaji breeds are the dominant dairy breed found in the northwest part of Nigeria. The pastoralists own and maintain the majority of the cattle and the cattle are fed on natural grass under the traditional system with little supplementary feeding regimes in some places (Annatte *et al.*, 2012).

Control of BVDV involves removing persistently infected (PI) animals from the herd, ensuring biosecurity and vaccination of susceptible animals (Moennig *et al.*, 2005). Several countries, including Nigeria, have initiated eradication programmes and many of these countries are close to or have achieved BVDV-free status (Presi *et al*, 2011). To achieve the proper control programme of the BVDV there is a need to ascertain the presence of BVDV infection among cattle rearing in the Daura zone, Katsina State, Northwest Nigeria, which can provide baseline data for future surveillance in the area. Therefore, this study aimed at molecular detection of BVDV infection among rearing cattle in the Daura zone, Katsina State, Northwestern Nigeria.

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MATERIALS AND METHODS

Study Area

The study was carried out in the Daura Zone Katsina State, Nigeria, located in Northwest, Nigeria at Latitude 13.03639°N and Longitude8.31778°E. The Zone has a relative humidity ranges from 22 to 52 (Wikipedia, 2020). Daura zone is the spiritual home of the Hausa and Fulani people that farming and cattle rearing being their major occupations.

Study Population

A total of 125 cattle rearing in the Daura zone of Katsina State, Northwest Nigeria, that has evidence of severe signs of BVDV infection were enrolled in the study.

Sample Collection and Processing

Five miles (5ml) of blood samples were collected through a jugular venous puncture in the sterile plain vacutainer tubes from cattle that showed evidence of severe signs of BVDV infection after consenting the farmer reared the animal. The samples were stored at -20^{o}C until analysis.

Molecular Detection Method

The molecular assay was carried out using Reverse Transcription Polymerase Chain Reaction PCR (RT-PCR). The PCR is a primer-mediated, temperature-dependent technique used for the enzymatic amplification of a specific sequence of DNA or RNA template.

RNA Extraction of Bovine Diarrhea Virus

Viral RNA in the serum samples of animals was obtained using a commercial extraction kit. Extraction was performed following the manufacturer's instructions (RNeasy Mini Kit, Qiagen, Germany).

Carrier RNA and RNA binding beads A were added to the binding buffer according to the table below and mixed briefly. The mixture is called Binding Solution.

Table 1 Equipment and reagent preparation

Binding Solution	Per Test
Binding Buffer	500ul
Carrier RNA	6ul
RNA Binding Beads A	20ul

Reverse Transcription (RT) PCR Protocol

The master mix was prepared, by mixing the 19.75µL PCR Mix and 0.25µL Enzyme mix to give the total volume of 20µL as the master mix. Using a sterile micropipette 20µL of the master mix was pipetted into a sterile real-time PCR reaction tube. Then, 5µL of RNA sample was added to each. Both positive and negative control were included in each set of reactions. The tubes were closed immediately to avoid contamination. The plate was loaded into a real-time PCR machine and set the amplification protocol as indicated in Table 2.

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Table 2 the following protocol was performed in the machine.

Temperature (^oC)	Time	Number of Cycles
45	10 Minute	1
95	15 Minute	1
95	15 Second	
60	1 Minute	40

Fluorescence measured at 60^0C) for 40cycles

Table 3 Selected fluorescence channels

Component	**Reporter**
Internal Control	HEX/JOE
Target Nucleic acid	FAM

Selection of fluorescence channels

Interpretation of Results

For the assay to be valid, the positive control must give a signal in both the FAM and HEX channels with a CT* <36. The Negative Control must not give a signal in the FAM channel.

Table 4 Interpretation of Results

Sample Result	**Result Interpretation**	
	FAM (Tested Sample)	**HEX/JOE (Internal Control)**
BVDV Positive	X	X
BVDV Strong Positive	X	
BVDV Negative		X

Data Analysis

The data obtained in this study were analysed using the statistical package for social sciences statistical software (SPSS) version 25.0. Categorical variables were presented as frequency and percentage. Chi-square or Fisher's test was used to obtain the relationship between all the associated variables. P values ≤ 0.05 was considered significant.

RESULT

From the entire 125 non-duplicated cattle serum samples process for the molecular detection of BVDV infection, an overall 10.4% (13.125) was obtained (Table 5). The highest BVDV RT-RNA prevalence of 11.4% (8/70) was obtained in female cattle and 9.1% (5/55) from male cattle. Calf cattle had the highest RT-RNA positive 15.2% (5/33), followed by 10.0% (5/50) and 7.1% (5/42) in adults and young adults, respectively. Statistically, the study showed no significant association between the occurrence of RT-RNA and sociodemographic variable as *p-value* >0.005 (Table 6). Comparison between the RT-RNA positive result and possible risk factors shows sharing feeding material can serve as a great risk factor and history of abortion. Although, all the observed possible risk factors showed no statistically significant association p>0.05 (Table 7). The result of the study indicated a great association between BVDV RT-RNA positive with the presence of diarrhoea, nasal discharge, and eye discharge

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(p<0.05). But, a decrease in milk production and failure to nurse showed a statistically significant difference (p>0.05) as shown in table 8. The total mean± standard deviation of BVDV RNA viral load of 26846.15±10753.65 per ml was obtained in the serum of BVDV RNA positive animals by RT-PCR (Table 9).

Table 5 Distribution of BVDV among Cattle in Daura Zone, Katsina State, Nigeria

PCR Result	Frequency	Percentages
Positive	13	10.4
Negative	112	89.6
Total	125	100.0

Table 6 Relationship between BVDV RT-RNA positive and Socio-Demographic Variables

Socio-Demographic Variables	**No. Tested**	**RT-RNA**		***P-value***
		Positive (%)	**Negative (%)**	
Sex				
Male	55 (44.0)	5 (9.1)	50 (90.9)	0.905*
Female	70 (56.0)	8 (11.4)	62 (88.6)	
Total	**125 (100.0)**	**13 (10.4)**	**112 (89.6)**	
Age (Years)				
Calf (<1)	33 (26.4)	5 (15.2)	28 (84.9)	0.526
Young Adult (1 – 2)	42 (33.6)	3 (7.1)	39 (92.9)	
Adult (>2)	50 (40.0)	5 (10.0)	45 (90.0)	
Total	**125 (100.0)**	**13 (10.4)**	**112 (89.6)**	
Breed				
Sokoto Gudali	37 (29.6)	4 (10.8)	33 (89.2)	0.773
White *Fulani*	49 (39.2)	4 (8.2)	45 (91.8)	
Mixed Breed	39 (31.2)	5 (12.8)	34 (87.2)	
Total	**125 (100.0)**	**13 (10.4)**	**112 (89.6)**	

Table 7 Relationship between BVDV RT-RNA positive and Possible Risk Factors

Possible Risk Factors	**No. Tested**	**Positive (%)**	**OR**	**95% CI**	**P-Value**
Sharing Feeding Material					
Yes	92 (73.6)	11 (12.0)	1.973	0.461 – 8.437	0.556
No	33 (26.4)	2 (6.1)			
Total	**125 (100.0)**	**13 (10.4)**			
History of Abortion					
Abortion	20 (30.8)	2 (10.0)	0.900	0.191 – 4.252	0.999
No Abortion	45 (69.2)	5 (11.1)			
Total	**65 (100.0)**	**7 (38.9)**			

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History of Vaccine					
Yes	0 (0.0)	0	-	-	-
No	125 (100.0)	13 (10.4)			
Total	**125 (100.0)**	**13 (10.4)**			

Table 8 Comparison between BVDV PCR positive and Possible Sign of the infection

Possible Sign	No. Tested	Positive (%)	OR	95% CI	P-Value
Diarrhea					
Yes	39 (31.2)	10 (25.6)	7.350	2.141 – 25.230	0.000
No	86 (68.8)	3 (3.5)			
Total	**125 (100.0)**	**13 (10.4)**			
Nasal Discharge					
Yes	42 (33.6)	11 (26.2)	10.870	2.524 – 46.810	0.000
No	83 (66.4)	2 (2.4)			
Total	**125 (100.0)**	**13 (10.4)**			
Eye Discharge					
Yes	31 (24.8)	9 (29.0)	6.823	2.258 – 20.610	0.000
No	94 (75.2)	4 (4.3)			
Total	**125 (100.0)**	**13 (10.4)**			
Decrease in Milk Production					
Yes	10 (18.9)	3 (30.0)	6.450	1.237 – 33.630	0.082
No	43 (81.1)	2 (4.7)			
Total	**53 (100.0)**	**5 (10.4)**			
Failure to Nurse					
Yes	2 (10.5)	0 (0.0)	-	-	0.400
No	17 (89.5)	3 (17.7)			
Total	**19 (100.0)**	3 (15.8)			

Table 4.9 Viral Load of BVDV RNA positive cattle in Daura Zone, Katsina State

	Positive BVDV RNA	Viral Load
Total BVDV RNA	13	26846.15±10753.65
Gender		
Male	5	26800.00±7463.24
Female	8	26875.00±12900.03
Age		
Adult	5	23400.00±6655.83
Young Adult	3	29000.00±19697.72
Calf	5	29000.00±9192.39
Shearing Food Material		
yes	11	25363.64±8476.71
No	2	35000.00±22627.42

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Chart I: Distribution of BVDV among Cattle in Daura Zone, Katsina State, Nigeria

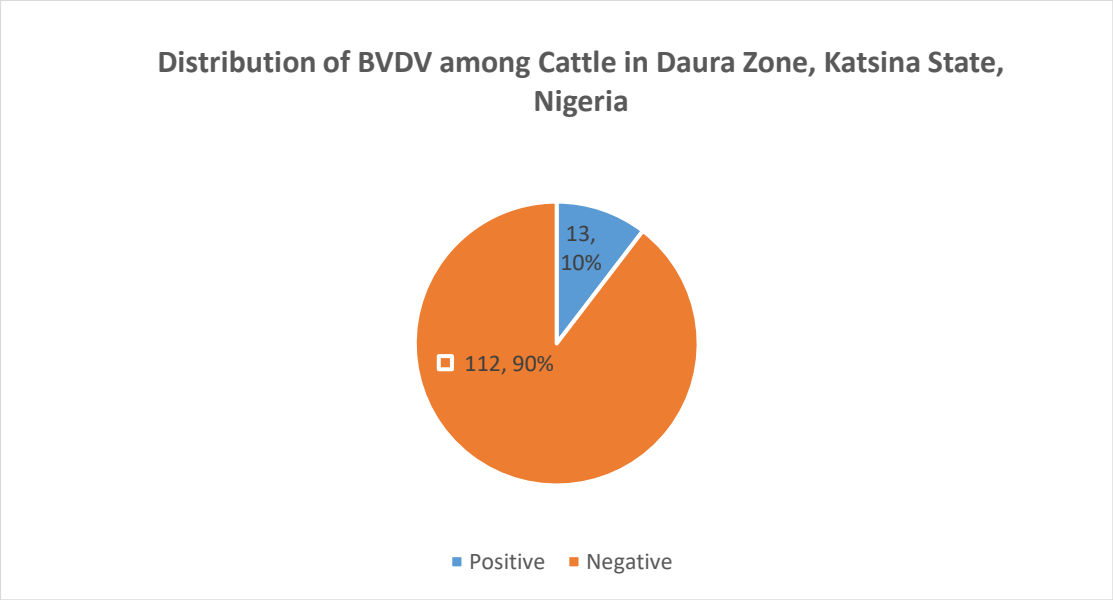
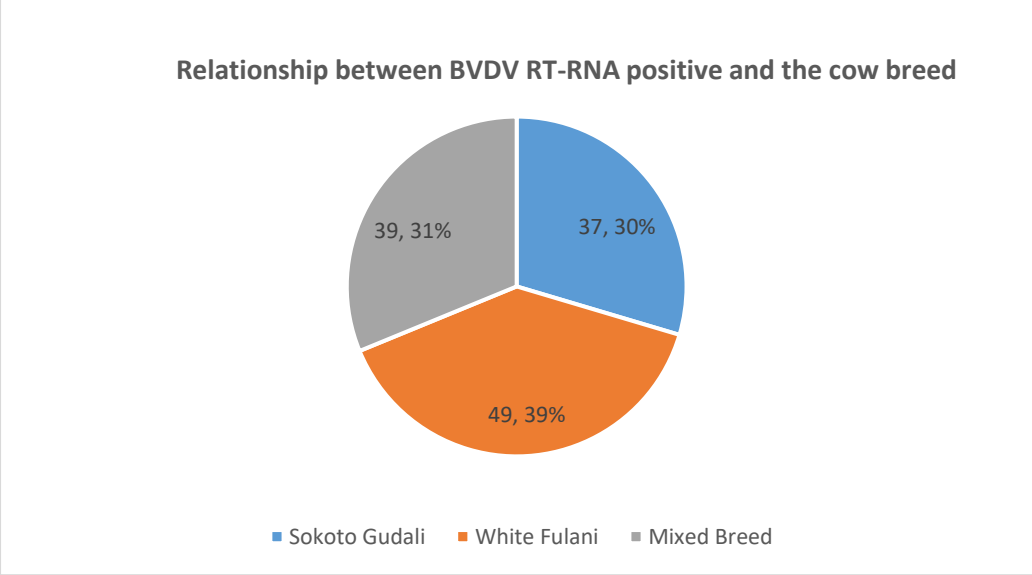


Chart II: Relationship between BVDV RT-RNA positive and the cow breed



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DISCUSSION

The RT-PCR analysis of BVDV RNA is the most sensitive and specific than the other detection method. In this study, the RT-PCR analysis of BVDV RNA is restricted to only 47 BVDV seropositive. Our result demonstrated an RT-PCR prevalence rate of 10.4% (13/125). Previous studies indicated a lower BVDV RNA prevalence rate of 8.7% by Ochirkhuu *et al.* (2016) from Mongolia and 0.0% by Ince and Sait (2022) from Konya Central Anatolia Region. However, higher BVDV RNA prevalence rates of 82.4% were reported by Chang *et al.* (2021), 25.7% by Peddireddi *et al.* (2018), 22.6% by Deng *et al.* (2015), and 100.0% were reported by Mahlum *et al.* (2002).

The present study reported diarrhoea as an important sign of BVDV infection (OR=7.350, 95%CI = 2.141 – 25.230; P=0.000). This report disagrees with the report of Demil *et al.* (2021) in their study done at Gondar city Northwest Ethiopia among dairy cattle and that of Uddin *et al.* (2017) in Bangladesh which both reported the non-significant association between the diarrhea and occurrence of BVDV, as diarrheic animals might have developed diarrhoea from other microbial pathogens. In the present study, nasal and eye discharge also served as possible signs of BVDV infection with (OR=10.870, 95%CI=2.524 – 46.810; P=0.000) and (OR=6.823, 95%CI=2.258 – 20.610; P=0.000), respectively. Several authors were reported respiratory diseases (nasal and eye discharge) could possible sign of BVDV infection (Duong *et al.*, 2008, Kampa *et al.*, 2009; Peter *et al.*, 2015). The total viral load obtained among the BVDV RNA positive in this study was 46846.15±10753.65 (mean±SD) per ml of serum by RT-PCR. This indicated the high concentration of the BVDV virus in the infected animals.

CONCLUSION

The result of this study reported a molecular prevalence rate of 10.4% (13/125) among cattle in the Daura zone Katsina State, by RT-PCR molecular detection techniques. The study suggested that sharing feeding materials among cattle can serve as and risk for transmitting BVDV infection, although the study showed no statistically significant association between all the observed risk factors and the prevalence of BVDV infection. Diarrhoea, and nasal and eye discharge can serve as great signs of BVDV infection.

RECOMMENDATIONS

Based on the outcome of this study, the following recommendation was made

1. There is a need for improving sanitary politics in the veterinary sector to prevent potential transmission the BVDV infection among the cattle and other domestic animals in the study area.

2. There is also a clear need to look at the role of BVDV in co-infections in the whole Nigerian setting to fully understand its importance and evaluate the need and importance for its control.

3. Studies should also be carried out to isolate and genetically characterise the strains of BVDV to determine the disease burden caused by this virus in Nigeria.

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4. Establishing eradication programs within the framework of principles, including identifying and eliminating persistently infected animals, increasing immunity against BVDV infection with vaccination, and implementing biosafety strategies, is a known reality already.

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