Journal of Veterinary and Biomedical Sciences
Vol. 5 (1). PP 24 - 33, July, 2023
***ISSN**: 2659-0743*
DOI: 10.36108/jvbs/3202.50.0140

Bovine Sero- surveillance of Foot- and - Mouth Disease in Four States in Nigeria

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Accepted June, 2023 and Published July, 2023

ABSTRACT

Animals with cloven hooves are susceptible to highly contagious foot and mouth disease (FMD). FMD epidemics have affected several underdeveloped countries, including Nigeria. The migration of pastoralist animals has been linked to FMD transmission in Nigeria, and surveillance is essential for assessing the threat and impact of FMD, as well as the emergence of PCP-FMD. Here, we report the sero-surveillance of FMD in cattle in four states in Nigeria in 2019. Sera collected from four states (Anambra, Enugu, Imo, and Katsina) were tested for antibodies against FMD non-structural protein (NSP), with an overall prevalence of 45%. Significantly higher seroprevalence was recorded in Imo (63%), followed by Kastina (56%), Anambra (29%), and Enugu (6%). An overall serotype-specific prevalence of 81%, 35 %, 50 %, and 35% was recorded for serotypes O, A, 50% for SAT 2, and SAT 1, respectively. Furthermore, circulating antibodies against four serotypes of FMDV were found in 16% of sera samples, 20% had antibodies against three circulating serotypes, 33% had antibodies against two serotypes, and 25% showed exposure to only one serotype, indicating exposure to multiple FMD serotypes. These findings demonstrate the presence of FMDV antibodies in cattle across all surveyed states, which may be related to the presence of multiple FMDV serotypes (O, A, SAT1and SAT2) and concurrent co-infection in these states. Therefore, there is a need for the continuous surveillance of FMD in Nigeria. This will help ascertain the burden of the disease and enable the implementation of necessary control measures to stop its potential transmission and further spread. This sero-surveillance data expands our understanding of the FMD situation in Nigeria and is crucial for determining the country's FMD risk in order to advance the PCP-FMD.

Keywords 3ABC ELISA, Antibodies, Foot and Mouth Disease, Nigeria, Sero-surveillance, Solid Phase ELISA, States

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease that affects cloven-hoofed animals causing economic and social threat to livestock farming [1][2]. The FMD virus (FMDV) has damaging effects on the international trade of livestock and animal products [3] and is detrimental to livelihood, productivity, and food security [4]. FMDV is a prototype member of the genus Aphthovirus in the family *Picornaviridae [5]. There are* seven serotypes of FMDV: A, O, C, Asia1, Southern African Territories (SAT) 1-3 and multiple subtypes for each serotype [6]. However, serotype C has not been reported since 2004 [7]. One of the major means of transmission of FMD is international animal movement through porous borders in West Africa and Nigeria [8]. Currently, five of the seven serotypes of FMDV are present in Africa (O, A, SAT-1, SAT-2, and SAT-3) [9]. FMD is spread extensively in less developed countries, specifically in West Africa, and has four serotypes (A, O, SAT-1, and SAT-2) circulating in Nigeria [10]. In West Africa, there is widespread pastoralist agriculture accompanied by free national and international movement of livestock, which has contributed to the continual spread of FMDV and makes control efforts in Nigeria very challenging [11].

FMD is also considered a potential bioterrorism agent, as it could be deliberately introduced into an FMD-free country to destabilize the economy, and the Food and Agriculture Organization (FMD has recommended and identified the control and global eradication of FAO). Accordingly, the FAO and World Organization for Animal Health (OIE) have proposed a strategy for the global control of FMD. Consequently, a progressive control pathway for foot and mouth disease (PCP-FMD) has been established to support local and regional control efforts (13). However, in Nigeria, where FMD outbreaks and disease burden are still being tracked, surveillance, a key element of disease control, has been restricted and complicated by problems such as insufficient sickness reporting and disjointed national monitoring (14). Disease surveillance is required to assess the risk and effect of FMD, advise control plan, and identify disease patterns for epidemiological analysis in order to enable the generation of information on FMD status [15[16]. Therefore, the main objective of this study was to estimate the seroprevalence of FMD and determine the FMD serotypes circulating in four States of Nigeria.

MATERIALS AND METHODS

Sample Collection

Based on limited information on FMD in the four states of Anambra, Enugu, Imo, and Kastina in Nigeria. Whole blood (6 mL; n = 441) was collected from the cattle live market through the jugular vein using a 10 ml syringe and needle by federal and state epidemiologists in collaboration with National Veterinary Research Institute (NVRI) Vom surveillance officers. The samples were maintained for 6-8 hours at room temperature, slanted at 45 °C, and allowed to clot to facilitate the separation of sera. The sera were decanted into a 5 ml sample collection tube and placed on ice packs for transportation to NVRI, Vom, Nigeria. Aliquots of the serum samples were heat-inactivated at 56°C for one hour at NVRI and shipped to the National Center for Foreign Animal Disease (NCFAD) in Winnipeg, Manitoba, Canada for testing. The sera were stored at -20 °C until use.

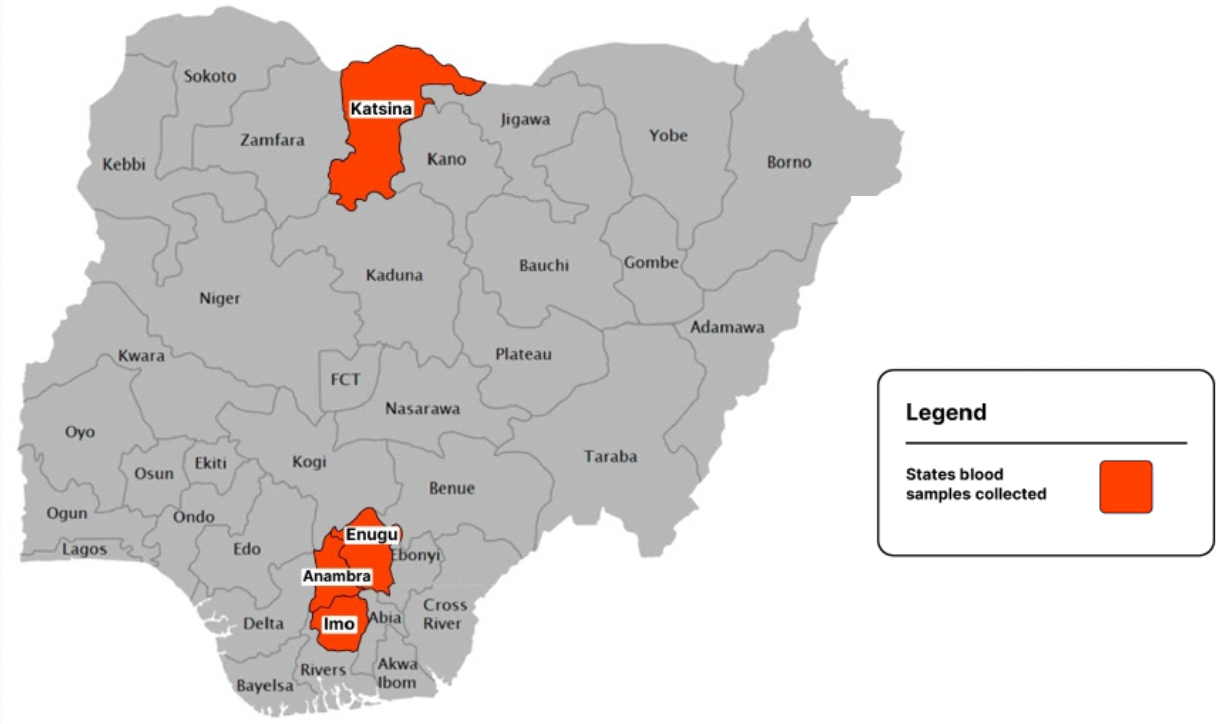

Figure 1: Map of Nigeria showing states where samples were collected

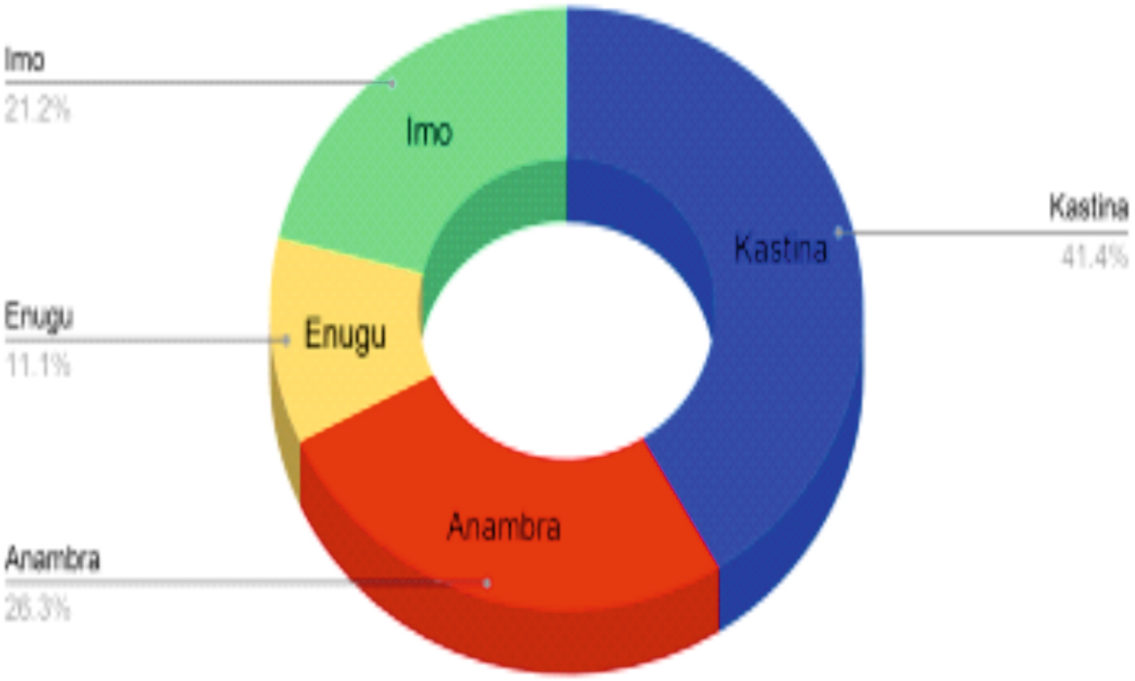

Figure 2: Distribution of serum samples and the percentage of samples collected

Imo (n=93), Katsina (n=183), Anambra (n=116), Enugu (n=49)

Detection of Antibodies to FMD Nonstructural Proteins in Serum Samples

Sera were tested at the NCFAD using an in-house 3ABC competitive ELISA (3ABC cELISA) as previously described Yang *et al* [17] with modifications. Briefly, 96 well plates (VWR, Mississauga, Ontario, Canada) were coated and incubated overnight at 4°C with His-3ABC recombinant antigen (100 µl per well) diluted in carbonate bicarbonate buffer (Sigma Aldrich, Oakville, Ontario, Canada). The next morning the plates were left on the bench for 10-15 minutes to reach room temperature and washed three times with 0.01M phosphate buffer saline containing 0.05% tween-20 (PBST). The samples and controls were immediately added followed by anti 3B monoclonal antibodies (F83Bp 1-3) conjugated to horseradish peroxidase (HRP) in all wells, incubated for 1 hour at 37°C on an incubator shaker. Plates were then washed five times and tetramethylbenzidine substrate (TMB) (100 µL per well) dispensed in all wells and incubated for 10 minutes at room temperature. The color development was stopped by adding TMB stop solution (100 µL per well) to all wells. Thereafter, the optical density (OD) was read at 450 nm absorbance with background subtraction. Test results were expressed as percentage of inhibition and calculated based on the mean optical density values of duplicate samples, compared with a standard negative reference serum. Test results were derived by the following formula previously described by Clavijo *et al* [18]: PI = [(negative reference serum OD–test sample OD)/ (negative reference serum OD–positive reference serum OD)] × 100%.

Detection of Serotype-specific Antibodies using Solid Phase Competitive ELISA (SPCE)

The sera positive for antibodies to FMD NSP by the 3ABC competitive ELISA were further tested at NVRI, Nigeria for antibodies to serotypes O, A, SAT 1 and SAT 2 using a solid phase competitive ELISA (SPCE) (IZSLER Brescia, Italy) as previously described Atuman *et al* [2]. Serum antibodies endpoint titer was expressed as the highest dilution producing 70% inhibition, with endpoint titer ≥70% considered as positive for antibodies to the specific FMDV serotype.

RESULTS

Seroprevalence of FMDV in Four States Surveyed in Nigeria

The seroprevalence of FMD in the study area based on antibodies to FMD NSP was 45% (199/441). Antibodies to FMD NSP were detected in all four states surveyed with prevalence of 63.0% (59 of 93) for Imo, 56.3% (103 of 183) for Katsina, 29.0% (34 of 116) for Anambra and 6.1% (3 of 49) for Enugu states (figure 3).

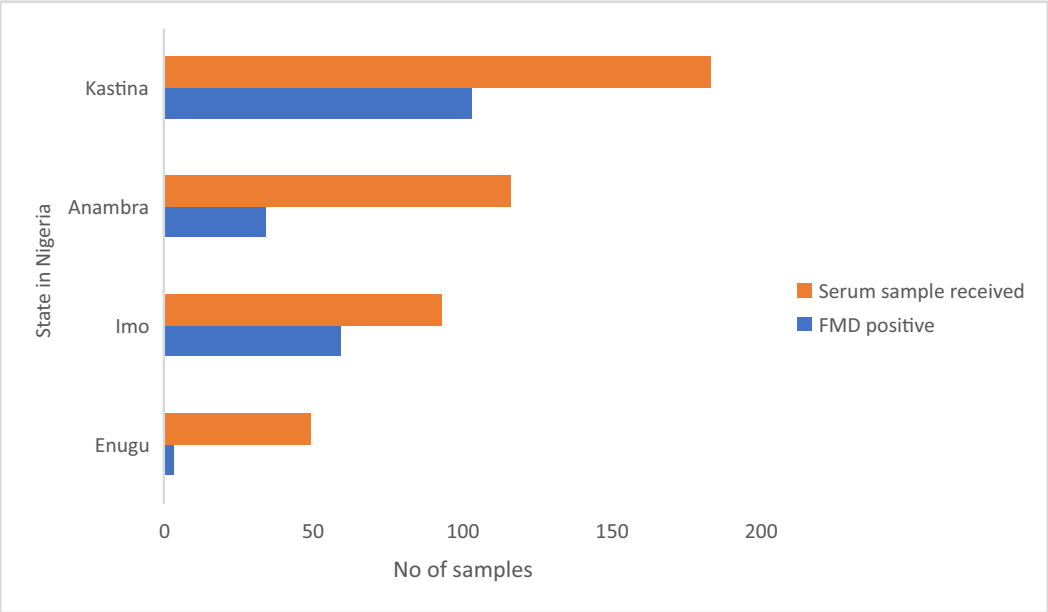

Figure 3: Horizontal bar charts showing the seroprevalence of foot and mouth disease in Nigeria for four states: Anambra (29%), Enugu (6%), Imo (63%), and Katsina (56%), in which sera were collected and the number of positive cases of foot and mouth disease in cattle.

FMDV Serotyping Profile in Four States Surveyed

Antibodies to all the four FMDV serotypes were detected in three states (Anambra, Imo and Katsina), while antibodies to two serotypes (A and O) were detected in Enugu. Serotype O had the highest seroprevalence followed by serotype SAT 2 in three States (Table 1). The percentages of the different serotypes are shown in (figure 4). Seropositivity to combinations of two or more serotypes was observed in multiple serum samples (Table 2).

Table 1: Prevalence of the different serotypes of FMD in circulation in the four states surveyed in Nigeria

Number of positive for the different serotypes				
States	**O**	**A**	**SAT1**	**SAT2**
Katsina	93(90%)	22(21%)	21(20%)	58(56%)
Anambra	23(68%)	12(35%)	22(65%)	23 (68%)
Enugu	1(33%)	1(33%)	0(0%)	0(0%)
Imo	44(75%)	35(59%)	26(44%)	18(31%)
Total	**161 (81%)**	**70(35%)**	**69 (35%)**	**99(50%)**

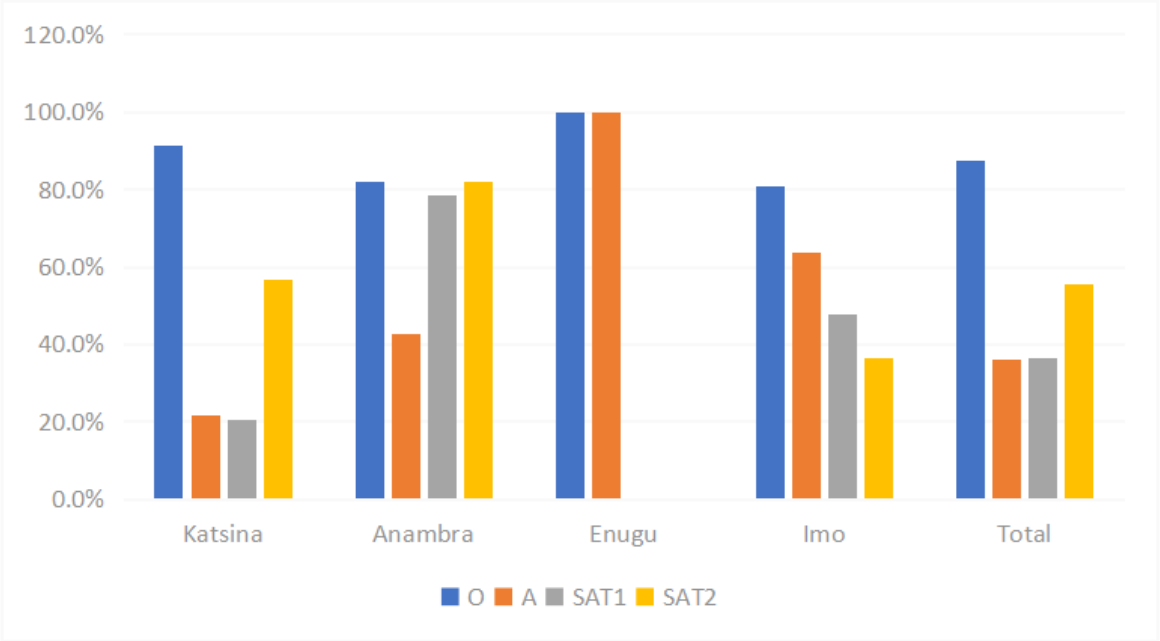

Figure 4: Bar charts showing the percentage of the different serotypes detected in the different regions surveyed; Anambra, Enugu, Imo and Katsina states.

Table 2: Seropositivity to combinations of two or more serotypes

States	1 Serotype	2 Serotypes	3 Serotypes	4 Serotypes
Katsina	29 (28%)	43 (42%)	17(17%)	7 (7%)
Anambra	1 (4%)	10 (36%)	9(32%)	8(29%)
Enugu	0(0%)	1 (100%)	0(0%)	0 (0%)
Imo	14 (32%)	3(7%)	9 (20%)	13 (30%)
Total	**44(25%)**	**57 (33%)**	**35 (20%)**	**28 (16%)**

DISCUSSION

Passive surveillance of FMD was performed in four States in Nigeria: Anambra, Enugu, Imo, and Katsina in 2019, with an overall seroprevalence of 45 % (199/441) of FMD in the study area. Antibodies against FMD NSP were detected in all four states surveyed, with a prevalence of 63.0% (59/93) for Imo, 56.3% (103/183) for Katsina, 29.0% (34/116) for Anambra, and 6.1% (3/49) for Enugu (figure 3). This indicates that cattle from these states are naturally exposed to FMDV, as antibodies to FMD NSP are only produced after viral replication in the animal host [19]. The overall FMD seroprevalence reported in this study is lower than that of other surveys conducted in Nigeria, which reported FMD seroprevalences of 64.7% in a study in Nigeria's border states [20], 55.9% in the study area of northern plateau state [21], 65.7 percent in some wildlife and cattle from Bauchi state [2], 75 percent from cattle in Kwara state [14], and 70.98% in North-Central Nigeria [22] but higher than 29% prevalence just for Plateau state [23]. The

size of the study sample pool and the number of states covered contributed to the prevalence differences between this study and other studies that have been previously reported. The findings of this study are in line with those of other studies showing that FMD is still an enzootic disease in Nigeria as a result of unrestricted local and transboundary trade, as well as the pastoral agricultural system and lack of vaccination programs. The results of this study show that outbreaks in the study area in Nigeria can be attributed to four FMDV serotypes and the possibility that co-infection with different FMDV serotypes can occur in Nigeria. Antibodies to all the four FMDV serotypes were detected. Earlier studies indicated that in an outbreak in 2012 serotypes O, A, SAT1, and SAT2 were detected in Nigeria [8]. Similarly, A, O, SAT 1 and SAT 2 serotypes were also responsible for FMD outbreaks in Northern Nigeria in 1999 [24] and serotype O and SAT2 were reported from outbreaks in 2008 [25]. Serotype O had the highest seroprevalence followed by serotype SAT 2 (Figure 4). The Seropositivity to combinations of two or more serotypes was observed in multiple serum samples (Table 2). This information is important for proper implementation of national and regional FMD control programs Our findings were unable to determine whether the individual sera's numerous serotype reactions were the result of several infections or serological cross-reactivity. However, multiple serotype infections that occurred in succession or simultaneously have been previously reported [1]. Co-infection with FMDV is frequent but may be spurred on by cross border cattle movements, sharing of common service providers, farm management systems and interaction with wildlife [26][27][22]. According to some investigations [28][29] FMD carrying buffalo may serve as a source for SAT virus reinfection with either the same viral strain or a different viral strain. Consequently, Multiple sequential infections with different serotypes and/or co-infections are possible [30]

Conclusion

The study has established the foot and mouth disease (FMD) endemicity in cattle and reaffirm the co-circulating of FMDV serotypes A, O, SAT 1, and SAT 2 in bovine within Nigeria. Consequently, research is necessary to identify how pastoralist animal movements contribute to FMD outbreaks in Nigeria and the best course of action for management which includes the use of multivalent vaccines that include the four local circulating serotypes.

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