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Field investigation of Foot and Mouth Disease (FMD) virus infection in cattle in the northern states of Nigeria.

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Abstract

The prevalence of FMD virus serotypes SAT 1 and SAT 2 among Nigerian cattle was determined using Complement Fixation (CF) and Serum Neutralization (SN) Tests in 2000 cattle sera obtained from nine northern states. The disease prevalence by CF and SN were 46.79% and 53.15% respectively. These figures were lower than that obtained in 1987 (55%). Plateau and Bauchi States maintained the highest positive cases with SAT 1 virus serotype using CF test (44.8% and 43.6%) and with SN test, 51.2% and 46.8% respectively. For SAT 2 virus, Borno and Adamawa States had the highest prevalence with CF, 41.00% and 30.50% and with SN maintained the highest prevalence; 46.50% and 29.50% respectively. The two serological tests were very specific and sensitive enough to detect and quantify the antibody levels in the infected animals. These results were very significant since these animals were not vaccinated against FMD. It showed evidence of FMD virus SAT 1 and SAT 2 serological types circulation in the country and may be responsible for set backs and the low output in the livestock sector in Nigeria. Further studies on other FMD virus serotypes in other states are suggested. Other details of the findings have been discussed.

Key word: Foot and Mouth Disease, Virus, Antibodies, Prevalence.

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Introduction

Foot and Mouth Disease Virus (FMDV) is a non-enveloped, single stranded RNA virus generally causes highly contagious vesicular disease in nearly all cloven-footed livestock. The virus is a nonenveloped , piconavirus (genus Aphthovirus) that contains seven serotypes The FMD is endemic in continents of Asia, Africa, and some regions in the South America. Transmission has been reported to occur by direct contact with infected animal, aerosol, contaminated semen, food products, persons and formites (Callis, 1996; Chukwuedo *et al.,* 2005). The disease in animals has been diagnosed using Enzyme Labeled Immunosorbent Assay (ELISA) (Abegunde *et al*.,1988; Chukwuedo *et al*.,2005; Alonso *et al*., 2008) complement fixation (Rweyemamu *et al.,*1978; Brooksby,1982; Reid *et al*., 20007) and serum neutralization tests (; Rweyemamu, 1978; Vosloo *et al*, 2003).

In Nigeria the Migrant Fulani herds are not routinely or year vaccinated against foot and mouth disease hence there are yearly outbreaks of foot and mouth disease in Nigeria cattle. Since the initial

documented outbreaks, the disease has become one of the major important economic diseases of livestock in the Country. The occurrence causes very significant losses and hardship to the animal owners since their livelyhood depends on the products (Chukwuedo *et al*., 2007). The disease occurred more often from September (on set of dry season) through to April (end of early dry season in the new year). During this period the animals migrate towards the middle belt and the Southern part of the country (Asagba, 1982; Abegunde,1989). In this present study efforts were made to determine the present status of FMD through the detection of the different virus serotype antibodies distribution in the cattle using Complement Fixation (CF) and Serum Neutralization (SN) tests.

Materials and Methods:

Virus strains: The 146s FMD virus isolates of SAT 1 (Nig 1/98) and SAT 2 (Nig 2/97) from Nigeria were grown in BHK-21 cL13 and used for the tests.

Cell culture monolayer: Baby hamster kidney (BHK-21, CL13 monolayer cells were prepared and propagated in T25 cm^2 flasks with Eagle's medium or Hanks containing 10% tryptose phosphate broth, 10% Fetal Calf Serum (FCS), 0.025% sodium carbonate and 100 units/ml of penicillin and streptomycin tissues culture grade (Flow Lab., ICN Bio-medicals Inc. U.K.).

Preparation of FMD virus 146s antigen: Each FMD virus isolate of SAT 1 (Nig 1/98) and SAT 2 (Nig 2/97) were grown separately in BHK-21 cells monolayer, in T25 cm^2 flasks and harvested at above 80% Cytopathic Effect (CPE).The flask contents were freeze thawed 3 times, centrifuged at 3000 rpm for 20 minutes (Universal 320R, Hettich 1406, Germany.) They were concentrated with polyethyleneglycol (PEG), Mol WF 6000, sedimented and pelleted at 30,000rpm for 2 hours (BECKMAN, COULTER, J6-MI Centrifuge, USA) The pellets of each FMD virus were resuspended in tris-buffer with 0.5m Nacl containing PBS pH 7.4 and purified by centrifugation). The purified (146s) virus was divided into aliquots and stored away of -80^{0}C REVCO (Ultra Low Temperature Freezer, HPZ, NIGERIA).

Complement (C^1): The complement used was normal guinea pig serum. Adult male guinea pigs were starved for 24 hours and bled and the pooled serum stored at -70^{0}C. The haemolytic system was prepared and used as described by Rweyemamu *et al.,(*1978). The complement and the haemolysin were standardized before use.

Samples: Seven to ten milliliters (7-10ml) of blood were collected from each Fulani migrant cattle through the jugular vein. The animal's age ranges from 1 to 5 years. The blood were allowed to clot and retracted at +4^{0}C. The serum was separated, heat inactivated at 56^{0}C for 30 mins in a water bath and store at -20^{0}C ready for assay. The field data and serum samples came from the Ten (10) northern state of the country: Taraba, Niger, Kaduna, Kebbi, Plateau, , Borno, Gombe, Adamawa, FCT and Bauchi.

Veronal Buffered Diluent (VBD): Veronal buffered saline was freshly prepared from oxoid tablets (Oxoid Ltd. U.K.)

Sheep Red Blood Cells (SRBC): The sheep red blood cells was collected freshly from NVRI sheep in alservers solution, washed three times in VBD and packed at 2000 rpm for 5mins using a bench centrifuge (Universal 320R Hettich 1406, Germany). An approximately 4% cell suspension was prepared.

Haemolytic cell indicator system: Rabbit anti-sheep red blood cells serum (haemolysin) was obtained from Buoroughs (welcome, U.K.) The haemolysin was reconstituted and titrated in a checker board format against complement. Aliquots of 4% Sheep Red Blood Cell (SRBC) suspension was mixed with equal volume of varying (10-fold) dilutions of haemolysin sensitization was for 15mins at 37^{O}C in a water bath, shaking every 5 minutes.

Complement fixation test: Complement dilutions were prepared in a two fold serial dilutions from 1:10 and held at +4^{0}C until used. 50µl each of the various dilutions of the antigen and the antibody were

added to the corresponding wells of the microtitre plate. This was incubated at 37^0C for 15 minute followed by 50µl of the complement dilution and incubated at 37^0C for 1hr. The 50µl of sensitized sheep red blood cells suspension was finally added and the reagents incubated at 37^0C for 1hr. after which the result was read (3,4).

Microneutralization test: Microneutralization tests were carried out with the method described by Rweyemamu (1978). The antiserum were diluted 1:4 in serum free cell culture medium containing 10% tryptose phosphate broth, 0.025% ($^w/_v$) sodium biocarbonate and 100iu/ml each of polymyxin, neomyxin and mycostatin, pH 7.4. Two fold serial dilutions of the antiserum in the tris buffere growth medium were made in flat bottomed, tissue culture grade microtitre plates (Flow Laboratories Inc. Virginia 22102, USA) using 0.05ml diluting loops. The test samples were prepared in duplicates. Pre-titrated virus (SAT1-Nig 4/81 and SAT 2-Nig 6/81) also diluted in serum free cell culture medium containing an estimated $100TCID_{50}$ virus doses per 0.05ml was dropped in each well. The antiserum-virus mixture was incubated at 37^0C for 1hr. after which 0.05ml of BHK-21 cell suspension containing 10^5 cell per ml in cell culture medium containing 2% calf serum was dropped in each well. The plates incubated at 37^0C for 72 hrs in 5% CO_2 condition. Various controls: antiserum tested in the absence of virus for cytotoxic activity; antigens in the absence of antiserum for virus infectivity and cell culture suspension in the absence of antiserum and antigen for cell viability were set up along with the test proper. Wells with 50% or greater evidence of CPE were considered to the infected and neutralization titres are expressed as the reciprocal of the final dilution of test serum with over 80% inhibition.

Results

The geographical distribution of FMD virus serological types (SAT 1 and SAT 2) in the Northern zones of Nigeria under study are presented in Table 1. Virus serotypes SAT 1 and SAT 2 were identified in states (Borno, Bauchi, Gombe, Kaduna and Plateau) while SAT 1 was responsible for outbreaks in Adamawa, Taraba, Federal Capital Territory, Kebbi and Niger states. The results showed that 4222 animals were involved, 2442(57.84%) animals were affected while 1547(36.64%) calves and 233(5.52%) adult cattle died.

Table 1: Overall results of Foot and Mouth Disease distribution in the northern zones

Zones	Location States	No. of animal involved	No. animal affected	No. of calf dead	No. of adult dead	Virus Type
	Adamawa	136	70	57	9	SAT2
	Borno	114	62	43	9	SAT1&2
NE	Bauchi	754	362	335	35	SAT1&2
	Gombe	518	327	171	20	SAT1&2
	Taraba	325	208	107	10	SAT1
	FCT	411	273	117	21	SAT1
NC	Kaduna	776	435	308	38	SAT1&2
	Plateau	796	467	251	43	SAT1
	Kebbi	103	69	33	1	SAT2
NW	Niger	289	149	135	5	SAT2
	Total	4222	2442(57.84)	1547(36.64)	233(5.52)	

Key: ( ) = Percentage; FCT = Federal Capital Territory; NE = North East, NC = North Central; NW = North West

The animal mortalities in the different states are showed in Table 2. The distribution showed that Plateau had the highest animals affected 467 (58.67%). Bauchi had 362(48.01%) animals affected and the highest number of animals dead among the calves 335(44.43%) while Plateau had the highest mortality in adult cattle, 43(5.40%). Kaduna had 308(39.69%) mortality in calves, and 38(4.50%) mortality in adult cattle. Niger and Kebbi States had the least calf mortalities with 1.73% and 0.97% respectively.

Table 2: Distribution of Foot and Disease in the different locations (states)

Location States	No. of animal involved	(%) animal affected	(%) calf mortality	% adult mortality	Virus Type
Adamawa	136	70(51.47)	57(41.91)	9(6.62)	SAT2
Borno	114	62(54.39)	43(37.72)	9(7.90)	SAT1&2
Bauchi	754	362(48.01)	335(44.43)	35(4.64)	SAT1&2
Gombe	518	327(63.13)	171(33.01)	20(3.86)	SAT1&2
Taraba	325	208(64.00)	107(32.92)	10(3.08)	SAT1
FCT	411	273(66.42)	117(28.47)	21(5.11)	SAT1
Kaduna	776	435(56.06)	308(39.69)	3(4.50)	SAT1&2
Plateau	796	467(58.67)	251(31.53)	43(5.40)	SAT1
Kebbi	103	69(66.99)	33(32.04)	1(0.97)	SAT2
Niger	289	149(51.56)	135(46.71)	5(1.73)	SAT2
Total	4222	2442(57.84)	1547(36.64)	233(5.52)	

Key: (%)= Percentage; FCT = Federal Capital Territory

In Table 3 the distribution of FMD virus antibodies based on the total animal population involved in the study, gave 57.84% prevalence, 36.64% mortality in calves while the adult cattle had 5.52% mortality. The data showed that Plateau had the highest prevalence of affected animals (11.06%) followed by Kaduna with 10.30%, Bauchi with 8.57% and Gombe with 7.75%. The least prevalence of the disease came from Borno with 1.47%, Adamawa with 1.56% and Kebbi 1.63%.

Table 3: Foot and Mouth Disease Distribution Based on Animal Sample Population

Location States	No. of Animal Involved	(%) Animal Affected	(%) Calf Mortality	% Adult Mortality	Virus Type
Adamawa	136	70(1.56)	57(1.35)	9(0.21)	SAT2
Borno	114	62(1.47)	43(1.02)	9(0.21)	SAT1&2
Bauchi	754	362(8.57)	335(7.94)	35(0.83)	SAT1&2
Gombe	518	327(7.75)	171(4.05)	20(0.47)	SAT1&2
Taraba	325	208(4.93)	107(2.53)	10(0.24)	SAT1
FCT	411	273(6.47)	117(2.77)	21(0.50)	SAT1
Kaduna	776	435(10.30)	308(7.30)	38(0.90)	SAT1&2
Plateau	796	467(11.06)	251(5.95)	43(1.02)	SAT1&2
Kebbi	103	69(1.63)	33(0.78)	1(0.02)	SAT2
Niger	289	149(3.53)	135(3.19)	5(0.12)	SAT2
Total	4222	2442(57.84)	1547(36.64)	233(5.52)	

Key: (%)= Percentage; FCT = Federal Capital Territory; N=4222

Table 4: SAT1 and SAT2 FMD Virus Antibody Distribution in Animal Serum

State Location	No. of animal Infected.	By CF Test. SAT1	By CF Test. SAT2	By SN Test. SAT1	By SN Test. SAT2
FCT	200	51(2.55)	11(.55)	58(2.0)	17(0.85)
Niger	200	72(3.60)	31(1.55)	78(3.9)	37(1.85)
Kaduna	250	94(4.7)	19(.95)	94(4.7)	26(1.30)
Gombe	200	67(3.35)	0(0.00)	71(3.55)	5(0.25)
Bauchi	250	109(5.45)	42(.84)	117(5.85)	45(2.25)
Plateau	250	112(5.60)	23(1.15)	128(6.40)	39(1.95)
Borno	200	45(2.25)	82(4.10)	44(2.20)	93(4.65)
Adamawa	200	53(2.65)	61(3.05)	59(29.50)	59(2.95)
Taraba	250	41(2.05)	48(2.40)	38(1.90)	55(2.75)
Total	**2000**	**317(14.59)**	**644(32.2)**	**687(34.35)**	**376(18.80)**

Key: ( ) = percentage values. CF= complement fixation. SN= Serum neutralization.

The CF and SN tests results of sera from clinically infected FMD cattle are presented in Table 4. The results gave 317(14.59%) SAT1 and 644(32.20%) SAT2 CF Antibodies. Similarly, SN tests gave 687(34.35%) SAT1 and 376(18.80%) SAT2 respectively. The sera contain more neutralizing antibody for SAT1 than complement fixing antibody while there are more complement fixing antibody than neutralizing antibody for SAT2.

Discussion

Foot and mouth disease (FMD) is an endemic contagious disease of cattle in Nigeria and it infects both calves and adult cows. The disease has substantially contributed to economic loss in the livestock sector of the economy (Ezeokoli *et al.,* 1988; Chukwuedo *et al.,*2003). Many researchers in the past have used various laboratory methods to assay and identify FMD in different cloven-footed animals globally. Complement fixation tests (Rweyemamu *et al*.,1978; ABU-Elzein and Crawther,1979) and neutralization tests (Rweyemamu *et al*.,1978; MaCkay *et al.,* 2001;) Chukwuedo et al., 2005) have been used in subtyping, typing, characterization and serological identification of FMD virus serotype in infected animals. These serological tests are specific and sensitive enough to detect and identify foot and mouth disease in fields and experimental samples.

In this present studies CF and SN tests were used to detect foot and mouth virus antibodies in Nigeria cattle. These animals have not received any previous vaccination against the disease. Some of the animals had lesions evidence of previous vesicular virus infections or diseases. Samples from the nine states under study had antibodies against SAT 1 FMD virus and SAT 2 serotype except Gombe state where no SAT 2 antibody was detected (Table 4). The results on Table 1 may suggest that SAT 1 may be responsible for most of the FMD virus outbreaks in Nigerian cattle. The high prevalence of SAT 2 in the northern part of the country agreed with Abegunde *et al*.,(1988) who reported 33% using indirect ELISA on SAT 2 outbreaks in Adamawa and Taraba states. But many disagreed that the virus may have entered the country from the neighboring country of Cameroon since previous studies have shown that serotypes O and A are predominantly causing FMD outbreaks in Cameroon (Ekue *et al,*1994; Megersa *et al*.,2009)

However, the SAT 1 and SAT 2 FMD virus outbreaks in Nigeria cattle may have entered the country through the country border with Chad and Niger and Benin Republics. It has been estimated that

over 200,000 cattle enter Nigeria from its northern border annually and all cases are traced to movement of cattle from Cameroon, Niger, Chad and Benin Republic (Asagba, 1982; Ezeokoli *et al*.,1988; Chukwuedo *et al*,2003).

The neutralization test detected more antibody prevalence than the CF test but the pattern of the results with CF remains the same with respect to highest and lowest prevalence in the various states. The neutralization antibodies to SAT 1 and SAT 2 FMD virus prevalence were 34.35% and 18.80% respectively (Table 3). Therefore the total prevalence of FMD virus either SAT 1 or SAT 2 using complement fixation test and neutralization test were 46.79% and 53.15% respectively. These values however, are lower than the 55% prevalence obtained by Abegunde (1989) in North Central part of Nigeria. This may be as a result of improvement on the management practices by the cattle owners following the World Organization for Animal Health (2002; 2008)

The low positive results from Taraba state disagreed with Nawathe and Goni (1976) who obtained a higher prevalence (35%). The lower prevalence obtained may suggest a down trend of the disease due to farmers awareness of the disease or improvement in the management and handling of the animals. Also the result showed that FMD virus SAT1 and SAT2 are still endemic in Nigeria cattle in the northern part of the country and will continue to pose serious problems in the improvement and growth of the livestock sector of the economy. This result reconfirmed the report of Sangare *et al*. (2003) and Ekue *et al.* (1994) that SAT1and SAT2 serotypes are in West Africa. It may continue to reduce the farmer's income and the national protein supply from livestock since FMD has economic consequences on both the cattle owners and the economy of the country (Armstrong *et al,*2004; Chukwuedo *et al*; 2007). Further studies in the other northern states of the country and possible in other livestock like sheep goats and pigs are hereby suggested.

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