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DIAGNOSTIC INVESTIGATION OF FOOT AND MOUTH DISEASE NATURAL INFECTION USING ANTIGEN DETECTION ELISA

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ABSTRACT

FMD virus SAT1 and SAT2 were found concurrently causing disease in Friesian cattle cross bred with N'dama breed of cattle in a dairy farm at Obudu Nigeria. Further investigation showed that the animals have been exposed to FMD virus serotypes A and O. Diagnosis was made using antigen detection ELISA a test with deviation from Liquid Phase Blocking ELISA (LPBE) and Solid Phase Competitive ELISA (SPCE). Most common FMDV serotypes reported to cause infections in Nigeria has been consistently Types A, O, SAT1, SAT2 as described in this study. Clinical diagnosis was made using signs of oral and feet lesions causing severe anorexia and lameness respectively in affected animals and calves. Mortality among calves was about 60%. There was a striking lymphocytosis from haematology of affected animals; other blood parameters were within normal physiological range and no concurrent condition was detected using haematology. The control of FMD in endemic area has to be adapted to local requirements which in this study has to be achieved by control of SAT1 and SAT2 which was the cause of the disease and was seen in weak reaction with other sampled animals. Though clinical diagnosis has to be complemented with serological test and molecular techniques it will be important to carry out local control. Antigen ELISA is found as very good diagnostic alternative for FMD in endemic area.

Key words: Antigen detection ELISA- diagnosis- Foot- and- mouth disease- Nigeria.

INTRODUCTION

Cattle are the single most important livestock species in Nigeria in terms of output of meat and milk and the capital value. They are primarily produced by pastoralists herdsmen who use the natural vegetation of communal range (Oyedipe, 2011). Cattle production has been hampered by factors including inadequate nutrition, poor genetic base of the indigenous breeds, poor growth rate, and low reproductive capacity, non-infectious and infectious diseases (Nuru, 1982). The breeding policy to improve livestock in Nigeria concentrated on the locally available breeds of animals. Afterwards a modification to include exotic breeds of cattle was introduced to upgrade the local stock; as such South Devon cattle were introduced at the Obudu Ranch (Oyedipe, 2011). This policy still has great challenges with the presence of diseases such as Foot-and-

Mouth disease which stands as a strong drawback to cattle production in the country. FMD is a primary cause of bovine lameness which is a major challenge facing the dairy industry, with negative impacts on milk production, fertility and longevity of animals (Sophie M. and Nick B. 2015). It affect animal growth rate, feed consumption reproductive capabilities

Foot-and-mouth disease (FMD) is a highly contagious viral disease of even-toed ungulates caused by a single stranded positive-sense RNA virus (Picornaviridae, genus *Aphthovirus*) and is one of the most economically important diseases of livestock (B. M. de C. Bronsvoort et al, 2006). Although often referred to as a single disease, there are seven serotypes of the virus distributed around the world: serotypes O, A, C, Asia1 and South African Territories (SAT1, SAT 2 and SAT 3). They are indistinguishable clinically, although they may have different geographical distributions (Bastos and Sangare, 2001) and epidemiologies (Hunter, 1998). Although mortality is rare in adult cattle, loss of productivity has been estimated to be as high as 25% in affected animals manifesting as reduction in milk production, loss of body condition, stunted growth in recovered animals. An important feature of FMD is that the virus may be recovered from the oropharynx for a varying period after an animal has been infected; if this period is longer than 28 days the animal is classified as persistently infected or a 'carrier'. The epidemiological role of persistently infected or 'carrier' animals is still controversial, but the possibility that they may become the focus of new outbreaks greatly influences control strategies and trade regulations (Macpherson, 1995; Nicholls et al; 1983). FMD is endemic in much of sub-Saharan Africa and FMD virus identification and characterization remain an important challenge to many of these countries because test could not be efficiently locally. Most often there is need to chip specimen to other countries for proper diagnosis with the incumbent risk of disease spread and introduction of new serotypes to FMD free countries.

MATERIALS AND METHODS

Clincal findings and sample collection:

In affected animals, the febrile stage passed unnoticed, animals were anorexic and showed various degrees of lameness which was very severe in some young and older animals. On farm visitation most oral lesion were healing but feet lesions were still obvious causing severe lameness. Mortalities were recorded in calves and 11 out of 19 died during the course of the disease. Blood samples were collected from sick and apparently healthy animals for haematology and blood parasite; aliquoted in EDTA bottles and remaining allowed to decant in slanted syringes at -4^{0c}, serum was harvested within 24hours post collection and stored at -20^{0c} till when needed.

Saliva and scrapings of the mucosa of healing ulcers used for ELISA test were collected in glycerol saline and preserved at -70^{0c} till when used. All samples were transported from the field to the laboratory on ice pack (Kitching & Donaldson, 1987).

PLATE A

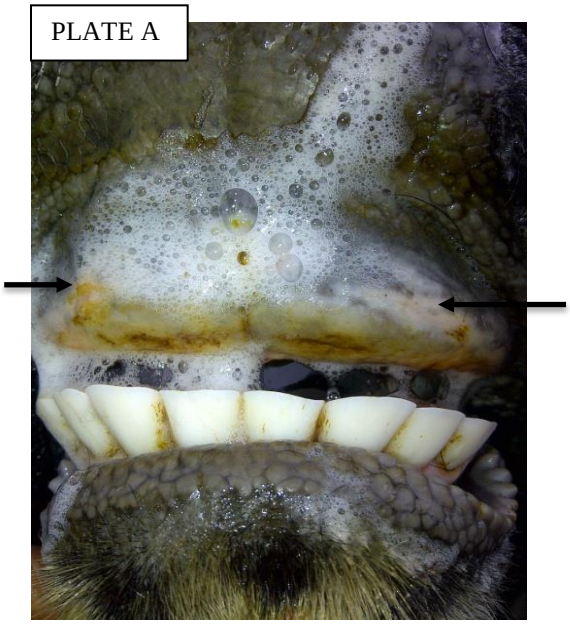


PLATE B

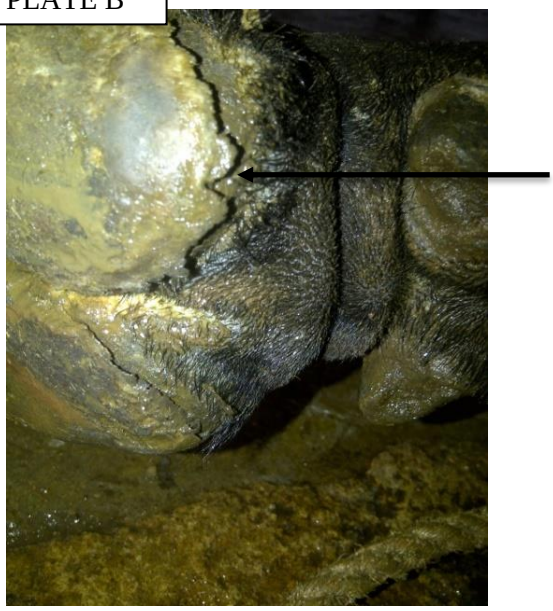


PLATE C



PLATE A: Healing oral lesions and foaming saliva on the palate of animal with FMD.

PLATE B: Partially sloughed hoof in clinical FMD.

PLATE C: Lameness in calve with lesion on the hind right hind limb in clinical FMD. The animal has difficulty in bearing weight on the affected limb.

SAMPLE ANALYSIS

HAEMATOLOGY

PCV: Tubes were filled by capillary diffusion to about 2/3 of its length, the outside carefully dried with a piece of gauze and the opposite end of the tube sealed with special clay. The sealed tubes were then placed in a high speed micro haematocrit centrifuge with the sealed edge near the outside rim, and spined at 2000 rpm for 5 minutes. The spun micro-haematocrit tube was then placed on microhaematocrit reader and PCV read off in percentage.

Total Erythrocyte Count: The haemocytometer method was used for the determination of total red cell count. A chemically clean red cell pipette was used to draw blood to the 0.5 mark of the pipette. The later was now plunged into a test tube containing diluting fluid. Normal saline solution was used (0.85% Nacl). This was aspirated to the 101 mark of the pipette and blood and fluid mix properly by inversion of the tube. Thus aspirated blood was diluted at the proportion of 1:200. Diluted blood was discharged into haemocytometer counting chamber, after allowing several minutes for red cells to settle to a single layer. A cover slip was applied and total number of cells in five squares in the centre of the counting chamber was evaluated and this value was multiplied by 10^6 to have total erythrocytes per 1µl of blood ($ax10^6$/ml).

Total leukocyte count: Haemocytometer method was used. The procedure was the same as in total erythrocyte count except that; the diluting fluid was solution of glacial acetic acid tinted with methylene blue, the white cell pipette was used (it allows a 1:20 dilution instead of the 1:200 dilution in erythrocyte count), the cells are counted in the 16 squares within the larger ruled area in the corner and total number of cells counted multiplied by 10^3 to obtain total number of leukocytes per µl of blood ($bx10^3$/ml).

ELISA

Oral samples with cell debris was homogenized using mortar and pestle with small amounts of sterile sand then suspended dilution of normal saline (10%) then centrifuged at 2000rpm for ten minutes. Supernatant fluid was harvested and stored. Each specimen was diluted and dispensed in 12 wells of a row with two replicates for each type specific MAb and the pan-FMDV MAb. Plates were incubted to allow reaction with the test sample antigen if present with the corresponding MAb and the pan-FMDV MAb. Repeated cycles of washing were done to remove unbound material. Conjugates A and B were added in their respective wells and incubated. All incubations were done at room temperature; the substrate was added after another cycle of washing to remove unbound conjugate. The colorimetric reaction was then discontinued by adding a stop solution and colour density read using spectrophotometer at 450NM wavelength. samples were classed as positive if the mean optical density (OD) reading, after subtraction of the background, was greater than 0·1 interpretation was made using the software provided by kit manufacturer (see table 2). Samples with ambiguous reading were retested and result confirmed.

RESULTS

Differential White blood cell count in 10 randomly selected animals from the affected herd showed high lymphocytosis above 65%. Other haematological parameters (PCV, Total Erythrocyte Count, HB, MCV, and MCHC) were within the normal physiological range. The striking lymphocytosis was as a result of cell mediated immune response to viremia see Figure 1:

Differential white cell count of cattle with FMD below. Generally under field conditions animals are often challenged with haemoparastic and other anaemia causing conditions but it was not the case in this investigation.

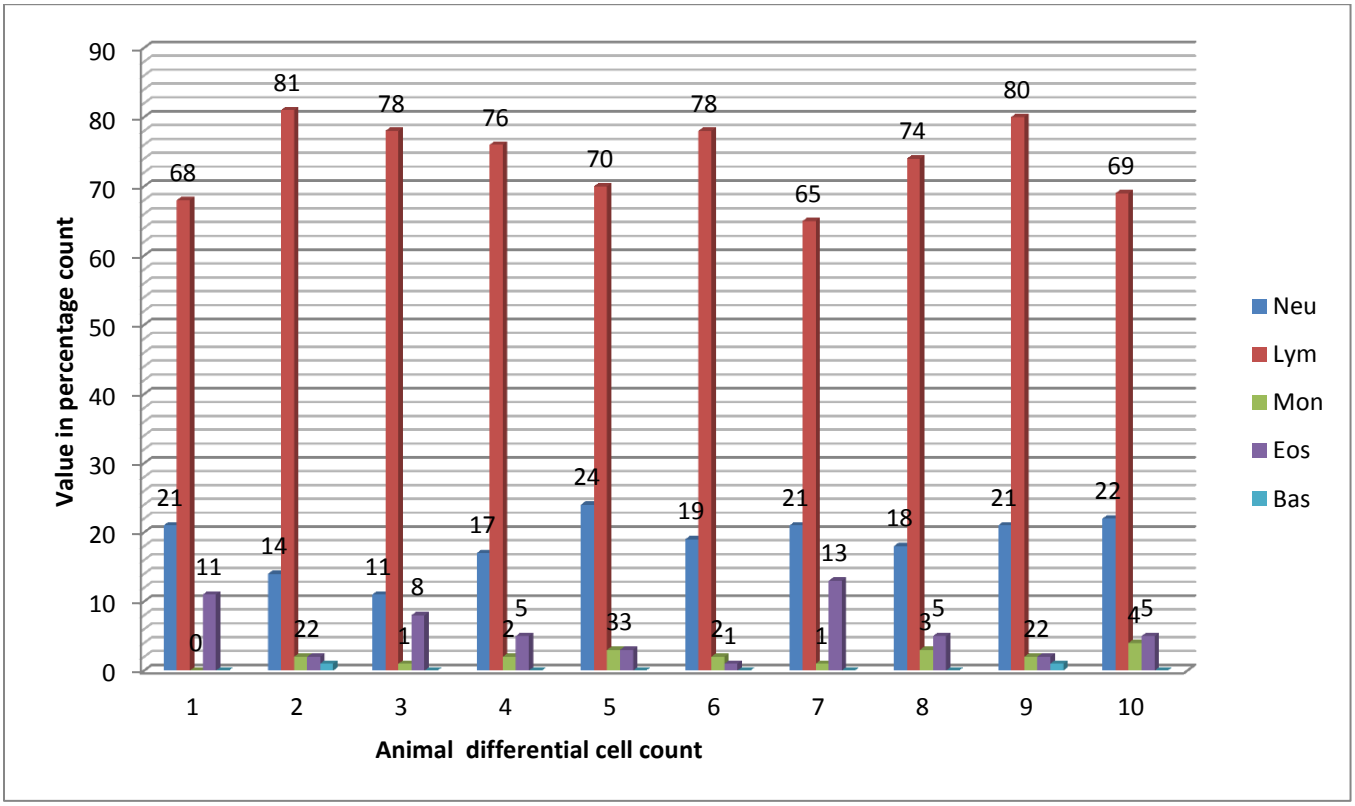
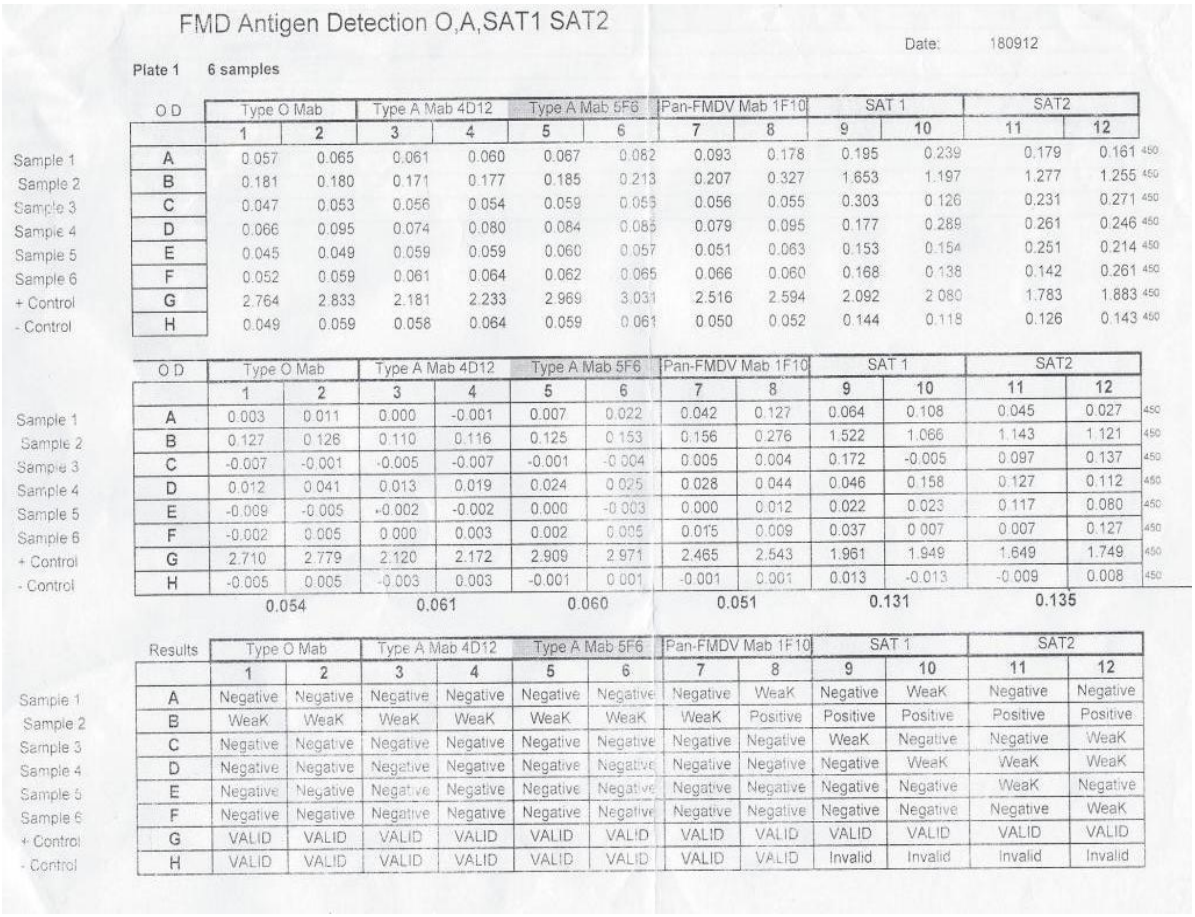


Figure 1: Differential white blood cell count in FMD infected cattle.

Antigen detection ELISA show on Plate D that FMD virus SAT1 and SAT2 were found to be present in same specimen in wells B9, B10, B11and B12. Other samples D (D11 and 12); E (E 11 and 12) show weak reactions to SAT1 and SAT2 virus serotypes. Using Solid phase Competitive ELISA for Serotypes A and O in a different study, there was evidence of antibodies to FMD virus types A and O in the specimens used in this study.

PLATE D: Antigen Detection ELISA Table for tested samples



FMD Antigen Detection O,A,SAT1 SAT2

Date: 180912

Plate 1 6 samples

O D	Type O Mab		Type A Mab 4D12		Type A Mab 5F6		Pan-FMDV Mab 1F10		SAT 1		SAT2		
	1	2	3	4	5	6	7	8	9	10	11	12	
Sample 1 A	0.057	0.065	0.061	0.060	0.067	0.082	0.093	0.178	0.195	0.239	0.179	0.161	450
Sample 2 B	0.181	0.180	0.171	0.177	0.185	0.213	0.207	0.327	1.653	1.197	1.277	1.255	450
Sample 3 C	0.047	0.053	0.056	0.054	0.059	0.055	0.056	0.055	0.303	0.126	0.231	0.271	450
Sample 4 D	0.066	0.095	0.074	0.080	0.084	0.085	0.079	0.095	0.177	0.289	0.261	0.246	450
Sample 5 E	0.045	0.049	0.059	0.059	0.060	0.057	0.051	0.063	0.153	0.154	0.251	0.214	450
Sample 6 F	0.052	0.059	0.061	0.064	0.062	0.065	0.066	0.060	0.168	0.138	0.142	0.261	450
+ Control G	2.764	2.833	2.181	2.233	2.969	3.031	2.516	2.594	2.092	2.080	1.783	1.883	450
- Control H	0.049	0.059	0.058	0.064	0.059	0.061	0.050	0.052	0.144	0.118	0.126	0.143	450

O D	Type O Mab		Type A Mab 4D12		Type A Mab 5F6		Pan-FMDV Mab 1F10		SAT 1		SAT2		
	1	2	3	4	5	6	7	8	9	10	11	12	
Sample 1 A	0.003	0.011	0.000	-0.001	0.007	0.022	0.042	0.127	0.064	0.108	0.045	0.027	450
Sample 2 B	0.127	0.126	0.110	0.116	0.125	0.153	0.156	0.276	1.522	1.066	1.143	1.121	450
Sample 3 C	-0.007	-0.001	-0.005	-0.007	-0.001	-0.004	0.005	0.004	0.172	-0.005	0.097	0.137	450
Sample 4 D	0.012	0.041	0.013	0.019	0.024	0.025	0.028	0.044	0.046	0.158	0.127	0.112	450
Sample 5 E	-0.009	-0.005	-0.002	-0.002	0.000	-0.003	0.000	0.012	0.022	0.023	0.117	0.080	450
Sample 6 F	-0.002	0.005	0.000	0.003	0.002	0.005	0.015	0.009	0.037	0.007	0.007	0.127	450
+ Control G	2.710	2.779	2.120	2.172	2.909	2.971	2.465	2.543	1.961	1.949	1.649	1.749	450
- Control H	-0.005	0.005	-0.003	0.003	-0.001	0.001	-0.001	0.001	0.013	-0.013	-0.009	0.008	450
	0.054		0.061		0.060		0.051		0.131		0.135		

Results	Type O Mab		Type A Mab 4D12		Type A Mab 5F6		Pan-FMDV Mab 1F10		SAT 1		SAT2	
	1	2	3	4	5	6	7	8	9	10	11	12
Sample 1 A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	WeaK	Negative	WeaK	Negative	Negative
Sample 2 B	WeaK	WeaK	WeaK	WeaK	WeaK	WeaK	WeaK	Positive	Positive	Positive	Positive	Positive
Sample 3 C	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	WeaK	Negative	Negative	WeaK
Sample 4 D	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	WeaK	WeaK	WeaK
Sample 5 E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	WeaK	Negative
Sample 6 F	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	WeaK
+ Control G	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID
- Control H	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	Invalid	Invalid	Invalid	Invalid

DISCUSSIONS AND RECOMMENDATIONS

Clinical FMD is described in this study as it occurs in animals on the field. Lesions as seen in Plates A, B and C are typical of the disease as described by Radostits et al, 1994. Blood picture of affected animals reflected an ongoing viremia with obvious lymphocytosis. There was no probable concurrent diseases as was suspected will be the case in most cattle diseases. The presence of positive results in wells B 9, 10 11 and 12, weak positive in wells B1, 2, 3, 4, 5, 6 and 7; D10, 11 and 12; E11 in this study highlights the complexity of FMD in the area based on multiple FMD virus serotypes being perpetuated in cattle populations. The weak positive reaction to FMD virus serotypes O and A in wells B1 and B2; B5 and B6 respectively was further investigated. The sera of those animals were tested using Solid Phase Competitive ELISA for serotypes O and A and such samples were positive. FMD remains a very important disease affecting beef and dairy production in the area. High mortality in young animals is an important source of economic loss. Most farmers have complained about the aftermath of FMD in their animals. From their explanation the animals that had suffered FMD become difficult to manage. They do not eat well and their production is seriously reduced. Because of its endemic nature FMD stands as a constant drawback in attempt to improving production. The rapid diagnosis using this indirect sandwich ELISA (Roeder and Smith 1987) with the modifications described by Ferris and Dawson (1988) and OIE, (2004) provides an edge for control base on specific virus

serotype identification. The serological typing using Solid phase competitive ELISA is directive but remains ambiguous because a good number of samples have antibodies to more than one serotypes and this cannot be conclusively attached to the ongoing infection. The practical FMD diagnosis has been a constant challenge to countries where the disease is endemic primarily due sample transportation from the field and its preservation in local laboratories, lack of equipments, reagents and trained personnel to carry out prompt laboratory diagnosis. More importantly the relative lack of concern from the authorities may be because the disease does not achieve its economic importance through high mortality as such its effects adverse effects on food security and economic development both at the level of village pastoralist and the more organized production systems continues to be underestimated. Concerned farmers opt for individual or small group solutions such as using measles vaccine for humans in controlling FMD in cattle (the local pastoralist) or vaccinating the animals with FMD vaccine not recommended by authorities. Although attempt at controlling FMD will needs a committed effort that is supported locally but that is rolled out across the whole of sub-Saharan Africa (15) if it is to have a chance to be successful. It is important to consider controlling the specific serotype in a given location after diagnosis. Different monovalent or polyvalent FMD can be recommended for use in specific location to progressively and effectively reduce FMV virus circulating load prior to developing suitable and sustainable control strategies that will allow traditional pastoralist/nomadic practices to continue.

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