www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i3.153

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Comparism of Presence of Foot and Mouth Disease Antibodies using Liquid Phase Blocking Elisa in Market and Ranch Cattle in Umuahia Abia State, Nigeria

Authors
Tchokote E. Y.[1]*, Ularamu G. H.[2], Ogbonnah, O. N.[3] Adeyefa C. A. O[4]
[1,3]Dept. of Veterinary Medicine, College of Veterinary Medicine Michael Okpara University of Agriculture, Umudike, Abia
[2]FMD Laboratory, Viral Research, National Veterinary Research Institute, Vom, Plateau State
[4]Dept. of Veterinary Medicine, Faculty of Veterinary Medicine University of Ibadan, Ibadan, Oyo
Email: **eugenyoungo@gmail.com*, +234 806 337 15 94

Abstract
A serological study was conducted to ascertain the presence of Foot and Mouth Disease (FMD) in cattle in three cattle farms and two cattle markets in Abia State. Eighty-eight sera were screened for FMD antibodies using the PrioCHECK® 3ABC NSP ELISA kit. The assay procedures were performed in accordance to the kit manufacturers' instructions. Test plate was read using the spectrophotometer (ELISA reader) at 450nanometer. The percentage inhibition (PI) of the optical density (OD) from each sample was calculated using the formula: PI=100-[OD_{450}test sample /OD_{450}max] ×100. Sample was said to be negative when PI is <70 and positive when PI is ≥ 70 as indicated by the manufacturer. Higher sero-prevalence was recorded in cattle samples from the market with a sero-prevalence rate (SPR) of 78.3%. There was a lower SPR of 40.5% from farm cattle; the overall SPR was 60.2%. There was no significant difference in seropositivity between farm and market cattle P > 0.05 using student t-test. The result confirmed the presence of FMD which stands as a production constraint to small cattle holders in the area; the epidemiological importance of cattle markets in FMD perpetuation base of high SPR in market animals.
Keywords: *Serological diagnosis, foot-and-Mouth Disease, cattle, Abia State.*

Introduction

Foot and mouth disease is a highly contagious viral disease and of high economic importance of cloven hoofed animals endemic throughout the country (Iyayi et al.; 2003) in recent years due to unavailability of specific field strain vaccines to prevent the infection. Cattle are highly susceptible to infection and also serve as carrier for FMD virus which can remain in the animal after a long period following an infection and holds the risk of an outbreak if such animals are not detected and measures taken for prevention. A study of the prevalence of the disease in cattle by the detection of antibodies which responds to the production of non structural proteins of the FMDV using liquid phase blocking ELISA becomes an epidemiological need for an eventual control of FMD. A complicating factor is the identification of so called 'carrier' animals where viruses can persist for a long time after an outbreak in the same species or in close proximity in another species. Carriers are produced only after infection which results in active multiplication of the virus in affected animal's tissues, but not all infected animals may show clinical disease. There is perpetuation and increase risk of disease from such animals. Though Abia State is not a major cattle producing State, an outbreak or a high

prevalence of FMD will cause a significant economic loss to small cattle producing population in the area.

Gloster *et al.*, in 1982 reported that FMD is transmitted by a variety of methods between herds, countries and continents but spread from one animal to another is by inhalation or ingestion. In the tropics, the most important mode of spread is believed to be by direct contact between animals moving freely across state and national boundaries as trade or nomadic cattle (Gloster *et al*., 1982). Spread from cattle to cattle is more likely by air borne means, and inhalation is the portal of entry. The virus can persist in aerosol from for long periods in temperate or sub-tropical climates. The speed and direction of the wind are important factors in determining the rate of air-borne spread. Humidity is also important but rain as such appears not to be.

Study Area

Abia state is a state located in the South-eastern part of Nigeria. The capital is Umuahia and the major commercial city is Aba, formerly a British colonial government outpost. Abia state occupies about 5,830 square kilometers. It is bounded on the north and north-east by Anambra, Enugu and Ebonyi. To the west is Imo state, to the east and south-east are Cross-river state and Akwa-Ibom State, and to the south is Rivers state. Abia state lies on within latitudes 5°25"North and 7°30"East and longitudes 5.417°North and 7.500°East (Hoiberg and Dale, 2010). Five areas of the state were conveniently selected based on the availability of cattle settlements and markets. The selection was done to cover as much cattle settlements and markets as much as possible. Two sample areas (Lokpanta cattle market and cattle settlement were purposively included based on the socio-economic distribution of cattle and also because Lokpanta is the only largest cattle depot in the South–East. Abia state has traditionally been infected with FMD through the borders as trans-state nomadic pastoralism is the most important type of cattle management system. The study population was grouped into two; ranched cattle (which include Michael Okpara University cattle farm, a cattle settlement within the environs of the state house of assembly and a cattle settlement situated around Lokpanta) and market cattle which include cattle from Lokpanta cattle market and Ubakala slaughter slab.

Materials and Methods

Blood Sample Collection

Cattle sampled were those within the ages one to four years old. This aging was done by firstly obtaining the herd history from the cattle owners and then followed by ageing them by a method known as ageing by dentition. The blood samples were collected by jugular puncture. Samples preserved slanted in a flask containing ice-packs from the field after which they were transferred to a refrigerator and kept at a temperature of -4°C to -8°C, serum was decanted and harvested 12hrs after collection and stored in cryovials at -20°C to -30°C till when needed. A total of 88 sera were collected from six different locations (table 1).

Serology

Liquid phase blocking ELISA (LPBE) using PrioCHECK® kits Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER Brescia Italy) for in-vitro detection of Antibodies specific to FMDV NSP independent of the virus serotype involved. Plates are precoated with MAbs specific to 3ABC FMDV nonstructural protein (NSP).

Test procedure

In the kit, the entire necessary reagents for the standard indirect ELISA technique were included with polystyrene microtiter plates pre-coated with recombinant FMD 3ABC protein. All reagents were brought to room temperature. Washing solution provided in the kit was diluted with demineralised water at 1:200 (1ml of washing fluid to 199 mls of water). Eighty µl of ELISA buffer was dispensed in every well of the plate, 20 µl each of the negative control, weak and strong positive controls were dispensed in the wells as

directed by the kit manufacturer. The test sera 20 µl was then dispensed in remaining wells; plates were covered with adhesive tape, gently mixed using a vortex shaker and incubated at room temperature overnight. Antibodies against FMDV NSP if present in the test sample will bind to the 3ABC antigen and prevent reaction with MAbs mapped with FMDV NSP.

On day two, plates were emptied and washed six times with 300 µl of the washing solution and this was done in order to remove unbound antigens from the test sample which are not specific to the 3ABC monoclonal antibodies of the FMD virus which was pre-coated in the test plates. The plate was tapped firmly after washing. One hundred µl of diluted conjugate each was dispensed into all the wells of the wells of the test plate (Plate 1). The test plate was sealed using the plate sealers and allowed to incubate for 60 minutes at room temperature. Washing was done as described above. A chromogen substrate (TMB) 100µl was added into the wells for colour development (Plate 2), the plate was incubated for 20 min, washed and a stop solution added 100µl in each well. Optical density was measured using a spectrophotometer at 450nm wavelength. Percentage inhibition of the sample was determined by: PI= 100-[OD sample/ODmax] x 100. ODmax being the average ODnegative controls. When PI < 70% There are no antibodies specific to FMDV NSP in the test serum. When PI > 70% then sample is positive for FMDV antibodies.

The contents of all the wells were mixed prior to measuring with a spectrophotometer using a filter size of 450 nanometers. The visual color change in wells is based on reaction for foot and mouth disease virus NSP

Results and Interpretation

Samples with PI ≥ 70% were considered positive indicating the presence of antibodies against the NSP of FMDV while samples with PI < 70% were negative indicating the absence of antibodies against the nonstructural proteins of FMDV. In this study 88 sera of cattle were examined from four areas of Abia state and one border between Abia and Imo states which were categorized into ranched and market cattle (Table 1 and 2) for the seroprevalence of antibodies to FMDV using the LPBE. The overall prevalence of FMD in the study areas was 60.2%. Seroprevalence rate (SPR) which is defined as the percentage of the positive sera samples present in a total number of sera collected from a group of animals. The formula for the calculation of the SPR is represented as follows: SPR= Number of positive sera samples / Total number of sera samples * 100%. There was higher seroprevalence in market animal (78.3%) than in ranched cattle (40.5%) and the specific (Figure 1). Using Student T test method of analysis at a confidence interval of 95%, p which is ≥ 0.06 and 0.09 is ≥ 0.05 (Table 3), was no significant difference in the seropositivity between the ranched and market cattle.

Table 1 Number of sera collected from the five areas sampled

Areas	Number of sera
MOUAU cattle farm	10
Lokpanta cattle market	32
Ubakala slaughter-slab	14
Fulani cattle settlement, Umuahia	17
Fulani cattle settlement, Lokpanta	15
Total number of samples	88

Table 2: Summary Result on the sero prevalence rate of foot and mouth disease in the study area

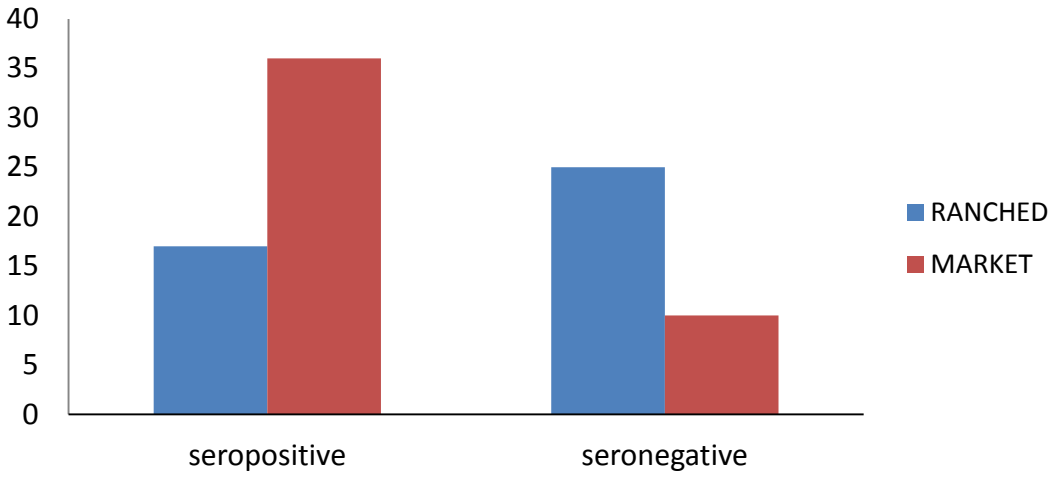
CATEGORY	No of Sera tested	No of seropositive	No of Seronegative	SPR (%)
RANCHED	42	17	25	40.5
MARKET	46	36	10	78.3
TOTAL	88	`53	35	60.2

Table 3: Analytical Table (Student T –Test)

One-Sample Statistics

	Statistic	Bias	Std. Error	Bootstrap[a] 95% Confidence Interval	
				Lower	Upper
Group N	46				
Mean	30.8478	.1204	2.5803	25.5886	35.9342
Std. Deviation	17.75508	-.25291	1.16325	15.23529	19.58066
Std. Error Mean	2.61784				
RANCH ANIMALS N	46				
Mean	.7965	-0031	.835	.6270	.9644
Std.Deviation	.57535	-00809	.03069	.50640	.62546
Std. Error Mean	.8483				
Market Animals N Mean	46				
Std.Deviation	.4771	-.0045	.0809	.3295	.6499
Std. Error Mean	.53682	-.01469	.09378	.34983	.71512
	.07915				

Figure1: Representation of FMD positive cattle from sampled animals



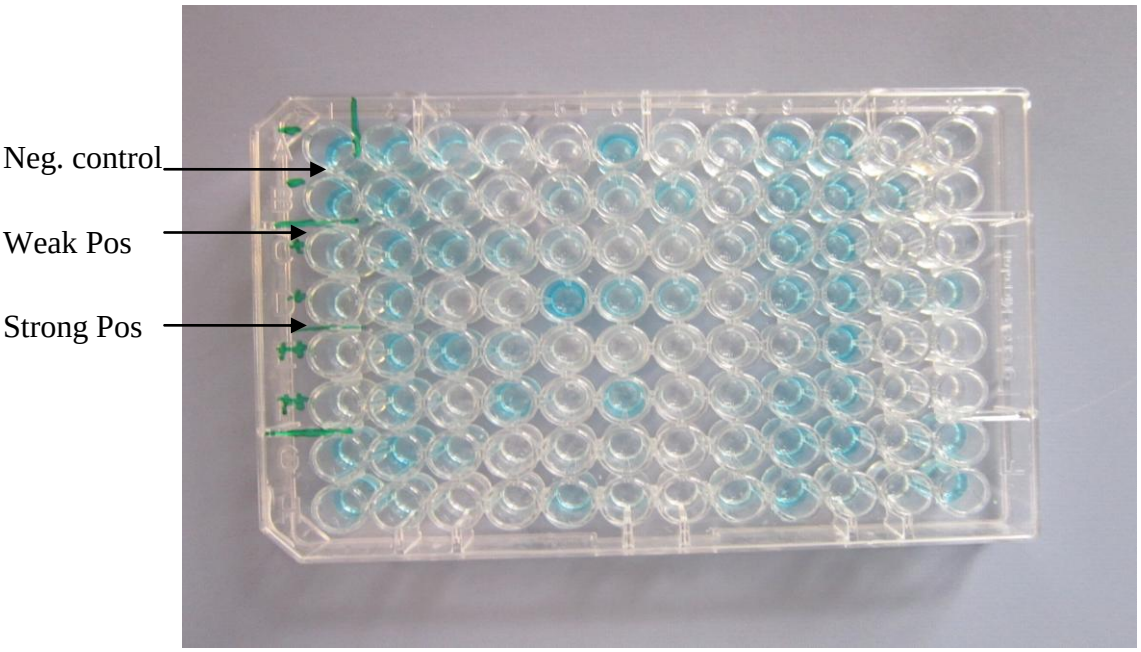


Plate 1: Titre plate showing bluish color change following incubation with Conjugate

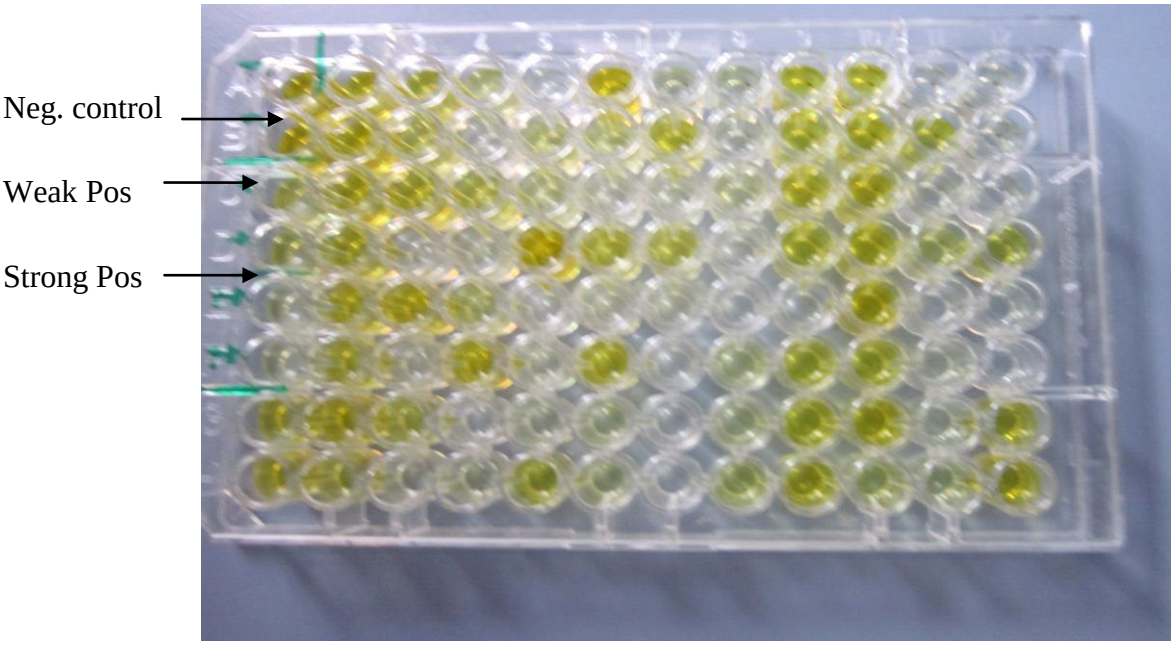


Plate 2: Titre plate showing color change following incubation with Chromogen (TMB) Substrate

Discussion

Based on the result of this serological study, 60.2% of the sampled animals have had FMD infection at least once before the age of four. This shows that the animals get infected with this disease very early in life due to the endemic nature the disease among the farm and market cattle. Comparison of the response between ranched and market cattle to the diagnostic test shows that market cattle have a higher seroprevalence of 78.3% of FMD virus as against ranched cattle which have a seroprevalence rate of 40.5% and this finding is similar to a survey reported by Lazarus *et al.*, 2011 and in which samples from abattoirs had a higher seroprevalence than samples from government ranches. This can be attributed to the fact that market cattle are an aggregation of cattle from different sources (such as from the Northern states and neighboring countries) as described by (Gloster *et al*., 1982). The lower seroprevalence in the farm animals can be attributed to the fact that most animals are born and reared in the state and because the endemicity of the virus is not is so high; the rate of infection is not as high as that in market cattle.

This study indicates that market as an area of high cattle population density is an important epidemiological indice for FMD control given that pastoralist activities revolve around the market and most often there is indiscriminate movement of animals and persons in and out of the market and across. Major source of infection into Abia state is probably from the market where infection revolves at a high prevalence rate. Radostits *et al*., 1997 that in most tropical and sub-tropical regions, the primary mode of transmission of the disease is through movement of animals across inter-state and international boundaries. Though after infection the virus could be isolated from tissues considering the endemic nature of the disease, infection may be maintained in the state of infected animal for a period of one year and above. Such animals could then serve as reservoirs and initiate new infections. Market animals have higher seroprevalence than farm animals.

Veterinary authorities in the state should be notified of any FMD outbreak so as to put in place technically efficient control measures though not very easy in underdeveloped nations like Nigeria.

References

1. Gloster. J., Sellers R.F, Donaldson A.I. (1982). Long distance transport of Foot-and-Mouth Disease virus over the sea. Vet. Rec. 110, 47-52
2. Ikede, B.O. and Onyekweodiri, E.O. (1977) "Pathology of foot-and-mouth disease."*Nigeria Journal of Animal Production* 4(1)
3. Iyayi E.A, Okoruwa V.O, Babayemi O.J, Busari A.A, and Peters O.F. (2003). Livestock production pattern of Agropastoralists in peri urban centers of South-West Nigeria. Nigeria Journal of Animal production 30(1): 87-92
4. James, A.D. and Rushton, J.(1947): The Economic of Foot and Mouth Disease. J. D. McLauchlan, W. M. Henderson 'The Occurrence of Foot-and-Mouth Disease in the Hedgehog under Natural Conditions', *The Journal of Hygiene*, Vol. 45, No. 4 (Dec., 1947), pp. 474-479.
5. Krystynak R.H, Charlebois P.A. (1987). The potential economic impact of an outbreak of Foot-and-Mouth Disease in Canada. Can. Vet J. 28(8)523-525
6. Lazarus D.D, Sclein, W.J.G, Wungak.Y, Kwange.D and Fasina, F.O. (2012)."Sero epidemiology of foot and mouth disease". African journal of microbiology Research. 6(8).pp,1756 -1761
7. Limoni, O. Fagbemi, A. Odegbo-Olukoya, Y. and Fabiyi, A.(1974). Outbreak of Foot and Mouth Disease in the West and Lagos States of Nigeria. Viological and Ecological Studies. *Rev. Roum. Virol*. 25, 361-364
8. Loeffler F, Frosch P. (1987). Summarischer bericht uber die ergebnisse der untersuchungen zur erforschung der maulund klauenseuche. Zentbl Bakteriol Parasitenkd Infektionskr. 22:257-259
9. Lu Z, Cao Y, Guo J, Q, S, Zhang Q. (2007) "Development and validation of a 3ABC indirect ELISA for differentiation of foot-and-mouth disease virus infected from vaccinated animals," Vet Microbiology, 125 (1-2); 157-169
10. Martinez, M.A., N. Vendaguer, M.G Mateu and E. Domingo. (1997). Evolution subverting essentially: dispersability of the cell attachment Arg-Gly-Asp motif in multiple passaged Foot-and Mouth Disease virus. Proc Natl. Acad. Sci. USA. 94:6798-6802
11. Nicholls, M.Y; Rwenyemanu, M.M; Okeke, E.N; Shidali, W.N. and Lamonde, A.G. (1983). The Control of Foot and Mouth Disease by Vaccination: Conside-ration for Nigeria. OIE, 2 (3), 771-780.
12. Nietzert E., E. Beck, P. A de Mello, I. Gomes, and I.E Bergmann. (1991). Expression of the aphthovirus RNA polymerase gene in Eschericia Coli and its use together with other bio-engineered nonstructural antigens in detection of late persistent infections . Virology, 184: 799-804
13. Martinez-salas. E, Saiz. M, Sobrino.F. (2008). FMD virus: Animal viruses; Molecular Biology. Caister Academic express. pp. 1-38
14. Marvin J. Grubman and Bary Baxt (2004). Clinical Microbiology Review. 17(2): 465-493.
15. Ninfa, O. (2010). Fundamental Laboratory Approaches for biochemistry and Biotechnology. Dearborn, M.I: University of Michigan.
16. OIE (2010). Foot and Mouth Disease, Nigeria. Information received on 25/08/2010 from Dr. Joseph Nylander, Director/CVO, Dept. of Livestock Ministry of Agriculture Abuja Nigeria.
17. Olabode, H.O.K. (2011). Foot and Mouth Disease in Nigeria. The Current Status and Control efforts, Pg 1-14.
18. Olaoku, E. (1976). Milk production in West Africa: Objectives and research approaches. Journal of the Association for

the Advancement of Agriculture in Africa, 3: 5 – 13.

19. Owolodum, B.Y. (1971). Foot and Mouth Disease and Virus types Distribution in Nigeria. Bull. Epiz. Dis. Afr. 19, 257-261.
20. Pattnaik, B. VenKataramanan, R .(1991).”Specificity of foot-and-mouth disease virus antigen in liquid phase ELISA,” *Indian Journal of Animal Science*, 59, 317.
21. Hoiberg, Dale H., ed. (2010). “Abia”. Encyclopedia Britannica. I: A-ak Bayes (15th ed). Chicago,IL: Encyclopedia Britanica Inc. p.32.