GROWTH COMPARISON OF NIGERIAN STRAINS OF FOOT AND MOUTH DISEASE VIRUS (FMDV) TYPES SAT 1 AND SAT 2 IN BHK, BK, VERO AND LK CELL CULTURE SYSTEMS

[1]CHUKWUEDO, AA*. AND [2]GOMWALK, N.E.

1. National Veterinary Research, Institute, FMD Laboratory P. O. box 207 Vom Plateau State Nigeria.
2. Department of Medical microbiology, faculty of Medical Science University of Jos, Jos Plateau State Nigeria

Corresponding Author*.

ABSTRACT

The growth characteristics of different strains of two stereotypes of foot and mouth disease virus (FMDV), SAT 1 and SAT 2, were compared in four cell culture systems (BHK, Vero, BK and LK cells). SAT 1 Strains yielded the highest virus titres (6.85 $\log_{10}$ TCID 50/ml) in BK cells followed by BHK cells (5.6log TUD_{50}/ml) while the lowest titres were obtained in LK cells (3.5 $\log_{10}$ $TCID_{50}$/ml). The SAT 2 strains yielded lower virus titres in BK cells system compared to the SAT 1. The SAT 2 had its highest titre in BK –21 (6.25 $\log_{10}$ $TCID_{50}$/ml) followed by vero cells (5.85$\log_{10}$ $TCID_{50}$/ml) but lowest in LK cell (3.8$\log_{10}$ $TCID_{50}$/ml). All the virus strains grew in all the cell culture systems but their growth vary from one cell to another and also from strain to strain within the serotypes. The virus – cell adaptation and sensitivity or susceptibility improves with repeated passages of virus in the cell monolayers. Although BHK – 21 cell are recommended for FMDV assay, these cells cultures that supported the virus growth may serve as alternative with further investigations hence it is advocated that more studied be conducted with more cell culture systems.

Key words: Foot and mouth disease, cell culture, SAT 1, SAT 2

INTRODUCTION

Foot and Mouth Disease Virus (FMDV) is the cause of foot and mouth disease, a highly contagious infectious disease of cloven hoofed domestic livestock, wild and animals of agricultural importance (Sherry *et al* 2000; Abegunde *et al* 2003, Samson 1994. the disease is characterized by vesicular lesions and subsequently by erosion of the epithelium of the mouth (Ferris Danaldson 1992). The disease however is assuming increasingly importance in Nigeria because of the present government efforts to upgrade and improve the indigenous cattle with the exotic breeds for increased meat and milk productions (Chukwuedo *et al*; 2003).

Since 1931 virus types O. A. SAT 1 and SAT 2 have been isolated in series of outbreaks (Abegunde *et al*, 1988; Ezeokoli *et al*, 1988). In most countries of the world, the disease is controlled by slaughter and immunization with chemically inactivated whole virus vaccine (Eric

et al, 1998). This present study described the growth of Nigeria FMD virus serotypes SAT 1 and SAT 2 in different cell systems.

MATERIALS AND METHODS

Viruses: A total of 14 FMD virus isolates were used. These include:

Nig 1/94	Nig 2/94	Nig 3/94	Nig 6/95	Nig 3/96
Nig 5/97	Nig 1/98	Nig 2/2000	Nig 1/95	Nig 3/95
Nig 5/95	Nig 2/97	Nig 4/97	Nig 3/98.	

Standard Antigens And Antibodies

The standard antigens and antibodies used includes: virus infections antigen (VIA), 1465 antigen to SAT1 and SAT2. Antibodies to VIA and 146s antigens, specific type antibodies to SAT1 and SAT2. All the reagents were obtained from Animal Virus Research Institute (AVRI), Pirbright, U.K.

Phosphate Buffered Saline (Pbs)

The PBS consists of 8.0g NaCl, 0.2g KH_2PO_4, 2.9g Na_2PO_4. $12H_2O$, 0.2g KCL. The salts were dissolved in one litre of distilled water and the PH was adjusted to 7.4 with NaOH or HCL. The Saline was sterilized by autoclaving at 121°C and 101bs pressure for 10 minutes, allowed to cool and store at 4°C ready for use.

Complement (C^i)

Serum from male guinea pigs taken after 24 hours starvation was used as a source of complement. The guinea pigs were bled through cardiac puncture and the blood was allowed to clot, store at +4°C overnight and centrifuged at 2500 rpm for 15 minutes in cold (M.S.E England) centrifuge. The supernatant was carefully aspirated into sterile bijou bottles in aliquots and store at –70°C Revco.

Veronal Buffered Diluent (Vbd)

Veronal buffered saline was freshly prepared from oxiod tablets (Oxoid Ltd, UK).

Sheep Red Blood Cells

The sheep red blood cell was collected from N.V.R.I sheep through the jugular veins in Alservers solution, washed three time in VBD and packed at 2000 rpm for 5 minutes using a bench centrifuge (MSE Super Minor England) An approximately 4% cell suspension was prepared.

Haemolytic Cell Indicator System

Lyophylized Rabbit anti-sheep red blood cells Serum (haemolysin) was obtained from Burroughs Welcome, U.K. The haemolysin was reconstituted and titrated in a chequered board format against complement. Aliquots of 4% SRBC suspension was mixed with equal volumes of varying (2 fold) dilutions of haemolysin. Sensitization was for 30 minutes at room temperature.

Complement dilutions were prepared in a two fold series from 1/10 and held at +4°C until used. 25ml each of the various dilutions of the antigen and the antibody were added to the corresponding wells of the micro titre plate. This was incubated at 37°C for 15 minutes followed by 25 ul of the complement dilution and incubation at 37°C for 1 hour. The 25 ul of sensitized

SRBC suspension was finally added and the reagents incubated at 37°C for 1 hour after which the result was read. (Davie; 1964)

Counter Immuno Electrophoresis (Cie) Test

Two rows of paired wells were drilled on a glass slide coated with agarose gel. The right column wells contained the antibody while left column wells contained the antigen. The wells were 8mm in diameter. The distance between the two antigen and two antibody wells were 4mm while the distance between the antigen and antibody wells were 2mm. The two rows of paired wells were 1 cm apart and the wick is 1cm away from the adjacent wells.

The test slide was placed in the electrophoretic chamber. The slide was connected to the buffer with a wick. The chamber contained the electrodes, which were connected to the positive terminal while the antisera to the negative end. The antigen wells were filled with the sample to be tested while the other row of wells was filled with antisera specific to foot - and - mouth disease virus (FMDV). Electric field was placed across the slide such that the antigen were negatively charged and the antibody positively charged. The charged reagents migrate to opposite direction where the antigen and antibody are homologous or specific to each other, and react in optimum concentration, precipitin lines were formed. (Bellhouse, *et al,* (1982)

Tissue Culture Systems

Four cell culture systems were used; Baby hamster kidney (BHK), strain 21, clone 13 and vero cells (established cell lines) and Bovine kidney (BK) and Lamb Kidney (LK) cells (Primary Cells) were grown 10_6/ml) in Eagles medium with 10% trypose phosphate broth, 10% fetal calf serum and antibiotics (100 units per ml of penicillin, 100 units per ml polymixin B and 0.002g per ml streptomycin). The cells were grown in Roux flasks, incubated at 37°c units complete monolayer is formed.

Virus Infectivity Titration In Tube Cell Monolayer

1.0ml of each of cell suspensions of BHK – 21, vero, BK and LK cells were grown (3.0 x 10^5/ml) in cell culture screw capped tubes until they become confluent. The virus suspensions were diluted 10 – fold serially in PBS (10^{-1} – 10^{-8}). The cell culture tubes monolayer were inoculated with 1ml per tube of appropriated virus dilutions.

All infected tubes and controls were incubated at 37°c for 24 to 72 hours and observed daily for cytopathic effect (CPF). The presence or absence of CPE in infected tubes was scored and virus $\log_{10}$ $TCID_{50}$ titre was determined by Reed and Muench method, (1938).

RESULT AND DISCUSSION

FMD virus serotype, SAT 1 infectivity in the cell systems maintained the highest yield in Bovine kidney cells followed by BHK – 21 cells (Table1). A titre range of 5.60 – 6.85 $\log_{10}$ $TCID_{50}$/ml was obtained in BK cells as compared to 4.45 – 5.60 $\log_{10}$ $TCID_{50}$/ml in BHK – 21. However the virus grew in all the cell cultures tested but had a low yield in vero (35 – 5.25 $\log_{10}$ $TCID_{50}$/ml) and LK (3.25 – 4.5 $\log_{10}$ $TCID_{50}$/ml) (Table 1).

TABLE I: FMD Virus SAT 1 infectivity assay in cell culture systems

FMD Virus log10 TCID 50% End point Titre in cell systems

SAT 1 VIRUS STRAINGS	IN BHK – 21	IN VERO	IN BK	IN LK
Nig 1/94	5.20	4.25	5.80	3.25
Nig 2/94	5.50	4.35	6.5	ND
Nig 2/94	5.60	4.50	6.00	4.50
Nig 6/95	5.25	5.25	6.75	ND
Nig 8/96	4.50	4.00	5.60	ND
Nig 5/97	4.45	ND	5.63	3.25
Nig 1/98	5.25	3.50	6.85	ND
Nig 2/2000	5.40	3.75	5.75	ND

ND: Not determined

The lowest titre yielded in BK cells with SAT1 FMD Virus during the study was higher than the highest titre obtained with BHK –21 cells (5.60 $\log_{10}$ $TCID_{50}$/ml). The SAT 1 strains (Nig. 1/98 and Nig 6/95) gave relatively higher titres in BHK – 21 and BK Cells but not determined in LK cells (Table 1) due to contaminations. Meanwhile SAT 1 strains successfully cultured in LK cells generally yielded low titres compared to other cell cultures used.

The SAT 2 infectivity assay in the different cell culture systems yielded titres which are not much significantly different from each other in BHK – 21, Vero and BK cells (Table II).

TABLE II: FMD VIRUS SAT 2 INFECTIVITY ASSAY IN CELL CULTURE SYSTEMS

SAT 2 VIRUS STRAINS	IN BHK – 21	IN VERO	IN BK	IN LK
Nig 1/95	5.50	4.75	5.50	4.0
Nig 3/95	4.80	ND	5.00	ND
Nig 5/95	4.25	3.85	5.55	ND
Nig 2/97	6.25	5.85	5.05	ND
Nig 4/97	4.75	5.00	5.00	3.8
Nig 3/98	5.50	5.00	4.75	ND

ND: Not determined.

However, FMD virus SAT 2 strain (Nig 2/97) gave the highest titres in the three cell cultures (BHK – 6.25 log_{10} $TCID_{50}$/ml, vero 5.85 log_{10} $TCID_{50}$/ml, BK 5.65 Log_{10} $TCID_{50}$/ml) but was not cultured in LK cells due to contaminations. SAT 2 growth in growth in vero cells in overall assessment was better that SAT 1 growth in the same vero cells.

Comparing the growth of SAT 1 and SAT 2 in BHK and BK cells, there was a significant difference in titres of SAT 1 grown in BHK and BK cells while culturing SAT 2 in the same cell system does not show prominent differences in titre. This finding agreed with the result obtained by Ferris et al (1984), Abegunde (1987) and Ferris and Donaldson (1992) while growing SAT 1 and SAT 2 in BHK - 21, BK and Bovine thyroid (BTY) cells also obtained lower SAT 2 strains than from European strains. The high titres of SAT 1 in BK cells, may be attributed to its earlier adaptation in cattle since all the strains were the SAT 1 and SAT 2 generally yielded low titres in lamb kidney cells (Table 11). Eric et al (1998) found that the most sensitive cell culture type for detecting FMD virus can vary therefore, depending both upon virus strains and previous passage in the host.

Although BHK – 21 cells are recommended and recognized as the standard cell culture for FMD virus cultivation, Donaldson et al (1970) found that Bty and IBRS cells were the most sensitive assay system of detecting FMD virus adapted to cattle (Table III & IV).

TABLE III: TITRE OF SOME SAT 1 STRAIN OF FMD IN BTY IBRS AND BHK – 21 CELLS

VIRUS STRAINS	TITRE IN BTY CELLS. LOG TCID50	TIRE IN IBRS CELLS LOG TCI D50	TIRE IN BHK – 21 CELLS LOG TCI DR50
Nig 4/81	6.44	6.00	3.24
Nig 5/81	6.31	6.41	3.39
Nig 10/81	6.00	6.56	3.41
Nig 11/80	6.37	6.40	4.25
Nig 155/68	6.82	6.44	4.56
Nig 1/68	6.80	6.52	5.25
Nig 1/68	6.87	6.48	4.38

*Mean of titration

TABLE IV: TITRE OF SOME SAT 2 STRAINS OF FMD IN BTY IBRS AND BHK – 21 CELLS

VIRUS STRAINS	TITRE IN BTY CELLS. LOG TCID50	TIRE IN IBRS CELLS LOG TCI D50	TIRE IN BHK – 21 CELLS LOG TCI DR50
Nig 2/82	6.2	6.87	5.47
Nig 6/81	4.8	4.44	2.87
Nig 8/81	5.4	5.31	5.31
Nig 3/80	6.0	6.44	3.44
Nig 5/68	5.2	6.0	3.00

*Mean of 3 titration

These other cell systems that supports the growth of these virus serotypes may serve as adjoints when BHK – 21 cells are not available. The use of primary cell culture for FMD virus growth and isolation should be done with caution because of the problems of adventitious agents that may serve as contaminations. The titre of the virus in LK cells may be improved after adaptation through series of passages in LK cell monolayer.

The virus yields after cultivation vary from one cell system to another. All the strains of the SAT 1 and SAT 2 used presented a typical FMD virus CPE pattern in the cell cultures. In this work reported here the serological assay of cell culture supernatant revealed the presence of too major antigens produced as a result of the FMD virus replication in the culture; whole virus (146s) and virus infectious antigen (VIA). Similarly, Sellers (1980) reported the presence of 164s and 12s structural capsid proteins and VIA antigen in FMD infected tissue culture or

animals. Antibodies to 146s and 12s proteins is type specific while VIA antibody is not specific and antibody produced against VIA antigen do not react with purified virus or its submit. It has been reported that the VIA is shown to be virus polymerase VP 56 on polyarcylamide gel electrophoresis (Newman, et al 1979) while Brown (1981) identified VIA as RNA dependent RNA polymerase and show to contain major virus coded protein of mol. Wt. 56,000 by double immuno diffusion (DID) studies in sera.

The CF test has been the method most widely used to differentiate FMD virus strains (Pereira, 1978). Although it can not differentiate between non-immunogenic and immunogenic antigens (Cowan, 1973). In this study it was used to further confirm the serotypes, SAT 1 and SAT2 identified by CIE (Table V). The CIE and CF tests conducted on the antisera showed that over 50% of the VIA positive sera contained SAT 1 antibodies (145/229, 47.23%) as compared to SAT 2. Similarly over 50% of the CIE confirmed SAT 1 and SAT 2 antisera contained CF antibodies (32.25 out of 47.23 and 13.36 out of 21.50, Table V).

TABLE V: ANTISERA – FMD SPECIFIC ANTIGEN REACTION BY CIE AND CF TESTS

NNO. OF ANIMAL INFECTED	NO. OF ANIMAL INFECTED	NO. OF SERA COLLECTED	NO POSITIVE TO VIA ANTIGEN (%)	NO. POSITIVE TO SATI ANTIGEN (%)	NO. POSITIVE TO SAT 2 ANTIGEN (%)	NO POSITIVE TO CF TEST	
						SAT 1 (%)	SAT2)%)
Plateau	60	47	31	18	8	15	3
Niger	48	40	38	18	14	11	8
Igbeti	71	64	41	34	6	22	3
Bauchi	23	23	17	12	5	9	3
Gembu	51	44	35	26	9	17	7
Azare	39	31	29	23	2	15	2
Yola	74	45	29	11	16	9	11
Bokkos	15	13	9	3	6	1	4
	335	307	229(74.59)	145(47.23)	66 (21.50)	99(32.25)	41 (13.36)

( ) = Showed % Values.

This study results shows that a great deal of variation is found in the growth characteristics of a serological type of FMD virus. Therefore there could be more information in investigating further the use of other cell systems as an alternative to BHK cells in FMD virus studies.

ACKNOWLEDGEMENT

We wish to acknowledge the assistance of the technician, Mr. Obadiah Shuarkyes, Dr. J. Ibu who proof read the paper and Mr. William Aboyi who typed the Manuscript.

REFERENCES

Abegunde, A., (1987) Epidemiology of FMD, Ph. D. Thesis, Ahmadu Bello University, Zaria.

Abegunde, A., Crowther, J. R.; Ezeokoh, C.D; and Umoh, J. U. (1988). Antibodies to VIA antigens in Nigeria Cattle. Bulletin of Animal Health Production in Africa 20 (3) 62 – 67.

Abegunde, A.; Chukwuedo, A. A. and Gomwalk, N. E. (2003) Quality Evaluation of two FMD Vaccines prepared from local isolates of serotypes SAT 1 and SAT 2 antigens and Montanide ISA 206 Formulations. Nig - J. Biotechn. 14 (1) 29 – 35.

Bellhouse R; Baker K.B and Hedger, R.S. (1982) Counter immuno electrophoresis in the diagnosis and serological surveillance of swine vesicular disease. Vet. Rec. 110, 357-358.

Borwn, P. (1981) Application of agar gel precipitation tests to the study of the virus of Foot and Mouth disease Virology, 5, 133 – 144.

Chukwuedo, A. A.; Abegunde, A and Gomwalk, N. E. (2003) Studies on the current status of Foot and Mouth Disease (FMD) Virus outbreaks in Nig. J. Biotechn. 14(1) 22 – 28.

Cowan, K. M. (1973) Antibody response to Viral antigens. Advanced Immunology, 17, 195 – 253.

Davie, J (1964) A complement fixation technique for the quantitative measurement of antigenic differences between strains of the virus of foot-and-mouth disease journal of Hygiene 62,410 – 411.

Donaldson, A. I.; Herniman, K.A.J; Parker J and Sellers R.F. (1970) Further investigations on the airborne excretion of Foot and Mouth Disease Virus. J. Hyg. Camb. 68, 557 – 564.

Ezeokoli, C. D.; Abegunde, A. J. Umoh, J. U. and Addo P.B. (1988) Epidemiology of Foot and Mouth disease in Nigeria Livestock J. Virol Methods 14 (1) 121 – 127.

Eric, B., Noemi, S., Nuira V., Carmen M.R., Ewald B. and Esteban D. (1998) Multiple Virulence Determinants, of Foot and Mouth disease virus in cell culture. J. Virol Vol. 72, (8) 6362 – 6372.

Ferris N. P. Donaldson A.I, Barnett I. T. R. and Osborne, R. W. (1984) Inactivation, Purification and stability of 1465 antigens ,of Foot and Mouth Disease Virus for use as reagents in the Complement Fixation Test. Rev. Sci. Tech. Off. Int. Epiz. 3 (2) 339 – 350.

Ferris, N. P and Donaldson A. I. (1992) The World reference Laboratory for Foot and Mouth Disease, a review of thirty-three years of activity (1978 – 1991). Rev. Science off Int. Epiz. 11(3) 657 – 684.

Newman J. F. E, Cartwright, B., Doel, T. R. and Brown, F. (1979) Purification and identification of RNA dependent RNA polymerase of Foot and Mouth Disease Virus Journal of Gen Virology, 45 497.

Pereira, H. G. (1978) Antigenic variation in relation to epidemiology and control of foot and mouth disease. British Veterinary Journal, 134, 28 – 33.

Reed, L. T. and Muench M. (1938). Simple estimate calculation Am. J. Hyg. 27:403.

Samson R. L. (1994). The Epidemiology of Foot and Mouth Disease: Implication for new Zealand. New Zealand Vet. J. 42, 41 – 53.

Sherry N.; Peter; M. W. and Barry B. (2000) High efficiency utilization of the Bovine B3 as a receptor for Foot and Mouth Disease Virus is dependent on the Bovine B3 Submit J. Virol. 74 (16) 7298 – 7306.

Seller, R. F. (1980) Quantitative aspects of the spread of Foot and Mouth disease. Veterinary Bulletin, 41. 431 – 439.