**NIGERIAN VETERINARY JOURNAL**

**ISSN 0331-3026**

**Nig. Vet. J., September 2019** **Vol 40 (3): 178 - 185.**

https://dx.doi.org/10.4314/nvj.v40i3.1 **ORIGINAL ARTICLE**

# Isolation and Serotyping of Foot-and-Mouth Disease Virus in Cattle collected from North Central, Nigeria

**Wungak, Y.S.[1*]; Olugasa, B.O.[2]; Ishola, O.[2]; Lazarus, D.[1]; Chukwuedo, A.A.[1] and Ularamu, H.G.[1]**

[1]Division of Virology, National Veterinary Research Institute, Vom, Plateau State. [2]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.
*Corresponding author: Email: yiltex2@gmail.com; Tel No: +2348064563108

## SUMMARY

Foot-and-mouth disease (FMD) is a disease of socio-economic importance which affects cattle, swine, Sheep, goats, and more than 70 wildlife species causing loss of production and with high mortalities in the young animals.The aim of this study was to isolate and serotype foot-and mouth disease (FMD) viruses collected in north central Nigeria, using the goat tongue cell line (ZZ-R 127). ZZ-R 127 cell line was used for the virus isolation and antigen Enzyme linked immunosorbent assay (ELISA) for FMDV serotypes O, A, SAT 1 & SAT 2 was used for the serotyping of the viruses. The ZZ-R 127 continuous cell line yielded rapid results with cytopathic effect (CPE) within 24 hours post inoculation. FMD viruses were isolated from twenty samples (n=20) out of the twenty two (n=22) collected. Antigen capture ELISA (Ag-ELISA) revealed thirteen (n=13) strains of serotype O, three (n=3) strains of serotype A, and four (n=4) strains of serotype SAT 2 with no virus detected in two samples.

Therefore, the use of ZZ-R 127 continuous cell line yielded rapid results within 24 hours of post inoculation compared to BHK-21 that may not give result at first passages. The ZZ-R 27 cell line is relatively easy to handle, maintain and cheaper for FMDV diagnosis in endemic countries like Nigeria, compared to bovine thyroid gland (BYT).

This study has confirmed the suitability of ZZ-R 127 in the primary isolation of viruses from clinical specimens with less turnaround time to generate results. Therefore, for rapid sensitive and specific laboratory assays, the use of ZZ-R 127 and Ag-ELISA for FMD diagnosis in endemic countries is strongly recommended.

**Key words:** Isolation, Foot-and-Mouth Disease Virus, field samples, cytopathic effect, Nigeria

## INTRODUCTION

Foot and mouth Disease (FMD) is currently considered worldwide as the most economically important disease of viral origin affecting cloven-hoofed livestock, because of its highly infectious nature, ability that may lead to persistence infections and long term impact on the productivity and condition of the susceptible animal's species (Brehm *et al,* 2009). FMD is caused by Foot and mouth disease virus (FMDV) of the genus *Aphthovirus*, family *Picornaviridae* (Jamal and Belsham, 2013). Worldwide, seven serotypes have been identified which includes; Serotypes O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3 (OIE, 2012). FMD is generally associated with low mortality rates in adults; nevertheless, it is regarded as the most important farm animal's diseases because of the huge losses associated with it in terms of livestock trade and productivity (Longjam *et al.,* 2011). However mortality in young animals is usually very high due to severe lesions it causes in the myocardium of young animals (Longjam *et al.,* 2011).

FMD is endemic in Nigeria with serological evidence to non-structural protein antibodies detected in cattle, camel, sheep, goat and pig population in some parts of the country ( Ehizibolo *et al.*, 2016;Lazarus *et al.,* 2012; Olabode *et al.,* 2013;Ularamu *et al.,* 2015; Wungak *et al.*, 2016).

Four serotypes of FMD (O, A, SAT 1 and SAT 2) are known to be circulating in Nigeria (Chukwuedo, and Nimzing, 2012; Ehizibolo *et al.,* 2014; Fasina *et al.,* 2013; Olabode *et al.*, 2014; Wungak *et al.,* 2015).

FMD has caused significant unreported economic losses to farmers and these will affect the gross domestic product (GDP) as well as food security in a country.

Therefore, there is the need to always isolate viruses from outbreaks in order to ensure effective vaccine match for a sustainable control programme. Nigeria, like many other endemic countries needs to initiate a strategic control programme to ensure food security and sustainable livestock production.

Rapid, sensitive, cheaper and specific laboratory assays are required for FMD diagnosis to ensure effective and efficient control measure in order to contain and prevent the spread of infection to susceptible animal population (Goller *et al.,* 1997).

The need for a cheaper, easily to handle, convenient cell line for primary isolation of FMDV in endemic countries is very fundamental; especially for laboratories that do not have facilities to handle primary cell-lines (Goller *et al.,* 1997).

Even though , FMDV diagnosis is possible by ELISA and real-time PCR, virus isolation is still an important step to have isolate and antigenic vaccine match for the selection of a suitable vaccine candidate (Brehm *et al.,* 2009). Furthermore, for most of the countries which do not have facility for RT-PCR platform in their laboratories, virus isolation remain the most sensitive test for FMDV (OIE, 2012).

Presently, the bovine thyroid cell (BTY) has been described as the most sensitive cell line for primary FMDV isolation (Ferris *et al.,* 2006). However, primary BTY cells lack the ability to be passaged or crop reserved without impairing their sensitivity (Ferris *et al.,* 2002; Carol, Houes 1989). In addition, the steady supply and maintenance of a fresh and suitable batch of primary BTY cells for diagnostic work is quite expensive and laborious (Ferris *et al.,* 2002). There is also the problem of sourcing FMDV free thyroid gland from calves in disease-endemic countries (Brehm *et al.,* 2009). Thus, most FMD diagnostic laboratories use other cell-culture system which are rather less susceptible to FMDV infection, however more convenient to handle, such as baby hamster kidney BHK- 21, pig kidney cell lines IBRS-2, PK-15 and SK-6. However, these established cell lines are generally less sensitive than primary BTY for detecting low amounts of virus load in samples and do no yield overnight amplification(OIE, 2012).

The goat tongue cell line (ZZ-R 127) is a novel cell line developed by the Friedrich-Loeffler-Institute (FLI) and being maintained by the Collection of Cell Lines in Veterinary Medicine (CCLV) Germany. The cell has been found to be rapid, sensitive and convenient cell culture system for the isolation of FMDV and consequently, a useful alternative to BTY (Brehm, et al., 2009).

This paper present the report of virus isolation of field samples from FMD outbreaks in North central, Nigeria using goat tongue cell line (ZZ-R 127).

## MATERIALS AND METHODS

### Study Area

The North central zone of Nigeria covers 296,898 $Km^2$ land area, representing nearly 32% of Nigeria total area. It includes Niger, Benue, Plateau, Kogi, Kwara and Nassarawa States. It is situated between latitudes 6º 30'-11º 20'N. The zone is divided into southern Guinea, derived savannas, and montane subzones. The annual rainfall decreases generally from the south part to the north part of the region and ranges from 1000mm to about 2000 mm per annum with an average of 187-220 rainy days per annum. The zone is dominated by plains, with mountainous areas in the Jos, Plateau.(NARSP, 1997).

### Sample Collection

Twenty-two (n=22) tongue epithelial specimens were collected purposively from clinically sick animals between June, 2011 and October, 2014 from north-central states of Plateau, Kogi, Nassarawa and Benue. Animals were clinically examined for the presence of FMD lesions in the mouth and buccal cavity, teats of lactating cows, nostril, and feet. Clinical specimens of epithelial tissue were collected from animals that showed typical clinical signs of FMD. Epithelial tissues were collected from unruptured or freshly ruptured vesicles and placed in a bottle with virus transport medium composed of the equal amount of glycerol and 0.04 M of phosphate-buffered saline with antibiotics (1000 units/ml penicillin, 100 units/ml mycostatin,100 units/ml neomycin, and 50 units/ml polymyxin) solution with pH 7.2 to 7.6 (OIE, 2012).The samples were then transported to the laboratory on cold-chain and stored at -20°C until processed. Both field FMD epithelial samples as well as the cell-culture supernatant were screened using antigen detection ELISA for serotypes A, O, SAT1 and SAT2 at the National Veterinary Research Institute, Vom Nigeria.

### Tissue Preparation for Virus Isolation and Antigen Detection

The samples were prepared as described previously (OIE, 2012). Briefly, the epithelial tissue samples were taken from sample bottles containing virus transport medium and were blotted dry on absorbent paper to reduce the glycerol content, which is toxic for cell cultures. A 10% suspension of the samples were prepared by grinding the tissue with sterile sand in a sterile mortar using pestle and mortar with a small volume of PBS. This was then centrifuged on a bench top centrifuge at 2000 ***g*** for 10 minutes. Centrifuged sample were kept at –80ºC, for virus isolation. Sample supernatants for virus isolations were furthered filtered through a Millipore filter of 0.22 μm pore size.

### Virus Isolation using ZZ-R cell line

The goat tongue cell line (ZZ-R 127) was obtained from the Friedrich-Loeffler-Institute (FLI), Germany and prepared at the FMD Research Laboratory, National Veterinary Research Institute, Vom, Nigeria.Confluent cell monolayer's of ZZ-R 127 were obtained and washed with 10ml of Phosphate buffer saline, pH 7.2-7.4, after which they were inoculated with 0.5ml of filtered field epithelial sample supernatant and incubated for 30 minutes at 37 °C. Afterwards, fresh cell Eagles's Minimum Essential Medium (EMEM) free of foetal calf serum (FCS) was added and the cultures were incubated at 37. 8°C and monitored for a cytopathic effect (CPE) for 48 hour. When no CPE was observed after 48 hrs, the cells were

subjected to one freeze-thaw cycle, clarified and inoculated unto a fresh cell monolayer. A sample was considered negative if no CPE was observed after 48 hours of the second cell passage. If the CPE was observed, the culture was harvested and the viral suspension was stored at -80°C until used.

**Detection of FMDV Antigen using Ag-ELISA**

The Ag-ELISA was performed according to the manufacturer's instructions IZSLER, Biotechnology Laboratory (Brescia Italy). The assay is a sandwich ELISA that performs with selected combinations of anti-FMDV monoclonal antibodies (MAbs), used as coated and conjugated antibodies. The test can be applied for detection and typing of FMD viruses in vesicular fluid, and homogenates of epithelial tissues. Only in these clinical specimens, the FMD virus usually attends the concentration required to provide a positive signal in ELISA assays ( Grazioli *et al.,* 2008).The kit is designed for detection and typing of FMD viruses of type O, A, SAT1 and SAT2. A pan-FMDV test, detecting any isolates of type O, A, C and Asia1 and, in addition, some of the SATs serotype is also included in the kit to complement the specific typing and to detect FMD viruses which might escape binding to the selected type-specific MAb (Grazioli *et al.,* 2008). Briefly, samples were diluted in equal volume of diluent buffer (1/2). A 50µl of each sample was distributed across the 12 wells of a row, two replicates for each type specific catching MAb and for the pan-FMDV-MAb. 50µl of the diluent buffer was added in all wells of G and H rows of the microtitre plate which are positive and negative controls respectively.The plate was incubated at room temperature (temperature range 18-22$^{o}$C) for 1 hour. After the incubation period, the plate was emptied to remove all remaining residual fluid. A 200µl of washing solution was added and, incubated for 3 minutes at room temperature (18-22$^{o}$C). The plate was emptied and the circle of washing was repeated 3 times. After the washing, A 50µl of appropriately diluted conjugate A was added into columns from 1 to 8 and conjugate B from 9 to 12. The plate was incubated for 1hour at room temperature. After the incubation period, four cycles of washing was repeated as above leaving the last one for 5minutes. After which 50µl of the substrate-chromogen solution was added to all the wells. The plate was covered and left at room temperature (18-22$^{o}$C) in the dark for 20 minutes. The reaction was later stopped by the addition of a stop solution and the plates were read on a MultiSkan® spectrophotometer ELISA plate reader (Thermo Scientific, USA) at 450 nm wavelength. Results were interpreted according to the protocol criteria for test validity and interpretation based on the manufacture's instruction.

**RESULT**

In this study a total of 22 clinical specimens were used, and 91% of these samples demonstrated strong CPE within 24 hours on ZZ-R 127 continuous cell line (Table I). FMDV serotypes O accounted for majority of the isolates (65%), followed by SAT 2, (20%) and A, (15%). Three serotypes (O, A & SAT 2) were isolated in clinical specimens from Plateau, two serotypes (O & A) in specimens from Kogi and only serotype O, was isolated from Benue State (Table I& II). Two out of the twenty-two specimen collected did not show CPE even after the second passage at 48 hours interval and it was declared negative. All clinical specimens that demonstrated CPE were further confirmed by an antigen detection and serotyping ELISA.

TABLE I. **Distribution of FMDV Isolated in the study area Using ZZR cell line**

| | | Presence of CPE at | | FMD serotype |
|---|---|---|---|---|
| Sample ID | Description | 24 hour | 48 hour | |
| KG/OKE/BUKU/5 | Epithelia | + | | O |
| KG/M5 | Epithelia | + | | A |
| MKD/FMD2011/04E | Epithelia | + | | O |
| PL/DN/001E | Epithelia | + | | SAT 2 |
| PL/DN/006/E | Epithelia | + | | SAT 2 |
| NS/DM/008 | Probang | - | - | NVD* |
| PL/BK/08185 | Epithelia | + | | SAT 2 |
| PL/BK/08196 | Epithelia | + | | SAT 2 |
| PL/KA/12M | Epithelia | + | | O |
| PL/BLD/02B | Epithelia | + | | A |
| PL/BLD/01A | Epithelia | + | | A |
| NS/WAM/03 | Epithelia | - | - | NVD* |
| PL/JS/KA/1 | Epithelia | + | | O |
| PL/JS/KA2 | Epithelia | + | | O |
| PL/JS/KA03 | Epithelia | + | | O |
| PL/KA/4/14 | Epithelia | + | | O |
| PL/KA/06/04/A-2 | Epithelia | + | | O |
| PL/KA/06/04/B-2 | Epithelia | + | | O |
| JS/BI/8/7/14/c | Epithelia | + | | O |
| JS/BI/6/7/14 | Epithelia | + | | O |
| BL/GA/07/14/1 | Epithelia | + | | O |
| BL/GA/07/14/2 | Epithelia | + | | O |

NVD = No Virus detected; + = CPE positive; - = CPE negative

TABLE II **FMDV Isolates based on States In the North Central Nigeria using Ag-ELISA**

| States | Number FMDV Samples | Number of FMDV Isolates | FMDV Serotypes |
|---|---|---|---|
| Plateau | 17 | 17 | SAT 2, O, A |
| Kogi | 2 | 2 | A, O |
| Benue | 1 | 1 | O |
| Nassarawa | 2 | - | - |
| **Total** | 22 | 20 | |

## DISCUSSION

It is an established fact that the BTY is the most sensitive cell for the primary isolation of FMDV (OIE, 2012). However, the challenge of maintaining continuous availability of FMDV-free BTY for diagnosis in an endemic setting is a major limitation. Therefore, there is a need to source for alternative cell lines that would be able to generate good CPE typical of FMD viruses with less rigours and struggle for disease endemic countries. To achieve this, other less sensitive but more convenient to handle cell lines such as cells of bovine, ovine and porcine origin or continuous cell lines such as BHK-21 or IB-RS-2 have being employed in most diagnostic laboratories (Brehm *et al.*, 2009).

In this study, Isolation of FMDV using ZZ-R 127 has proven to be rapid, reliable, convenient and easy to handle with an overnight amplification and detection of the wild-type FMD viruses circuiting in north central Nigeria. This is a welcome development for most laboratories that have challenges with primary virus isolation using continuous cell lines in resource scarce conditions.

Although PCR is known to be more sensitive and rapid for diagnostic purposes than virus isolations, the later still remains the gold standard. Therefore, there is the need to ensure that a cell culture system that is rapid, highly sensitive, which can also be easily maintained and could serve as alternative to BTY, cell monolayer should always be in place especially in laboratories located in endemic countries.

Although virus isolation depends on the quality of the sample as well as the virus load in the samples, isolation was achieved in over 90% of the clinical specimens tested using ZZ-R 127 cell line. The susceptibility of ZZ-R 127 cell line to FMDV and its suitability for the virus isolation from the field samples collected from north central Nigeria is in agreement with the study conducted by Brehm *et al.*(2009), where they reported that ZZR,127 is only slightly inferior to BTY but more superior to other continuous cell line (BHK- 21, IB-RS2), for FMDV isolation.

This study has improved the capacity and turns around time for virus isolation and prompt diagnosis in our laboratory. It has proved to be rapid, reliable, convenient and easy to handle, yielding an overnight amplification and detection of the wild-type FMD virus from clinical specimen. From this observation, we recommend that the cell line should be adopted for the primary isolation of FMDV in other laboratories. More research should also be conducted on its use and application for the isolation of other viral diseases of ovine and caprine species such as PPR and Orf.

### Limitation

Following the challenges we had with isolating these viruses in other cell lines there was no data for comparison of these isolates to other cell lines, except that most of them could not be isolated in BHK-21 after several attempts of primary isolation. Also we were not able to determine the infectivity titre of the isolates during the study.

### Acknowledgement

The authors acknowledge the management of the National Veterinary Research Institute, Vom for the use of their facility. The contribution of Mrs Monica Onoja and Mr Agom D,for preparing the cell line is also acknowledged. We wish to thank Friedrich-Loeffler-Institute for the supply of this cell line.

## REFERENCES


BREHM, K.E., FERRIS, N.P. LENK, M., RIEBE, R., and HAAS, B. (2009);Highly sensitive fetal goat tongue cell line for detection and isolation of foot-and-mouth disease virus. Journal of Clinical Microbiology. 2009; 47: 3156–3160.

CHUKWUEDO, A.A., and NIMZING, A. ( 2012); Field investigation of Foot and Mouth Disease ( FMD ) virus infection in cattle in the northern states of Nigeria . *Nigerian Journal of Biotechnology*., 24: 20–26.

EHIZIBOLO, D.O., IJOMANTA J.O., MICHAEL, C.A., WUNGAK, Y.S. LAZARUS D.D., ULARAMU, H.G., MESEKO, C., and UMOH, J.U. (2016);Serological survey of foot and mouth disease virus antibodies in apparently healthy domestic pigs in Nigeria. *African Journal of Microbiology Research.*,10:87–92.

EHIZIBOLO, D.O., PEREZ, A.M., CARRILLO, C., PAUSZEK, S., ALKHAMIS, M., AJOGI, I. AJOGI I., UMOH, J.U., KAZEEM, H.M., EHIZIBOLO, P.O., FABIAN, A. BERninger, M,. MORAN, K, RODRIQUEZ, L.L., and METWALLY, S.(2014);Epidemiological Analysis, Serological Prevalence and Genotypic Analysis of Foot-and-Mouth Disease in Nigeria 2008-2009. *Transboundary and Emerging Diseases.* 500–510.

FASINA, F.O., CONNEL, D.R., TALABI, O. A., LAZURUS, D.D., AELEKE, G.A., OLUSANYA, T.P., and HEMANDEZ, J.A. (2013); Foot-and-mouth disease virus strains and examination of exposure factors associated with seropositivity of cattle herds in Nigeria during 2007 – 2009. *Preventive Veterinary Medicine.*,109:334–342.

FERRIS, N.P., HUTCHINGS, G.H., MOULSDALE, H.J., GOLDING, J., and CLARKE, J.B.(2002); Sensitivity of primary cells immortalised by oncogene transfection for the detection and isolation of foot-and-mouth disease and swine vesicular disease viruses. *Veterinary Microbiology*.,84:307–316.

FERRIS, N.P., KING, D.P., REID, S.M., HUTCHINGS, G.H, SHAW, E, PATON, D.J., GORIS, N, HAAS, B., HOFFMANN, B., BROCCHI, E., BUGNETTI, M. DEKKER, A., and DE CLERCQ, K. (2006); Foot-and-mouth disease virus: a first inter-laboratory comparison trial to evaluate virus isolation and RT-PCR detection methods. Veterinary microbiology., 117: 130–40.

GOLLER, K.V, DILL, V, MADI, M. (2017). Rapid and simple detection of foot-and-mouth disease virus: Evaluation of a cartridge-based molecular detection system for use in basic laboratories. *Transbound Emerg Dis.*, 00:1–7.

GRAZIOLI E, BROCCHI, V., and TRANQUILLO, S.P.(2008); Development of Solid Phase Competitive ELISA Bases on Monoclonal. The Global Control of FMD - Tools, Ideas and Ideals – Erice, Italy 14-17; 2008. pp. 259–268.

HOUSE, C., and HOUSE, J.A. (1989);Evaluation of techniques to demonstrate foot-and-mouth disease virus in bovine tongue epithelium: comparison of the sensitivity of cattle, mice, primary cell cultures, cryopreserved cell cultures and established cell lines. *Vet Microbiol.* 1989; 20:99–109.

JAMAL, S.M., and BELSHAM G.J.(2013); Foot-and-mouth disease : past , present and future.*VeterinaryReseaserch.*,5:1–14.

KNOWLES, N.J, and SAMUEL, A.R. (2003) Molecular epidemiology of foot-and mouth disease virus. *Virus Research*., 91: 65–80.

LAZARUS, D.D. SCHIELEN, W.J.G., WUNGAK, Y. KWANGE, D. And FASINA, F.O. (2011); Sero-epidemiology offoot-and-mouth disease in some Border

States of Nigeria. *African Journal of Microbiology Research.2011;* 6 (8):1756-1761.

LONGJAM, N., DEB, R., SAMAH, A.K., TAYO, T., AWACHAT, V.B., and SAXENAS, V.K..(2016); A Brief Review on Diagnosis of Foot-and-Mouth Disease of Livestock : Conventional to Molecular Tools. *Vet World*.,;9(1):32-7.

NATIONAL AGRICULTURE RESEARCH STRATEGY PLAN:1996-2010. Department of Agricultural Sciences Federal Ministry of Agriculture and National Resources Abuja, Nigeria Shaib, Aliyu and Bakshi, eds. 1997;49-51

OIE, (2012). Foot and Mouth Disease Chapter 2.1.5 in: *OIE Terrestrial Manual*. 2012;1-29.

OLABODE, H.O., MAKAJOULA, K., ABIOLA, R. DOGONGIGINYA I., and DANIEL, N. (2014). Geo-spatial distribution of serologically detected bovine Foot and Mouth Disease (FMD) serotype outbreaks in Ilesha Baruba, Kwara State-Nigeria. *Journal of Advanced Veterinary and Animal Research*. 2014;1: 1.

OLABODE, O.H.,KAZEEM, H.M., RAJI, M.A., and IBRAHIM, N.D. (2013); Seroprevalence of foot and mouth disease virus antibodies in trade cattle (Bos indicus) in Kwara state of Nigeria. *Veterinary World*. ; 6: 828–832

ULARAMU, H.G. WUNGAK, Y.S., LAZARUS, D.D., WOMA, T.Y., EHIZIBOLO, D.D., DOGONYARO, B.B., BWALA, D.G., BAKARI, D.G., AGOM, D., ONOJA, M.A., IBU, J.O., and SHAMAKI, D. (2015);. Serological evidence of foot-and-mouth disease virus (FMDV) in camels (Camelus dromedaries) in Nigeria. *Journal of Veterinary Medicine and Animal Health.,* 7, 261–264

WUNGAK, Y.S., LAZARUS, D.D., AGOM, D., and ULARAMU, H.G. (2015); Serological evidence of foot-and-mouth disease virus ( FMDV ) antibodies in pigs from northeastern Nigeria. *Journal of Veterinary Medicine and Animal Health*., 7, 237–260.

WUNGAK, Y.S., OLUGASA, B.O., ISHOLA, O.O., LAZARUS, D.D., and ULARAMU, H.G. (2016); Foot-and-mouth disease ( FMD ) prevalence and exposure factors associated with seropositivity of cattle in north-central , Nigeria .*African Journal of Biotechnology*.,15:1224–1232.