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# Foot-and-mouth disease virus isolates: Candidate strains for trivalent vaccine development in Nigeria

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**A quality foot and mouth disease (FMD) vaccine is a prerequisite for effective control in addition to other zoosanitary measures and effective biosecurity practices in disease endemic sub-Saharan countries like Nigeria. To ensure an effective control programme by vaccination, countries that practice mass vaccination campaigns need to conduct vaccine matching studies to establish a relationship between prevalent field isolates with available vaccine for effective control. To this effect, a research was conducted in order to select foot-and-mouth disease viruses (FMDV) that will give a quality vaccine containing relevant serotypes and matching strains as a pre-requisite for effective vaccine. The study was conducted using two dimensional virus neutralization assays to determine the antigenic relationship 'r' value between the candidate vaccine strains and the field isolates. A total of forty-two specimens (epithelial tissue) were send to the World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) for virus detection and antigen serotyping and some of the field isolates were selected for vaccine matching based on geographic location and topotypes/subtype. The isolates selected were two each of serotype O, A and SAT 2 from bovine species. The selected field isolates revealed high antigenic similarity with the vaccine strains tested showing 'r' value greater than 0.3 which suggests a close relationship between field isolates and vaccine strain tested. A potent vaccine containing the vaccine strain is likely to confer protection in vaccinated candidates. A vaccine match with 'r' value less than 0.3 suggests poor protection against challenge with the isolates. The result of this study has indicated that the selected field isolates could be used as vaccine strains for a candidate trivalent FMD vaccine production in Nigeria.**

**Key words:** foot and mouth disease (FMD) virus, 'r' value, vaccine strain, Nigeria.

## INTRODUCTION

Foot-and-mouth disease (FMD) is an economically challenging disease affecting all ruminants and cloven hoofed domestic and wild animal species (OIE, 2008). The disease is characterized by vesicles and ulcer in the

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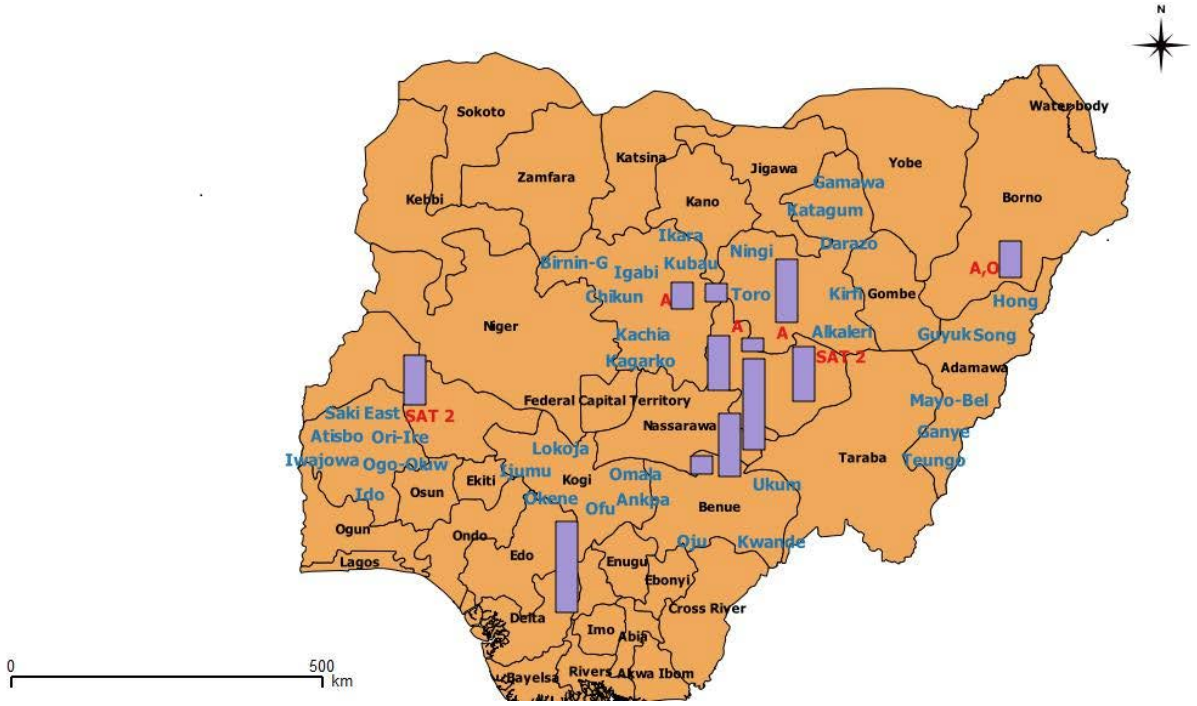


**Figure 1.** A Map showing the origin of the vaccine and field Isolates used in the study.

mouth, muzzle, feet and udder of lactating cows. FMD incurs huge economic losses to the livestock industry because of high morbidity in adult animals resulting in loss of production, loss of draught power, retarded growth, abortion in pregnant animals and mortality in young calves and lambs (Ayelet et al., 2009).

FMD is an endemic trans-boundary animal disease (TAD) in Nigeria with outbreaks occurring seasonally in both pastoral and sedentary husbandry. It is one of the major animal diseases that impact negatively on trades in livestock and livestock products in most developing countries (Jamal et al., 2014). To date, four of the seven serotypes had been found in circulation in Nigeria (Fasina et al., 2013). These include serotypes O, A, SAT 1 and SAT 2 and each serotype has many subtype variants. This antigenic variation is a cause of major setback in the control of FMD, as infection or vaccination with one serotype of FMDV does not protect against other serotypes and may fail to confer protection against other subtypes within the same serotype (Ayelet et al., 2009). The disease is caused by a small positive sense ssRNA virus (approx. 8.3 kb), which belongs to the *Aphthovirus* genus of the family *picornaviridae* (Ayelet et al., 2009).

A good quality vaccine containing relevant serotypes and matching strains is vital for effective control programme. However, the protective efficacy of FMD vaccine can be evaluated through vaccine matching using indirect serological methods (Rweyemamu 1984a; Rweyemamu 1984b) and it can also be calculated using the relatedness between the field isolate and available vaccine strains using *in vivo* challenge tests (Brehm et al., 2008; Goris et al., 2008). Selection of viruses for vaccine matching is very important and should be based on epidemiological information, including stages of an epidemic, geographical locations or range of host species (Alonso et al., 1993; Paton et al., 2005) by incorporating a minimum of two isolates from outbreak (OIE, 2008).

Since vaccination is important in the control of FMD in West Africa, Nigeria in particular, and the best vaccination program should involve those that target the topotypes, strains and serotypes circulating within the West and Central Africa sub-region (Knowles and Samuel, 2003). It is necessary to compare field viruses against vaccine strains as it has been shown that some variants within a serotype were unable to break through immunity and therefore more antigenic characterization is important (Knowles and Samuel, 2003). More so, the presence of multiple serotypes of FMD and the inability to cross protect among serotypes and subtypes has necessitated the need to develop a vaccine having strains that can confer sufficient immunity (Jamal et al., 2014). In this regard this study was designed to determine FMD vaccine strains that are most appropriate for serotypes/topotypes currently circulating in Nigeria.

## MATERIALS AND METHODS

### FMD virus candidate strains for trivalent vaccine

This study was conducted on virus isolates from different region of the Country of Nigeria (Figure 1) between 2011 and 2014. The samples were sent to the World reference laboratory for foot-and mouth disease (WRLFMD), United Kingdom for virus detection and

antigen serotyping. A total number of 42 specimens (epithelial tissue) were sent for virus detection and antigen serotyping, of which ten serotype O, nine serotype A, and 13 SAT 2 were detected respectively (Table 1). The vaccine candidates were selected according to specific geographic locations, endemic regions, topotypes/subtype, source species and the period of occurrence (OIE, 2008). Some vital determinants that may affect the efficacy of a vaccine and determine whether it may protect or not are: the ability of the vaccine strain to elicit antibodies that will cross-react and protect against the field or outbreak virus in question (defined as the vaccine or antigenic match), and the potency of the vaccine to elicit a strong and long-lasting immune response (Doel, 2005). Two isolates were selected from each of the field circulating serotype representing serotypes O, A and SAT 2. The isolates used were A NIG. 03/13, A NIG. 07/13, SAT 2 NIG 03/12, SAT 2 NIG 17/11, O NIG 03/14 and O NIG 04/14 (Table 2). All samples were isolated in primary cell culture and serotyped by ELISA (Table 2).

### Vaccine preparation

The preparation of inactivated monovalent FMD vaccine from selected vaccine strains was based on the OIE recommended protocol (OIE, 2008). The virus isolate after primary isolation using ZZR continuous cell line was adapted to BHK-21 cell line and passage to 7$^{th}$ passage on BHK-21 monolayer. The isolates were inactivated using 0.04% of 10% buffered Formalin and the effect of the formalin was neutralized by 2% each of sodium thiosulphate (20%) and sodium bisulphite (20%) (Iyer et al., 2000). After the inactivation process equal volume of Montanide ISA 206 was added and mixed thoroughly. The prepared vaccine was kept at +4 ° C until use.

### Antiserum preparation

A total of ten adult rabbits weighing averagely 2.5 kg were used after been screened for FMD Non-structural proteins antibodies by 3ABC-ELISA (PrioCHECK®FMDV NS prionics Lelystad B.V. Netherlands). The rabbits were inoculated intramuscularly using 1.0 ml of the inactivated antigen and four booster doses were given to achieve a high antibody titre. The rabbit were bled at day 56$^{th}$ and serum harvested and stored at -20°C until used.

### FMD virus titration

The FMDV vaccine candidates and field isolates were titrated using a tenfold serial dilution beginning with $10^{-1}$ by taking 0.5 ml of the isolate to 4.5 ml of the diluent minimum essential medium (MEM). Using a sterile pipette tips, 0.5 ml from the first dilution was taken and transferred to the next and continued serially to the tenth dilution using different sterile pipette tips at each transfer. Fifty microliter of each virus dilution ($10^{-1}$ to $10^{-8}$) was distributed in the wells of respective rows on microtiter plates containing established cell layers of baby hamster kidney (BHK-21). Then 100 µl/well MEM was added and incubated at 37° C for 24 h and the titer was determine (Ayelet et al., 2013)

### Vaccine matching by two-dimensional virus neutralization

The vaccine matching was performed at the WRLFMD by using the two-dimensional virus neutralization test according to protocol of WRLFMD United Kingdom. Briefly, both field isolates and vaccine strains were passaged on monolayer of BHK-21 cell culture until adapted to give 100% CPE within 24 h. The infected BHK-21 monolayer cells were subjected to three times freeze-thaw cycles to release the viral particles from the cells. Fifty microliter of serum raised against the reference vaccine strain was added on row A wells (1-10) and serially diluted starting with ½ in microtiter plates (OIE, 2008) and a constant amount (50 µl) of pre-titrated field isolates of 100 $TCID_{50}$ dose was added in each well using two columns for each antigen. Columns 1 and 2 of each microtiter plate were used for homologous virus of the candidate vaccine strain. After 1 h incubation at 37°C, 50 µl of virus/serum mixture was transferred into their respective microplate wells containing established monolayer BHK-21 cells, sealed with a semi permeable sealer and incubated at 37°C in a 5% $CO_2$ incubator for 48 h. After 48 h incubation, plates were observed for cytopathic effect using an inverted microscope. Finally, titers of the reference antiserum against the heterologous or field isolates and titer of reference antiserum against homologous virus was calculated for each test viruses and candidate vaccine strains (OIE, 2008).

### Statistical analysis

The data obtained in two dimensional sero-neutralization assays were used in order to calculate the antigenic similarity between candidates of vaccinal strains and field isolates. Antibody titers of the vaccine serum against the field isolate for each virus dose used were calculated using the Spearman-Karber method (OIE, 2008). The titer of the vaccine serum against 100 $TCID_{50}$ of each virus was then estimated by regression analysis. The relationship between the field isolate and the vaccine strain is then expressed as:

$$r = \frac{\text{Reciprocal arithmetic titer of reference serum against field virus}}{\text{Reciprocal arithmetic titer of reference serum against vaccine virus}}$$

It is generally accepted that in case of neutralization, r values greater than 0.3 indicates that the field isolate is sufficiently similar to the vaccine strain (OIE, 2008).

## RESULTS

The interpretation of the results for the vaccine matching suggests that there is a close relationship between field isolates studied and vaccine strains tested. And a potent vaccine containing the vaccine strain is likely to confer protection against challenge with a homologous or related virus. The isolates with ‘r’ value less than 0.3 suggest that the field isolate is so different from the vaccine strain and that the vaccine is unlikely to protect.

The results as shown in Table 3 indicates that there is a close antigenic relationship between the vaccinal A ERI98 and A TUR06 and the field isolates of A NIG 03/13 and A NIG 07/13. This is because the calculated ‘r’ value was greatly higher than the minimum required value (>0.3).

For serotype O (Table 4) the results indicated that O NIG 03/14 was antigenically related to O TUR 5/09.

The SAT 2 serotype of SAT 2 NIG 03/12 and SAT 2 17/11 were antigenically related to that of SAT 2 ERI and SAT 2 ZIM but the SAT 2 NIG17/11 showed slight decrease in ‘r’ value compare to SAT 2 NIG 03/12 as shown on Table 5.

## DISCUSSION

Foot-and-mouth disease is an endemic disease in West

**Table 1.** FMD detection and serotyping results.

| NIG. Reference | WRL Reference | Description of Sample | PCR Result | Serotyping Result by Cell Culture/ELISA |
|---|---|---|---|---|
| MKD/FMD2011/04E | NIG1/2011 | BOVINE, epithelium, collected 11/06/20111 | FMDV (GD) | O |
| KG/M5 | NIG2/2011 | BOVINE, epithelium, collected 26/06/2011 | FMDV (GD) | A |
| KG/OKEBUKU/5 | NIG3/2011 | BOVINE, epithelium, collected 26/06/2011 | FMDV (GD) | O |
| BN/MKD/18 | NIG4/2011 | BOVINE, probang, collected 07/07/2011 | (NGD) | NVD |
| PL/DN/001/E | NIG5/2011 | BOVINE, epithelium, collected 20/07/2011 | FMDV (GD) | SAT 2 |
| PL/DN/006/E | NIG6/2011 | BOVINE, epithelium, collected 20/07/2011 | FMDV (GD) | SAT 2 |
| KD/KCH/07 | NIG7/2011 | BOVINE, epithelium, collected 22/07/2011 | FMDV (GD) | SAT 2, O |
| PL/JS/005 | NIG9/2011 | BOVINE, epithelium, collected 02/08/2011 | FMDV (GD) | SAT 2 |
| PL/JS/001 | NIG10/2011 | BOVINE, epithelium, collected 02/08/2011 | FMDV (GD) | SAT 2, O |
| NS/DM/008 | NIG11/2011 | BOVINE, probang, collected 02/08/2011 | NGD | NVD |
| PL/TNK/01 | NIG12/2011 | BOVINE, epithelium, collected 06/08/2011 | FMDV (GD) | SAT 2 |
| PL/TNK/05 | NIG13/2011 | BOVINE, epithelium, collected 06/08/2011 | FMDV (GD) | SAT 2 |
| 14/UD/EP/2/1/11 | NIG14/2011 | BOVINE, epithelium/homogenate, collected 10/08/2011 | FMDV (GD) | NVD |
| TR/IB/29/P | NIG15/2011 | BOVINE, probang, collected 09/09/2011 | FMDV (GD) | NVD |
| PL/BK/08185 | NIG16/2011 | BOVINE, epithelium, collected 03/11/2011 | FMDV (GD) | SAT 2 |
| PL/BK/08196 | NIG17/2011 | BOVINE, epithelium, collected 03/11/2011 | FMDV (GD) | SAT 2 |
| OY/IGB/4 | NIG1/2012 | BOVINE, epithelium, collected 22/06/2012 | FMDV (GD) | SAT 2 |

**Table 1.** Cont'd.

| | | | | |
|---|---|---|---|---|
| OY/IGB/02C | NIG2/2012 | BOVINE, epithelium, collected 22/06/2012 | FMDV (GD) | SAT 2 |
| OY/IGB/03b | NIG3/2012 | BOVINE, epithelium, collected 22/06/2012 | FMDV (GD) | SAT 2 |
| AD/MDG/2012 | NIG4/2012 | BOVINE, epithelium, collected 03/08/2012 | FMDV (GD) | O |
| AD/SH/6 | NIG5/2012 | BOVINE, epithelium, collected 03/08/2012 | FMDV (GD) | O |
| PL/SH/2012 | NIG6/2012 | BOVINE, epithelium, collected 08/08/2012 | FMDV (GD) | NVD |
| PL/KA/12M | NIG7/2012 | BOVINE, epithelium, collected 09/09/2012 | FMDV (GD) | NVD |
| PL/BLD/02B | NIG8/2012 | BOVINE, epithelium, collected | FMDV (GD) | A |
| PL/BLD/01A | NIG9/2012 | BOVINE, epithelium, collected 06/11/2012 | FMDV (GD) | A |
| PL/BLD/01B | NIG10/2012 | BOVINE, epithelium, collected 06/11/2012 | FMDV (GD) | A |
| NS/WAM/03 | NIG 11/2012 | BOVINE, epithelium, Collected 07/11/2012 | FMDV GD | NVD |
| KD/KAU/01 | NIG 12/2012 | BOVINE, Epithelium, Collected 13/1/2012 | FMDV GD | A |
| ABJ, TISS/03 | NIG 1/2012 | BOVINE, epithelium,/homogenate, Collected 03/01/2013 | FMDV GD | NVD |
| KD/KAU/1 | NIG 2/2013 | BOVINE, epithelium, Collected 26/06/2013 | FMDV GD | NVD |
| PL/BLD/02/13 | NIG 3/2013 | BOVINE, Lab isolated sample/cell culture, Collected 17/09/2013 | FMDV GD | A |
| PL/BLD/04/13 | NIG 4/2013 | BOVINE, Lab isolated sample/cell culture, Collected 17/09/2013 | FMDV GD | NVD |
| PL/BLD/03/13 | NIG 5/2013 | BOVINE, Lab isolated sample/cell culture, Collected 17/09/2013 | NGD | NVD |
| BAU/T/B2/13 | NIG 6/2013 | BOVINE, Epithelium, Collected 21/11/2013 | FMDV GD | A |
| BAU/T/C3/13 | NIG 7/2013 | BOVINE, Epithelium, Collected 21/11/2013 | FMDV GD | A |

**Table 1.** Cont'd.

| | | | | |
|---|---|---|---|---|
| BAU/T/A1/13 | NIG 8/2013 | BOVINE, Epithelium, Collected 21/11/2013 | FMDV GD | A |
| PL/JS/KA/1 | NIG 1/2014 | BOVINE, Epithelium, Collected 03/01/2014 | FMDV GD | O |
| PL/JS/KA/2 | NIG 2/2014 | BOVINE, Epithelium, Collected 03/01/2014 | FMDV GD | O |
| PL/KA/03 | NIG 3/2014 | BOVINE, Epithelium, Collected 03/01/2014 | FMDV GD | O |
| PL/KA/4/14 | NIG 4/2014 | BOVINE, Epithelium, Collected 14/01/2014 | FMDV GD | O |
| PL/KA/7B | NIG 5/2014 | BOVINE, Epithelium, Collected 18/01/2014 | NGD | NVD |

NVD, No virus Detected; FMDV GD, FMDV Genome Detected; NGD, No Genome Detected.

**Table 2**. FMDV candidate vaccine strains selected for vaccine matching.

| Name of candidate vaccine | Site of isolation | Year of isolation | Serotype | topotype |
|---|---|---|---|---|
| O NIG 03/2014(PL/KA/03) | Plateau | 2014 | O | EA-3 |
| O NIG 04/2014(PL/KA/4/14) | Plateau | 2014 | O | EA-3 |
| A NIG 03/2013(PL/BLD/02/13) | Plateau | 2013 | A | Africa |
| A NIG 07/13(BAU/T/C3/13) | Bauchi | 2013 | A | Africa |
| SAT 2 NIG 03/12(OY/IGB/03b) | Oyo | 2012 | SAT2 | VII |
| SAT 2 NIG 17/11(PL/BK08196) | Plateau | 2011 | SAT2 | VII |

Africa and Nigeria in particular which has remained a major economic challenge for livestock production. FMD has hampered the export of livestock and livestock products to more international market due to strict zoosanitary regulations. The genetic diversity of FMD and its endemic nature in Nigeria and the current circulating serotypes/subtypes in the Country have necessitated research into the production of trivalent vaccine. Therefore, in order to have good quality, FMD vaccine that will have a protective capacity, the candidates vaccine need to be evaluated through vaccine matching using indirect serological methods (Rweyemamu, 1984b; Paton et al., 2005). The viruses selected for vaccine matching in this research was based on epidemiological information, including the stages of an epidemic and the geographical locations as reported by (Alonso et al., 1993; Paton et al., 2005).

From the findings, the A NIG 03/13 ('r'=0.41) and A NIG 07/13('r'=0.46) field isolates showed a good serological matching with A ERI 98 but a decrease in 'r' value A NIG 03/13 (0.35) and A NIG 07/13(0.28) to A TUR 06. The serological match of the field isolates to A ERI 98 could be attributed to the topotype similarities which suggest that there is close relationship between field isolate and the vaccine strain. But the low 'r' value recorded with the vaccine strain of A Iran 2005 and A22 IRQ suggest that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect (Ayelet et al., 2009).

The O NIG 03/14 and 04/14 also showed a good serological match with O TUR 5/09 which also suggest that there is a close relationship between the field isolates and the vaccine strain. But the result with O Manisa vaccine strain was far lower and these suggest that the Nigeria serotype O 2014 field isolate is so different from the vaccine strain and the vaccine is unlikely to protect.

The 'r' value obtained for SAT 2 serotype indicate that the SAT 2 Eri and SAT 2 ZIM showed a close relationship between field isolate and vaccine strain. However, the result of SAT 2 Nig17/11 indicated 'r' value less than 0.3 which suggest that the field isolate of SAT 2 NIG17/2011 is so different from the SAT 2 ZIM strain and that the vaccine is unlikely to protect (Rweyemamu, 1984a). The likely explanation of this difference in 'r' value (0.25) of

**Table 3.** 'r' values obtained between serotype A field isolates and vaccine strains.

| Field Isolates | 2dm VNT | | | |
|---|---|---|---|---|
| | Vaccines | | | |
| | **A ERI98** | **A IRAN 2005** | **A22 IRQ** | **A TUR06** |
| A NIG 03/13 (mean) | 0.41 | 0.07 | 0.26 | 0.35 |
| A NIG 07/13 (mean) | 0.46 | 0.06 | 0.17 | 0.28 |

**Table 4.** 'r' values obtained between serotype O field isolates and vaccine strains.

| Field Isolates | 2dm VNT | | |
|---|---|---|---|
| | Vaccines | | |
| | **0.3039** | **O Manisa** | **O TUR. 5/09** |
| O NIG 03/14 (mean) | 0.65 | 0.10 | 0.40 |
| A NIG 07/13 (mean) | 0.53 | 0.06 | - |

**Table 5.** 'r' values obtained between serotype SAT 2 field isolates and vaccine strains.

| Field Isolates | 2dm VNT | |
|---|---|---|
| | Vaccines | |
| | **SAT 2 ERI** | **SAT 2 ZIM** |
| SAT 2 NIG 03/12 (mean) | 0.36 | 0.47 |
| SAT 2 NIG 17/11 (mean) | 0.37 | 0.25 |

the Nigerian isolate (SAT 2 NIG 17/11) with that of SAT 2 ZIM strain could be as the differences in strain, because the two Nigerian isolates are from different geographical locations and could have different strain.

In conclusion all the tested candidate vaccine strains that had 'r' value greater than 0.3 indicate strong antigenic match. This also suggests that there is a close relationship between the field isolate and vaccine strain. But the candidate vaccine strains that had 'r' value less than 0.3 suggest that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect. Therefore the candidate vaccine strain selected could be included in the trivalent vaccine formulation so as to effectively control FMD outbreaks in Nigeria. Finally vaccine matching should be routinely carried out in order to have a potent vaccine strain that is likely to confer protection in FMD endemic Country like Nigeria.

## Conflict of interests

The author(s) did not declare any conflict of interest.

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