MOLECULAR CHARACTERIZATION OF FOOT AND MOUTH DISEASE VIRUS AMONG SMALL RUMINANTS IN MIXED LIVESTOCK SYSTEMS IN GOMBE STATE, NIGERIA

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ABSTRACT

In assessing molecular characterization of foot and mouth disease virus among small ruminants in mixed livestock systems, three (43) epithelium samples (30 from cattle, 8 sheep and 5 goats) were collected and processed for RT – PCR targeting the conserved VPI/ID of the FMD virus. The amplicons were separated in a 1.5% agarose gel electrophoresis, stained with ethidium bromide and documented using gel documentation system. Amplicons size of approximately 328 base pair were considered positive. Eight outsourced samples were sent to FAO World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) for sequencing. The sequencing was analysed using TREE VIEW soft wares. The tree – view was produced from sequence using basic local alignment search tool (BLAST) pairwise alignments and fast minimum evolution. The result showed that 7 (23.3%), 1 (14.3%) and 0 (0%) were positive for cattle, sheep and goats respectively. The phylogeny indicated that the Nigerian Foot and Mouth Disease virus isolate obtained from this study (NIG/1/2024) was more closely related to NIG/3/2023 and TUN/2/2023. With an identity rate of 97.63%, the Nigerian Foot and Mouth Disease virus isolate also forms a paraphyletic relationship with Foot and Mouth Disease virus in Tunisia. It also forms a polyphyletic relationship with isolate from Ethiopia, Sudan, Iran, Ghana, Burkina Faso and Senegal.. In conclusion, the Phylogenetic analysis revealed that the Nigerian FMDV isolates from this study (NIG/1/2024, NIG/2/2024, NIG/3/2024 and NIG/4/2024) shares a close genetic relationship with NIG/3/2023, TUN/2/2023, Ethiopia, Sudan, Iran, Ghana, Burkina Faso and Senegal with a sequence identity of 97.63%. Movement-controlled measure, use of multivalent vaccines comprising the three local circulating serotypes and public education on FMD was recommended.

Keywords: FMD, Epithelium, Molecular, RT PCR, Phylogeny, Nigeria.

INTRODUCTION

Small ruminants (sheep and goats) are sometimes preferred by farmers compared to large ruminants because of the small space they occupy and less fodder requirement. Small ruminant production has been described as one of the fastest growing agricultural subsectors in developing countries. Its share of agricultural gross domestic product (GDP) has been reported as 33% and is rapidly increasing (Delqado, 2005; Thornton, 2010). In addition, goats have high adaptability to harsh climates which makes them suitable for husbandry in marginal areas (Kosgey, *et al.,* 2008; Wanyoike, 2009). They are veritable sources of income generation, household consumption, and hobby and as security against crop failure. Lebbie (2004) reported that sheep and goats play a significant role in the food chain and overall livelihoods of rural households, where they are largely the property of women and their children. Rearing of SR plays a very important role in the lives of households in developing countries.

In Nigeria, the role of SR farming in poverty alleviation for the common people cannot be overemphasized; it has become an integral part of the socio-economic life of most rural dwellers. SR production is an immense benefit to livestock farmers in the areas of meat and milk production, hides and skin, manure income generation, cultural and religious ceremonies and festivals, they also serve as a source of ready cash to small farmers in emergency situation (Al-khaz'leh *et al.,* 2005; Nwachukwu and Berenidu, 2020). Other advantages include lack of social and religious barrier to its production and consumption (Yusuf *et al.,* 2018). Infectious diseases are the major constraints in SR production (Nyariki and Amwata, 2019).

Foot and mouth disease (FMD) is an acute highly contagious, transboundary, disease caused by foot and mouth disease virus (FMDV). It affects cloven-hoofed domestic ruminants such as cattle, swine, sheep and goats as well as cloven-hoofed wild ruminants (Arzt *et al.,* 2011). It severely affects livestock production leading to disruption of trade in animals and their products at regional and international level. A global strategy for the control of FMD was endorsed in 2012 to minimize the burden of FMD in endemic settings and maintain free status in FMD-free countries (OIE,

2018). About 77% of the global livestock population is affected by the disease, mainly in Africa, the Middle East and Asia, and some few areas in South America (OIE, 2018). This is coupled with the possibility of disease incursion in countries which are currently free (OIE, 2018).

The FMDV is classified into the *Picornaviridae* family and the genus Apthovirus. It is a small non-enveloped virus with an icosahedral capsid and a positive sense RNA consisting of a large open reading frame encoding for four structural proteins and ten nonstructural proteins (Mason *et al.,* 2003). The FMDV exists in seven immunologically distinct serotypes; A, O, C, Asia 1, SAT 1, SAT 2 and SAT3; all with distinct lineages except SAT 1 and SAT 2 which have unresolved clades (Yoon *et al.,* 2011). The disease is among the World Organisation for Animal Health (OIE) listed diseases which are transmissible with serious potential to spread across national borders and which require immediate reporting in order to control their spread (OIE, 2018). The incubation period for foot-and-mouth disease virus is between one and 14 days in cattle (Arzt, *et al.,* 2010), 3-8 days in small ruminants (Mahmoud *et al.,* 2019). The disease in cattle is characterized by high fever within two to three days, formation of vesicles inside the mouth leading to drooling of saliva. Vesicles are also on the nose, teats and when on the feet may rupture and cause lameness. It also causes weight loss in adults and significant reduction in milk production which sometimes fails to return to normal even after recovery. Morbidity rate can be as high as 100% though mortality rate is low in adults but high in neonates due to myocarditis. About 50% of infected ruminants remain asymptomatic carriers in the oropharynx. In cattle the virus can persist for 3-5 years, in sheep up to nine months, in goats up to six months and in the African buffalo up to five years. Pigs do not become carriers (Stenfeldt *et al.,* 2014). Viral excretion in carrier animals is intermittent and declines over time and the risk of transmission from the African buffalo to cattle exists (Bengis *et al.,* 1986). Foot and mouth disease in adult sheep and goats is frequently asymptomatic, but can cause high mortality in young animals (Mahmoud *et al.,* 2019). Lameness is a significant feature characterized by unwillingness to rise or move (Donaldson *et al.,* 2000; Mahmoud *et al.,* 2019). The disease can easily be missed unless individual animals are carefully examined for disease lesions. Small ruminants can therefore be responsible for the introduction of FMD into previously disease-free herds (Kitching *et al.,* 2002). The mortality rate in sheep and goats is generally less than 1% in adult animals. Clinical disease in young lambs and kids is characterized by death due to heart failure without the appearance of vesicles (Barnett and cox 1999).

Although FMD may be suspected based on clinical signs and post-mortem findings, it cannot be differentiated clinically from other vesicular diseases. Confirmation of any suspected case through laboratory tests is therefore essential. The richest source of virus in diagnosis is vesicular fluid or epithelium from fresh lesions (Radostits *et al.,* 2007). Serum is used for antibody detection where lesions are not fresh and also in epidemiological surveys. Differential diagnosis for FMD in small ruminants includes Peste des petit ruminantium (PPR) which can be ruled out by signs of pneumonia and diarrhea, Bluetongue disease (atypical signs are facial oedema and nasal ulceration), Capripox (which is ruled out by presence of pock lesions), Contagious ecthyma is also ruled out because of lacks of vesicular stomatitis and lameness which are characteristic in FMD), Pneumonic Pasteurellosis and Contagious Caprine Pleuropneumonia (CCPP) are characterized by respiratory illness alone (Radostits *et al.,* 2007).

FMD is widely distributed in the developing world, in particular Africa, Asia, Middle East and South America, where livestock farming forms the backbone of rural economies that supports approximately 70% of the world's poor (Maree *et al.,* 2014). FMD outbreaks particularly affects vulnerable individuals such as women and children in rural areas since approximately 75% of livestock in Africa are raised under the pastoral systems for sustainable livelihoods (Scoones *et al.,* 2010; Ferguson *et al.,* 2013; Miguel *et al.,* 2013). The lack of veterinary infrastructure, human resources, movement controls, and appropriate vaccines render many developing countries particularly exposed to the spread of FMD (Doel, 2003; Sutmoller *et al.,* 2003; Perry and Rich, 2007). In sub-Saharan Africa, two transmission cycles of FMD occur: one in which FMDV circulates between wildlife and domestic animals and the other in which the virus spreads among domestic animals. The cycle between wildlife and domestic animals occurs in southern and eastern Africa, but due to the low populations of wildlife in West Africa, the disease is maintained mainly in domestic animals (Fasina *et al.,* 2013).

In Africa, the diversity of circulating field strains of FMDV makes the selection of sufficiently cross-protective FMD vaccines a challenge. There is a need for risk-based surveillance to determine endemic areas and factors that influence disease dissemination, to assist the design of targeted, area-wide, or ecosystem-based disease control strategies, as most African regions adopt the Food and Agriculture Organization of the United Nations (FAO)-OIE Progressive Control Pathway (PCP) for the Control of FMD (Rweyemamu *et al.,* 2008). Also, the efficiency of FMD surveillance and control programmes in developing countries is often challenged by the issue of underreporting (Madin, 2011; Bellet *et al.,* 2012). However, FMD is known to cause significant financial losses for small scale producers, making it a threat to the livelihood and food security of the poorest communities (Bellet *et al.,* 2012). The seroprevalence of between 50 and

78% have been reported in cattle population (Lazarus *et al.,* 2012; Wungak *et al.,* 2015) with serotypes A, O, SAT 1 and SAT 2 among currently circulating strains in Nigeria (Wungak *et al.,* 2017; Ularamu *et al.,* 2016; Vandenbussche *et al.,* 2017).

Four serotypes of FMD (O, A, SAT 1 and SAT 2) are known to be circulating in Nigeria (Chukwuedo, and Nimzing, 2012; Fasina *et al.,* 2013; Ehizibolo *et al.,* 2014; Olabode *et al.,* 2014; Wungak *et al.,* 2015). The 3ABC competition antibody ELISA which has high sensitivity and specificity can deliver same-day results when using the short protocol and is routinely applied for general screening FMD (Parida, *et al.,* 2007; Parida, *et al.,* 2009).

Sheep and goats form a substantial proportion of the global FMD-susceptible livestock population. However, these species have not been studied with regard to their epidemiological role and significance in the spread of FMD. Unlike cattle, sheep and goats are not included in vaccination control programs in Study area. In spite of their potential infection, though all species are subject to the normal quarantine measures in disease outbreak. Thus, the present study was to investigate the sero-prevalence of FMD in small domestic small ruminants in Gombe state and to investigate potential risk factors associated with FMD occurrence in these animals.

MATERIALS AND METHOD

Ethical Approval

Ethical approval for this study was obtained from the Animal Use and Care Committee of Ahmadu Bello University, Zaria. The approval reference number is ABUCAUC/2025/050

Study area

The study was conducted in Gombe State, Nigeria. It is located on longitude 11° 10‘ E and latitude 10° 15′ and situated in the north eastern part of Nigeria. Being located within the expansive savannah allows the state to share common borders with the states of Borno, Yobe, Taraba, Adamawa and Bauchi. Gombe State has an area of 20,265 km^2 and a population of about 2,353,879 million people (NPC, 2006). Gombe state has two distinct climates, the dry season (November – March) and the rainy season (April – October) with an average rainfall received of 850mm/annum. Administratively the state is made up of 11 local Government Areas and 14 traditional chiefdoms (GSG, 2013). The state has an estimated cattle population density of 1 million and 2.5 million small ruminants (GSBS, 2018). The savannah vegetation as well as the present of Dadin-kowa dam, Cham dam, Balanga dam and Wawa Zange grazing reserve makes the state suitable for livestock rearing. The state comprises of different ethenic groups which include Fuifulde, Tera, Tangale, Babur, Kanuri, Waja, Hausa, Bolewa, Jukun, Cham, Tula, Dadiya, Pero, Lunguda, Awak and Kamo.

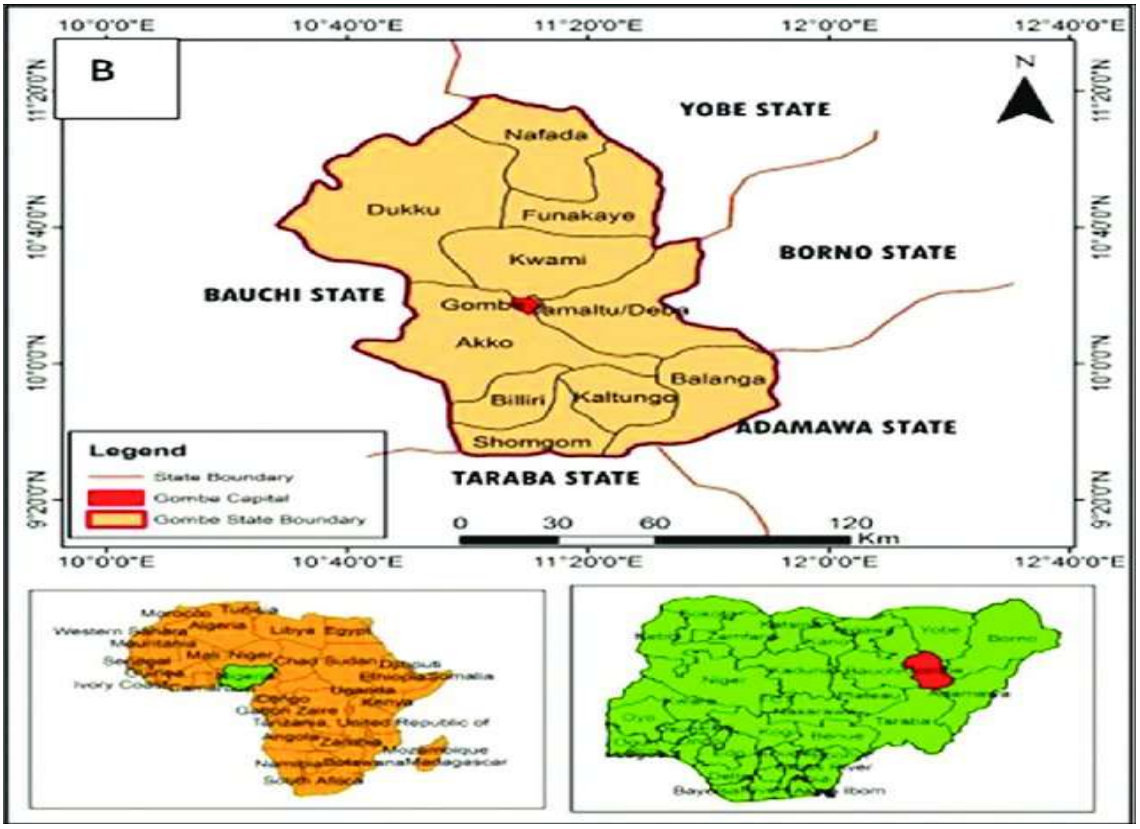

Figure 1. Map of Gombe State showing the study area (Source: Adamu and Saidu 2015)

Study Design

The study was a cross-sectional study, combining field observation, serological testing, molecular analysis, and survey-based risk assessment. The study population included animals: Sheep and goats in mixed herds with cattle and humans: Farmers and livestock handlers of the Gombe region. A multistage cluster sampling was employed to select sampling units.

A semi-structured closed ended questionnaire was developed and administered to the herders immediately after sample collection. These questions were deployed via KoBoToolbox, using the KoBoCollect mobile app on Android phones, to enable both offline data collection and online data submission (Lakshminarasimhappa, 2021) by trained field assistants.

Physical examination of the animals

The physical examination was conducted to gather essential information for assessing the health status of the animals. This information, combined with the history obtained from the farmer or herdsman, enabled us to identify specific signs of disease and helped localize the disease process to particular organ systems.

Initial observations were made from a distance, focusing on the animal's posture, gait, behavior, and general physical condition. Observing other members of the flock or herd helped differentiate normal from abnormal characteristics within the specific management system, as what is considered "normal" may vary from farm to farm. Additionally, the owner's or herdsman's perception of normal may actually reflect abnormal conditions. This comparative observation was valuable for assessing the incidence of diseases or disorders linked to management practices.

Vital parameters including respiratory rate, pulse rate, and body temperature, were recorded. As more animals across different herds were examined, a broader understanding of management-related deficiencies was developed, allowing for more reliable assessments.

With the animal properly restrained, the physical examination progressed to specific body parts. Clinical signs were checked on the limbs, dental pad, coronary band, and interdigital spaces for any lesion's indicative of Foot and Mouth Disease.

Molecular Diagnosis

Collection of Epithelial Tissue Samples for antigen detection

A total of 43 epithelial tissue samples were purposively collected—two samples (one from a small ruminant and one from a cattle) from each household herd. Animals were clinically examined for the presence of Foot and Mouth Disease (FMD) lesions on the mouth, teats, nostrils, and feet.

Epithelial tissue was collected from unruptured or freshly ruptured vesicles and placed in sterile bottles containing virus transport medium. The medium consisted of equal parts glycerol and 0.04 M phosphate-buffered saline (PBS) with antibiotics, including penicillin, streptomycin, gentamicin, and amphotericin B, adjusted to a pH of 7.2–7.6 (OIE, 2012).

Samples were transported to the laboratory under cold chain conditions and stored at −20°C until processing.

Sample processing

Samples were centrifuged to pelletize tissues for homogenization and supernatant was kept, the obtained suspensions were stored for RNA extraction, and subsequently real-time PCR, rRT-PCR and sequencing. These protocols were conducted in accordance with World Reference Laboratories (WRL), Pirbright, UK and Office International des Epizooties (open information extraction [OIE], Paris, France) guidelines (OIE, 2012) at the Foot-Mouth-Disease Laboratory, National Centre for Animal Diseases, UK.

RNA extraction using RNeasy spin-columns method.

Total RNA template was extracted following OIE/WRL Pirbright protocol as described (Knowles and Samuel, 1998), using RNeasy mini kit (Qaigen, Germany) in accordance with manufacturer's recommendation. Each tissue sample suspension was lysed using an equal volume of 460 µl lysis buffer, and 70% alcohol was added and mixture loaded on RNeasy spin column and washed twice. The RNA was eluted with 50 µl DEPC-dH2O into clean collection tube and spin in a micro centrifuge for 60 s at 10, 000 rpm. Extracted RNA was kept on ice for immediate use or stored at −20°C until use for real-time reverse transcription polymerase chain reaction (rRT-PCR) and RT-PCR.

Real-time PCR

Reversed transcription of FMDV RNA and PCR amplification of reversed transcribed RNA was conducted using automated real-time PCR as prescribed by OIE, (2012) and regarded as smart cycler II and beta-actin rRT-PCR as described (Moniwa, *et al.,* 2007). This method amplified a conserved segment of the FMDV RNA polymerase gene (3D). The following primers was used 5′-ACTGGGTTTTAYAAACCTGTGATG-3′ and 5′-TCAACTTCTCCKGATGGTCCCA-3′ (FMDV 1186F sequence and FMDV 1237 R sequence respectively) alongside with the probe 5′-FAMTCCTTTGCACGCCGTGGGAC-TAMRA-3′ (labeled with 6-carboxyfluorescein at the 5' end, and the quencher tetramethyl rhodamine at the 3' end). Reactions was performed using, 2 µl and 5 µl of the RNA sample template added onto 23 µl FMD master mix and 20 µl beta-actin master mix respectively in reaction tubes (Cepheid, Sunnyvale, CA) and inserted into the small cycler machine slots. TE buffer (Tris EDTA was used as a negative control. The real-time PCR reactions was carried out in a smart cycler II thermocycler (Cepheid Inc.). The one-step real-time

PCR amplification started with reverse transcription (RT) at 50°C for 30 min; followed by activation at 95°C for 15 min; followed by 50 cycles of 95°C for 1 s and 60°C for 1 min. One positive and 1 negative control were included in each reaction. The automated smart cycler machine connected to a computer loaded with "smart cycler" software displaced the cycling profile as FAM (FMDV detection) and TET (surfactant protein C or beta-actin detection) graphs. Ct values <35.99 was considered positive. Amplication with Ct value between 35.99 and 40.00 and or low curve to the threshold sample was considered suspicious or doubtful while, Ct value >40 was considered negative. Acceptable Ct range for beta-actin positive was between 12 and 35.99.

RT of virus RNA

Oligonucleotide primers for RT-reaction and subsequent PCR amplification was used as previously published (Knowles and Samuel, 1998). Briefly, the mixture (11 µl) of 10 mM dNTPs, ×5 RT buffer (with dithiothreitol), Moloney murine leukemia virus (200 U/µl) RNasin (3.3 U/µl), DEPC-H2O, and primers (25 pmol/µl) was added to 14 µl of each RNA template to make up a total volume of 25 µl in sterile 0.75 ml eppendorf tubes. Then spin briefly in a microfuge before heating at 42°C for 60 min and at 94°C for 10 min in a thermocycler. "RT product" tubes were labelled and stored at −20°C.

PCR for FMDV detection

PCR amplification of reverse transcribed RNA reaction was optimized as previously described (Knowles and Samuel, 1998), for PCR using degenerative primers for O-A-C (Alfonso primers produced by North American Conservation Area Database [NCAD]), Canada (Moniwa, *et al.,* 2007) and SAT 1-3 serotypes primers based on published oligonucleotides (Knowles and Samuel, 1998). Briefly, in a sterile 0.75 ml tubes, 25 mM MgCl2 with concentrations 1.5 mM, 10 mM dNTPs (200 µM), ×10 buffer, primers (0.5 pmol/µl), Taq DNA polymerase (5 U/µl) 2.5U, RT product and DEPC-H2O was be added to make a final volume of 50 µl. Then 20 µl mineral oil was added to the top the mixture before it was spin. This mixture was heated in thermocycler at 94°C, 4 min, 1 cycle: 94°C, 60 s, 55°C, 60 s, 72°C, 90 s in 30 cycles and lastly, 72°C, 5 min, 1 cycle. The PCR product was stored until used.

Sequencing of PCR products

The PCR amplicons was directly sequenced for strain characterization of capsid region VP1/1D gene as described (Knowles and Samuel, 1998), using sequencing primers designed by the Pirbright Laboratory for SAT 1-3 serotypes and Alfonso Primers (NCAD, Canada) for serotypes O, A, C and promega F-Mol® kit based on manufacturer's instruction. Briefly, purification of PCR Products was done using Promega Wizard Preps™ and cloned into pGEM-T easy vector and stored −20°C in 0.75 ml eppendorf tubes. End-labeling of oligonucleotide primers was then carried out using T4 polynucleotide kinase. Isolates was labelled, and 1 µl of the appropriate dd/dNTPs was added, spin briefly in a microfuge and stored at 4°C. In a separate tube, template DNA, ×5 sequencing buffer, adenosine triphosphate labelled primers, sequencing grade Taq polymerase (5 U/µl) and DEPC-H2O was added for each reaction set, mixed and spin, then mineral oil was added to top reaction mixture. The mixture was placed in a preheater thermal cycler at 94°C, and 4 µl stop solution was added and spin down. The reaction mixture was then heated at 80-90°C for 2-5 min before gel electrophoresis. Sequenced recombinant plasmids data was compared to GenBank data base.

Gel electrophoresis of cycle sequencing products

Gel electrophoresis of cycle sequencing products was conducted as described (Knowles and Samuel, 1998), using serotype A-O-C (Alfonso NCAD, Canada) and SAT 1-3 (Pirbright, UK) sequencing primers. Auto-radiograph developed was labelled with the name of each sample.

Sequence analysis

Sequence analysis was conducted using TREEVIEW software as described (Page, 1996). The dendogram tree-view was produced from sequence obtained using basic local alignment search tool (BLAST) pairwise alignments and fast minimum evolution based on BLASTN program from NCBI. The BLAST computed a pairwise alignment between a query and the database sequences searched. In this sequence tree presentation will be an implicit alignment between the databases sequences constructed, based upon the alignment of database sequences to the query.

Data Analysis

Both data sets were then brought together in a Microsoft Excel Spreadsheet, cleaned and coded before being exported to Statistical Package for Social Science (SPSS) Version 20 for analysis. Analysis included descriptive analysis of the variables to generate means, medians, proportions and confidence intervals. Chi-squared test as recommended by Campbell (2007) and Richardson (2011) was used to compare proportions while the confidence intervals of the proportions was calculated using the method recommended by Altman *et al.* (2000). The test of crude association between risk factors (both individual animal and herd level) and FMD sero-positivity was done using chi-square test.

RESULTS AND DISCUSSION

Molecular Detection of FMDV in Sheep, Goats, and Cattle Using PCR

A total of 43 epithelial tissue samples were collected from sheep, goats, and cattle in Gombe State for molecular detection of FMDV using polymerase chain reaction (PCR). Out of these, only 8 samples (18.6%) tested positive for FMDV.

Among the 30 samples collected from cattle comprising, 15 tongue and 15-foot epithelial tissues; 7 samples (23.3%) tested positive, all of which were from tongue epithelium (2 males and 5 females). In contrast, 13-foot epithelial tissue samples were

collected from small ruminants. Only one sample (14.3%), obtained from sheep foot epithelium, tested positive. No positive result was detected from goat epithelial tissues (Table 1; Plate 1).

Phylogenetic Analysis

Out of the eight samples outsourced to the FAO World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), The Pirbright Institute, United Kingdom, only four passed quality control and were successfully sequenced. Phylogenetic analysis revealed that the Nigerian FMDV isolates from this study (NIG/1/2024, NIG/2/2024, NIG/3/2024 and NIG/4/2024) shares a close genetic relationship with NIG/3/2023 and TUN/2/2023, with a sequence identity of 97.63%.

The isolate forms a paraphyletic relationship with FMDV strains from Tunisia and a polyphyletic relationship with isolates from Ethiopia, Sudan, Iran, Pakistan, and India (Figure 1).

Table 1: Molecular Detections of Foot Mouth Diseases Virus in Sheep and Goat kept in close contact with Cattle in Gombe (n = 43)

Animal Species	No. Tested	No.Positive (%)	P – Value	Fisher's Exact Test
Sheep	7	1 (14.3)	0.464	0.597
Goat	6	0 (0.00)		
Cattle	30	7 (23.3)		

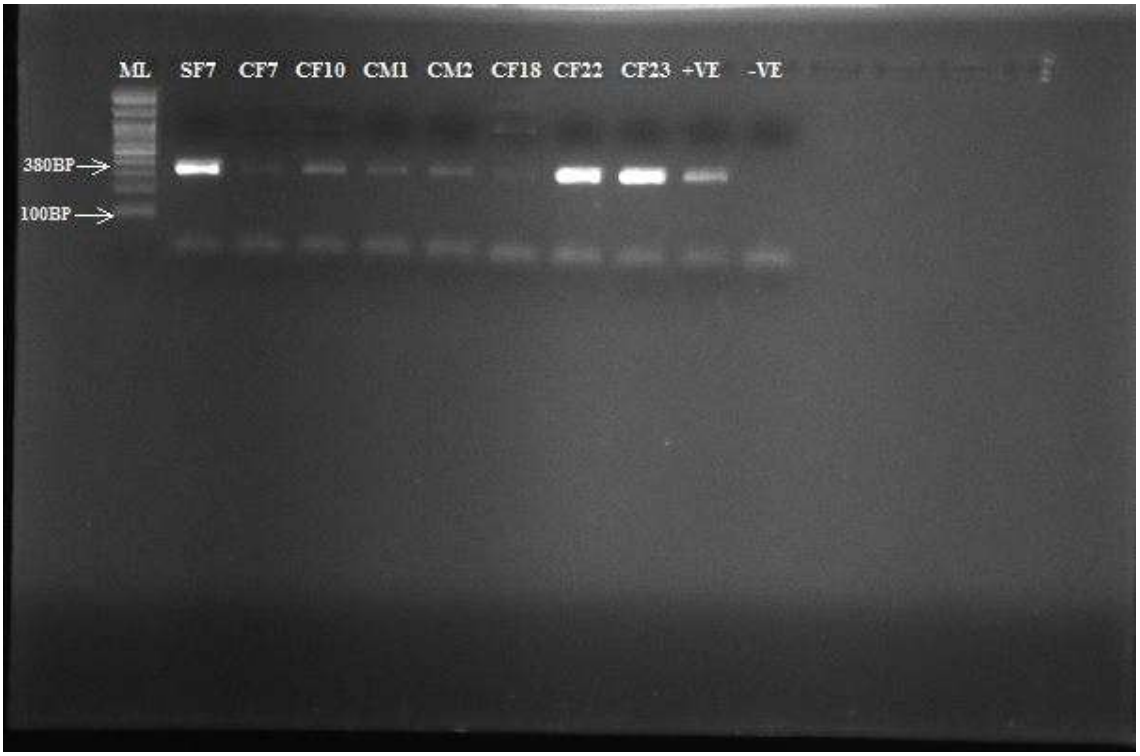

Plate IX: The electropherogram of RT-PCR products resolved on 1.5% agarose gel amplified FMDV RNA using O-A-C primers. Lane 1: 100 bp molecular ladder, Lane 2: Sample SF7 (Sheep #7), Lanes 3–4: Samples CF7 and CF10 (Cow #7 and #10), Lanes 5–6: Samples CM1 and CM2 (Bull #1 and #2), Lanes 7–9: Samples CF18, CF22, and CF23 (Cow #18, #22, and #23) faint bands observed, Lane 10: Positive control, Lane 11: Negative control. Amplicon size: ~380 bp, corresponding to the expected FMDV gene fragment

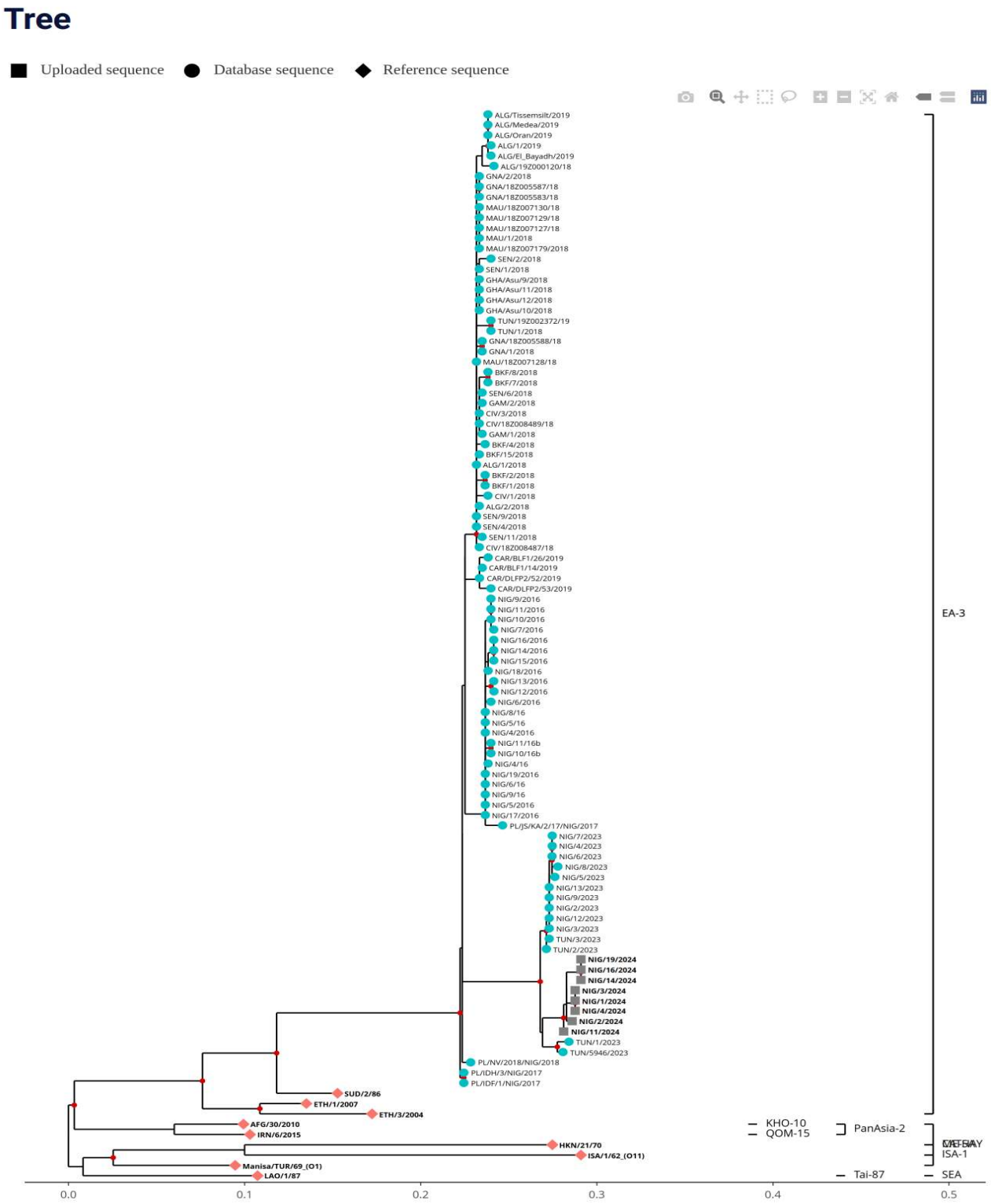

Figure-2: Phylogenetic tree of FMDV serotype O based on VP1 gene sequences, inferred using the Maximum Likelihood method and Tamura-Nei model. Branch lengths represent the number of nucleotide substitutions per site. Bootstrap values ≥60% are shown at the nodes. The isolates from this study (NIG/1/2024, NIG/2/2024, NIG/3/2024 and NIG/4/2024) is marked with a black square (■).

DISCUSION

The molecular detection results indicate that FMDV was more frequently identified in cattle, particularly in tongue epithelial tissues, suggesting a possible predilection of the virus for this site in bovines. The absence of positive results in goat samples may reflect lower viral load, sampling limitations, or species-specific differences in viral shedding.

The detection of FMDV in sheep, albeit at a lower rate, supports the role of small ruminants in the epidemiology of the disease, especially in mixed-species grazing systems. These findings underscore the importance of targeted sampling and species-specific surveillance strategies in molecular diagnostics and outbreak investigations.

The occurrence positive results from tongue epithelia, is in agreement with the earlier results (Islam *et al.,* 2001), who found that tongue epithelia samples tested positive for the virus in 51.43% of cases while foot tissue samples tested positive in only 30.67% of cases. The system for collecting samples, the area where samples were collected, and the seasons might all contribute to this difference (Ullah *et al.,* 2023). Reduced amounts of viral antigens may result from sample being exposed to higher temperatures, an inappropriate pH, or rancidity of the material (Saeed *et al.,* 2015).

Base on sex of the animals, seven (7) out of 43 samples collected were male animals in which two (2) were positive and thirty-six (36) were female animal in which six (6) were positive respectively (Table 26). The high prevalence FMD in female animals during the present study was consistent with the findings of previous studies (Mazengia *et al.,* 2010; Olabode *et al.,* 2013; Wungak *et al.,* 2016; Mesfine *et al.,* 2019; Chowdhury *et al.,* 2019; Atuman *et al.,* 2020; Afroz, *et al.,* 2023). The unequally distributed sample size, in which the sample size of female animals was higher than male animals, might be responsible for the observed variance in FMD prevalence between the sexes of animals. In most parts of the world including Gombe State Nigeria, the female animals are retained for milk production and breeding, so they have lower offtake rates than males (Mazengia *et al*., 2010; Olabode *et al.,* 2013; Wungak *et al.,* 2016; Atuman *et al.,* 2020; Afroz, *et al.,* 2023). Due to their propensity to remain in the herd for longer periods of time, the female animals are presumably more likely to be exposed to FMDV or its serotypes during their life span (Mesfine *et al.,* 2019). The present study that shows higher prevalence in female animals may also be attributed to the fact that females are in much more stress due to production, heat and breeding stress. Thus, using an RT-PCR-based molecular approach, the present study identified FMDV type O' from the collected viral sample. Moreover, this research also investigated tongue epithelia tissue more prominent for FMD exposure than foot tissue.

In this study, FMD serotypes and sequences were investigated in a rapid, unambiguously and cost-effective approach using automated real-time PCR and direct sequencing post optimized RT-PCR using sequencing primers for region VP1/1D gene as previously described (Knowles and Samuel, 2003). Out of the 8 samples outsourced and send to FAO World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD). The Pirbright Institute, Ash Road, Pirbright, Woking GU24 0NF, United Kingdom for sequencing only 4 passed quality control for this analysis. The phylogeny indicated that the Nigerian Foot and Mouth Disease virus isolates obtained from this study (NIG/1/2024, NIG/2/2024, NIG/3/2024 and NIG/4/2024) are more closely related to NIG/3/2023 and TUN/2/2023. With an identity rate of 97.63%, the Nigerian Foot and Mouth Disease virus isolate also forms a paraphyletic relationship with Foot and Mouth Disease virus in Tunusia. It also forms a polyphyletic relationship with isolate from Tunisia, Ethiopia, Sudan, Iran, Ghana, Burkina Faso and Senegal. (Figure 1). This observed multiple sequences are in line with previous documented heterogeneity amongst serotypes (Nagendrakumar, *et al.,* 2009) associated with antigenic variation and quasi-species characteristics of FMDV genome (Longjam and Tayo, 2011) causing diverse outbreaks in aberration to parental serotypes.

Must of the investigated animals were FMDV topotype O/EA-3. Based on the phylogenetic trees and nucleotide sequence alignment, the data of the present study suggest the continued circulation in 2024 in Nigeria of the FMDV topotype O/EA-3 virus lineage as described by Ehizibolo *et al.* (2017) in 2014 with ~1% VP1 nt change per year. This confirms previous observations from (Bertram *et al.,*2018; Ularamu, *et al.,* 2020) who suggested a pattern of continuous transmission of FMDV topotype O/EA-3 in the West African region. This % of change in VP1 nucleotide identity is in agreement with previous observations made by Knowles and Samuel (2003). The data of the present study also suggest that different sub-lineages of FMDV topotype O/EA-3 were circulating in Nigeria in 2024, in line with previous observations made by Ehizibolo *et al.* (2020) in 2016.

In this study, cattle were more significant in the maintenance of FMDV, as recovered cattle were not often removed from the herds. Thus, subclinical infections may constitute important viral reservoir precipitating carrier role associated with increasing outbreak occurrence in herds (Arzt, *et al.,* 2011). Also, co-habitation scenario of sheep and goats with cattle was observed amongst the Fulani herd settlements studied as this potential small ruminant could act as virus carriers for up to 6 and 9 months (Kitching, and Hughes, 2002)., militating against effective disease control. However, the observed limited serotype analysed by PCR and sequencing could be linked to the sample size and status submitted for the procedures as a result of delayed and under reporting of outbreaks, in sufficient logistics and absence of visible epithelial tissues

CONCLUSION AND RECOMMENDATIONS.

Conclusion

In conclusion, the Phylogenetic analysis revealed that the Nigerian FMDV isolates from this study (NIG/1/2024, NIG/2/2024, NIG/3/2024 and NIG/4/2024) shares a close genetic relationship with NIG/3/2023, TUN/2/2023, Ethiopia, Sudan, Iran, Ghana, Burkina Faso and Senegal with a sequence identity of 97.63%

Recommendations.

i Animal Movement-control measures should be put in place

ii use of multivalent vaccines comprising the three local circulating serotypes .

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