SPATIO-TEMPORAL AND MOLECULAR EPIZOOTIOLOGY OF FOOT-AND-MOUTH DISEASE IN CATTLE IN NORTH-CENTRAL NIGERIA, 2011-2014

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MATRICULATION NUMBER: 154349

A THESIS IN THE DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF IBADAN IN PARTIAL FULFILMENT OF THE REQUIREMENT OF THE DOCTOR OF PHILOSOPHY OF THE UNIVERSITYOF IBADAN, NIGERIA

CERTIFICATION

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ABSTRACT

Foot-and-Mouth Disease (FMD) caused by FMD Virus (FMDV) is an economic limitation to cattle production. The current epizootiological status of FMD and circulating serotypes of FMDV in north-central Nigeria is unknown. Spatio-temporal and molecular techniques are important to the study of FMD spread, ultimately leading to the prevention and control of the disease. This study was designed to determine the seroprevalence, associated risk factors of seropositivity, circulating serotypes and their spatial distribution, as well as isolate and characterise FMDV in cattle herds in North-Central Nigeria.

A cross-sectional study was undertaken from February 2013 to April 2014; using three-step multistage sampling, 1,206 sera were collected from 150 herds in Plateau (n=589) and Niger (n=617) states. For molecular study, tongue epithelial specimens (n=40) from lesions of clinically sick animals were collected purposively between June 2011 and October 2014 from north-central states (Plateau 26; Kogi 4; Nassarawa 6; Benue 4). Seroprevalence was determined using FMD 3ABC ELISA kit and associated risk factors were determined using pre-tested questionnaire (n=150) administered to participating farmers. Circulating serotypes were determined using FMDV serotypes-specific ELISA and antigen-detection ELISA. Spatial distribution was done using purely spatial cluster analysis. The FMDVs were isolated using foetal goat tongue cell line and bovine thyroid glands cell line. Virus characterization was done using PCR, sequencing and phylogenetic analyses of the *VP1* gene. Sequence comparisons were made with other country reference strains in gene bank. Multiple sequence alignment was done. Data were analysed using descriptive statistics, chi-square and logistic regression at $\alpha_{0.05}$.

Overall seroprevalence of 71.0% was recorded (Plateau 54.2%; Niger 85.4%). Risk factors associated with FMD seropositivity were management system (OR 9.31; CI 4.81-19.02), trans-boundary crossing (OR 5.12; CI 3.75-7.43), herd mixing at the watering point (OR 171.83; CI 23.82-1253.02) and age (OR 1.14; CI 0.83-1.48). The FMDV serotypes A, O, SAT 1 and SAT 2 were found to be diffusely distributed and co-circulating in north-central Nigeria. Sequence analysis of serotype A revealed that the virus was within Africa typotypes which belong to genotype G-IV. They were closely related with FMDV from Bauchi state (94.7%), Cameroon (93.0%) and Togo (90.0%). Serotype O isolates were

within the West Africa (WA) topotypes and East Africa-3 topotypes (EA-3). Isolates from Plateau state revealed close genetic relationship with sequences from Adamawa state (98.1%) and Cameroon (87.0%); isolates from Kogi state had sequence similarity with those from Togo (94.7%), Ghana (93.9%) and Benin (92.8%). Benue state isolates clustered with FMDV isolates from Plateau state (98.6%) and Sudan (94.2%).The VP1 region of FMDV SAT 2 showed that it belonged to topotype VII. The isolates had a close genetic relationship with SAT 2 isolates from Liberia (93.5%), Niger (92.1%), Senegal (91.5%), Sudan (91.1%) and Cameroon (91.4%).

The spatio-temporal pattern of Foot-and-Mouth Disease virus in north-central Nigeria indicated trans-boundary spread of serotype O East Africa-3 topotype. Use of Foot-and-Mouth Disease virus serotypes A, O, SAT 1 and SAT 2, with East Africa-3 topotype in vaccine production and animal movement restriction will enhance the control of this disease in the region.

Keywords: Foot-and-Mouth Disease, Serotype O, Topotype, Transboundary spread, VP1 gene.

Word count: 500

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Abbreviations

BHK-21 Baby hamster kidney cell line

BTY Bovine thyroid primary cell line

CFT Complement Fixation Test

CI Confidence Interval

CPE Cytopathic Effect

DNA Deoxyribonucleic acid

DPI days post infection

ELISA Enzyme Linked Immunosorbant Assay

FCS foetal calf serum

FMDV Foot and Mouth Disease Virus

FMD Foot and Mouth Disease

IB-RS-2 Pig kidney cell line

IgA Immunoglobulin A

IgG Immunoglobulin G

IgM Immunoglobulin M

TADs Transboundary Animal Diseases

LPBE Liquid Phase Blocking ELISA

MEM Minimum Essential Media

MLD50 Mice lethal dose 50

MAb Monoclonal antibody

NSPs Non Structural proteins

NVRI National Veterinary Research Institute

OD Optical density

OIE Office International des Epizootic

OR Odds ratio

OP Oesophageol Pharyngeal fluid

OPD OrthoPhyneyleneDiamine

PI Percentage of Inhibition

PBS Phosphate buffer saline PD50 Protective Dose 50

RIP Radio-Immuno Precipitation

RNA Ribonucleic acid

RPM Revolution per minute

RT- PCR Reverse Transcriptase Polymerase Chain Reaction

SAT South Africa Territories

SPCE Solid Phase Competitive Elisa

SNT Serum Neutralization Test

TCID50 Fifty tissue culture infective dose

µL Microliter

UV Ultra Violet

VNT Virus Neutralization test

VP Virus protein

ZZ-R Fetal goat tongue cell line

WRL World Reference Laboratory of Foot and Mouth Disease

WAHID World Animal Health Information Database

$\chi 2$ Chi square

DEDICATION

I dedicate this thesis to my father, late WOII Samuel SimwalWungak

ACKNOWLEDGEMENT

I wish to thank my supervisors Dr B.O. Olugasa and Dr. Olayinka O. Ishola for devoting their time and energy to attend to this work. Indeed, you have mentored me through the course of this work. My appreciation goes to the management of the National Veterinary Research Institute, Vom, for granting me permission to undertake this programme. My gratitude goes to the staff of Foot and mouth research centre, National Veterinary Research Institute, Vom, especially Dr Ularamu H.G, Dr. Lazarus D. D., Mr Agom D. and Mrs M. Onoja for your assistance in no small ways to the success of this work; I can never thank you enough for the various roles you have all played. I remain thankful to all the farmers for their cooperation and understanding during the sampling. My sincere gratitude goes to Dr. Alhaji Bida for the role he played during sample collection in Niger State. Worthy of appreciation is the staff of Pirbright Institute, UK, for analysing parts of the samples used.

I express my gratitude to my lecturers, friends and colleagues for always being there for me.

I wish to thank my Pastor, Stephen Anamah, for his unfailing moral support and prayers towards the success of this thesis. May the Lord your God bless you and your family.

I remain indebted to my family, especially my dear wife NenmanYiltaweWungak and my two sons Ponen and Zadok and daughter Rejoice for their patience and understanding during the entire course of this work.

I wish to thank my family members for their support in many ways toward the success of this work. To my mother, I can never thank you enough for your constant prayers and motherly care for me to succeed. To my late father WOII Simwal Samuel Wungak, though you are no longer alive to celebrate with me on the successful completion of my thesis, know that your desire and prayers for me has been answered by God Almighty and I believe strongly that much more of your prayers for me will all come to pass in the course of time.

Above all, I give thanks to God Almighty for making it possible for me to complete this work, despite all the challenges.

CHAPTER ONE

INTRODUCTION

1.1 Background

Nigeria has an estimated human population of over 174.51 million (Nigeria Population Statistics, 2013) and the animal population is put as follows: Birds 182,1093,43 Cattle 19,542,582 Goats 72,4666,98, Sheep, 41,326,780 and Swine 70,66905 (WAHID, 2014). Livestock production is a vital source of animal based protein for the rapidly growing human population; therefore, it is of paramount important to control infectious animal diseases which are a serious threat to the livestock industry and food security. Although Foot- and Mouth-Disease (FMD) causes one of the most economically important diseases in Nigeria, no serious attention has been given to it so far. This may be due to its low mortality rates as compared with other diseases that cause high mortality like Rinderpest, which was recently eradicated.

Foot and Mouth Disease is a trans-boundary animal disease (TAD). It is one of the major animal diseases that impact very negatively on trades and production in livestock and livestock products in the country. To date, four of the seven known serotypes have been found in circulation in Nigeria (Fasina *et al.,* 2013). These include serotypes O, A, SAT1, and SAT2.

Foot and Mouth Disease is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae* (Grubman and Baxt, 2004). There are seven serotypes of the virus namely:

A, O, C, SAT 1, SAT 2, SAT 3 and Asia 1(Klein, 2009). Infection with one serotype does not confer immune protection against another. Within a serotype, many strains can be identified through biochemical and immunological assays (OIE, 2012). The disease is associated with high fever, loss of appetite, salivation and vesicular eruptions on the feet, mouth and teats (Thomson, 1995a). Morbidity is usually high, but there is rare mortality in adult animals. Myocarditis has been seen in young animals which usually resulted in death. Recovered animals may remain in poor physical condition over a long period of time leading to losses for livestock industries (Sangare, 2002).

FMD was first detected in Nigeria in 1924 in sporadic outbreak which was attributed to serotype O virus, Subsequently, other serotypes (A, SAT 1 and SAT 2) were identified, and these were believed to be introduced from cattle population from neighboring countries entering Nigeria for grazing and trade (Ehizibolo *et al.*, 2014). FMD results in the death of calves, reduces milk production and causes psychological trauma for the farmers. It causes a huge loss and has limited the effort of the Nigerian herdsmen and poor farmers, to maximize the genetic potentials of indigenous breed of cattle (Fasina *et al.*, 2013).

It should be understood that pastoralist production system is the predominant system of animals management in the Sub-Saharan Africa and many of these herds move across national and international borders in search of pasture and water for their livestock without any need for quarantine and movement control measures. Furthermore, the poor and often grossly underfunded veterinary services and infrastructures in most African countries as well as in Nigeria, couple with porous international and inter-state border movement constitute a great limitation to the effective implementation of movement restrictions, national veterinary quarantine services and control posts and consequently, impede the implementation of effective disease policy.

In Nigeria, like in many other developing countries, where eradication of FMD seems too costly, FMD control can mainly be achieved through vaccination and control of animal

movements. Currently, limited vaccine development effort has been documented in Nigeria.

Vaccination is an important tool in the control of FMD in Nigeria. This should target the serotypes and strains/topotypes circulating within the Nigeria and West Africa region. Effective surveillance that will lead to vaccine production will be needed to identify all of the circulating strains and serotype available within the country.

Most studies of FMDV are done in countries where control measures are being implemented. In contrast, in areas such as sub-Saharan Africa, where FMDV is endemic, there are limited submissions to the Pirbright Institute, even where there submissions are made, these are usually from very limited geographical areas and as a result, it may give a biased information of the FMD situation and epizootiology. However, regions, where the disease is endemic, are where new strains are most likely to evolve (Bronsvoort et al., 2004). At the time of this study, Nigeria has no FMD control program and no license had been issued for vaccine importation, presenting an opportunity to study the natural ecology and epidemiology of FMDV.

The epizootiology of FMD is crucial for the proper implementation of good control measures. The identification of circulating serotypes as well as the possible origin of the virus is one of the major factors contributing to control of the disease. In recent years, the nucleotide sequences of viruses generated much attention in this regard and sequence data has been instrumental in identifying the origin of an outbreak (Sangar *et al.,* 1987). Furthermore, serological techniques through the determination of Nonstructural proteins (NSP) and Structural proteins (NP) have been used to determine Seroprevalence of FMD and FMDV serotypes respectively (Bronsvoort *et al.,* 2006).

A literature review has, however, shown that there is the paucity of information on FMD epizootiology in Nigeria.

Therefore, this study was designed to determine the seroprevalence and risk factors associated with FMD seropositivity, serotypes circulating and their spatial distribution as well as isolate and characterize FMDV in cattle herds in North-Central Nigeria.

1.2 Research questions

1.2.1 What is the annual seroprevalence of FMD in North-Central Nigeria during 2013 - 2014?

1.2.2 What are the risk factors associated with FMD endemicity in North-Central Nigeria?

1.2.3 What are the various serotypes of FMD virus circulating in North-Central Nigeria during 2011 - 2014?

1.2.4 What is the prevalence of FMDV serotypes circulating in the study area?

1.2.5 Is there any genetic diversity among the FMDV serotypes circulating in North-Central Nigeria?

1.2.6 Is there a difference in the topotypes of the FMDV circulating in North-Central Nigeria and those of other places around the world?

1.2.7 Is there a genetic difference between FMDV isolates from the study area and the ones previously isolated in Nigeria and other parts of the world?

1.3 Justification

1.3.1 FMD causes economic losses among cattle and pigs and in particular in intensive dairy and pig production systems.

1.3.2 The presence of FMD is a major constraint to international trade in livestock products and is an effective barrier to markets with the highest prices for these products.

1.3.3 There are dynamic changes in field status of FMD in Nigeria as a result; regular information update is required on the status of FMD to keep control at an effective level within Nigeria. (Owolodun, 1971; Nawathe and Goni, 1976; Durojaiye, 1981; Abegunde *et al.,* 1988 and Ishola *et al*., 2011).

1.3.4 FMD reduces productivity including milk yield, fertility rate, growth rate and traction power where beasts of burden are used; it also causes mortality in young stock;

1.3.5There is the need to improve upon the logical framework for the control of FMD in Nigeria.

1.3.6 There is the need for effective surveillance to generate FMDV isolates that will be used as vaccine candidates for the production of a more effective vaccine.

1.4 General and Specific Objectives

1.4.1 General Objective

To determine the seroprevalence, molecular and spatio-temporal epizootiology of FMD virus serotypes in North-Central Nigeria for use in achieving more effective and efficient control and prevention of the disease in Nigeria.

1.4.2 Specific Objectives

The specific objectives of this study were:

Study 1: Objectives

1. To determine the seroprevalence of FMD among cattle herds in North Central Nigeria;
2. To identify the risk factors associated with seropositivity of FMD in the North-Central part of Nigeria.

Study 2: Objectives

1. To identify the FMDV serotypes circulating in the study area.
2. To determine the prevalence of FMDV serotypes in North Central, Nigeria,
3. To determine the spatial distribution of FMDV serotype in the study area

Study 3: Objective

1. To carry out isolation and molecular characterization of FMDV in North-Central Nigeria.

1.5 Description of the study area

The North Central Nigeria is located at Central Nigeria (Middle belt). The zone is populated by mostly minority ethnic groups. It is characterized with Guinea savannah and marked by crystalline rock outcroppings and gently rolling hills such as the Jos Plateau. The major or notable rivers in Nigeria, River Niger, and Benue meet at the region precisely at Lokoja town in Kogi state. The two major seasons are the rainy season from the month of April through October and a dry season from November through March. The temperature is also relative from state to state as it is relatively cold weather in Jos Plateau while other states are predominantly hot weather conditions. The zone has six states namely; Plateau, Niger, Nassarawa, Kogi, Benue and Kwara states.

The geopolitical zone has an estimated human population of 20,266,256 and cattle population of 2,363,369 (Kogi 367,754, Kwara 66,905, Nassarawa 88,532, Niger 803,013 Plateau 976,029, Benue 61,136) (GLIPHA, 2011). The predominant economic activities are farming and fishing as a result of their fertile nature of land and the presence of river Niger and Benue around Kogi, Benue and part of Niger and some other related areas nearby the riverine environs, mining amongst Jos Plateau people.

Due to the abundance of grassland in the zone, it supports a massive population of livestock and serves as the major cattle trek routes to the Eastern and Southern Nigeria. The region also shares International boundaries to the west with Benin republic through Niger and Kwara States (Felix, 2009).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Foot-and-mouth disease virus (FMDV) was first recognized by Loeffler and Frosch in 1898 as the first filterable viral organism causing disease in animals. It is a highly contagious viral agent affecting over 60 species of cloven-hoofed domestic and wild animals (Hedger, 1981). The disease is associated with a high morbidity but mortality is usually low in adult animals, however, death usually occurs in young animals as a result of myocarditis. The recovered animals may remain in poor physical and reduced reproductive condition over long periods of time leading to sustained economic losses for the pastoralists and livestock industry (Sangare *et al.*, 2003).

An understanding of the epizootiology of FMD is imperative for the implementation of good and effective control program and the eradication of the disease. One of the important parts of combating foot-and-mouth disease (FMD) is virus characterization, where the possible origins of the disease are investigated by comparing the relationships between field isolates against reference and historical viruses. Unlike the southern region and some parts of central and East Africa, little is known about the FMD situation in Nigeria and West Africa. In addition, epizootiological studies in southern Africa were usually focused on the Southern African Territories (SAT) types and little studies were given to other FMD virus serotypes on the continent (Bastos, 1998; Bastos *et al.*, 2000; Van Rensburg and Nel, 1999; Vosloo *et al.*, 1995, 1992; Vosloo *et al.*, 2002).

The lack of interest in the research of FMD virus (FMDV) within West African countries was due to the competing prevalence of other major diseases of animal and such as rinderpest (Sangare, 2002). The recent FMD outbreaks in the United Kingdom and the evidence of

trans-continental transmission of the virus (Samuel and Knowles, 2001) have highlighted the need for a collaborative worldwide programmes to control the spread of the virus.

2.2. Definition

FMD is one of the most infectious viral diseases of animals and it has a great potential for causing huge economic losses in susceptible cloven-hoofed animals. It is characterized by fever, salivation, loss of appetite and vesicular eruptions on the muzzle, feet, mouth and teats (Thomson, 1995b). It belongs to category list A based on OIE disease classifications (OIE, 2012).

2.2.1 Etiology

2.2.2 Taxonomy

FMD virus was defined by the International Committee on Taxonomy of Viruses (ICTV) in 1963 as belonging to the genus *Aphthovirus*, of the family *Picornaviridae.* The name, *picornaviride* is gotten from the Latin word 'Pico' meaning small and 'rna' meaning RNA, which refers to the size and genome type, of the virus while 'Aphthovirus' refers to the vesicular lesions formed in all the susceptible cloven-hoofed animals (OIE, 2012).

2.2.3. Physicochemical properties

Picornaviruses are small RNA viruses that are enclosed within a non-enveloped protein shell (capsid). The capsid is composed of polypeptides that is devoid of lipo-protein, and therefore, is stable to lipid solvents like chloroform and ether (Cooper *et al.,* 1978). The FMD virus is sensitive to pH, and is normally inactivated when subjected to pH below 6.5 or above 11. However, the virion is protected in milk and milk products, and can survive at 70 °C for 15 seconds and at pH 4.6. The virus is known survive for long periods in chilled meat or frozen bone marrow and lymph nodes (Mckercher and Callis, 1983). Two percent solutions of Potassium Hydroxide (KOH) or Sodium Hydroxide (NaOH) and 4% Na_2Co3 are known to be effective disinfectants for FMD virus contaminated objects, however, the virus is known to be resistant to substances like phenolic, alcohol and quaternary ammonium disinfectants (Sahle *et al.*, 2004). The sizes and amount of aerosol droplets also

play an important role in the drying out or survival of the virus; droplet aerosol with a size of 0.5 - 0.7 μm is optimal for longer survival in the air, while smaller aerosols usually dry out easily. The virus survives longer in proteins in dry conditions e.g. in epithelial fragments (Donaldson and Ferris, 1975)

2.2.4. Virus Morphology

Picornaviruses genomes are icosahederal, non envelop and contain a molecule of positive sense, single stranded RNA (ssRNA), ranging from 7 to 8.5 kilobase in length. A viral genomic Protein (Vpg), which is enclosed by the 3B genome region, is covalently linked to the 5' end of the genome and a poly (A) tract of variable length is located at the 3' terminus. The diameter of 22 - 25 nm capsids is made up of 60 capsomers each consisting of four proteins (VP1, VP2, VP3 and VP4). VP1-VP3 are exposed on the surface, while VP4 is located internally at the pentametric apex of the icosahedrons and contains a myristic acid molecule attached to the amino terminal glycine (Robert & Bruce, 1981).

2.2.5 Genomic organization

FMD virus has single stranded, positive sense RNA (ssRNA) that is approximately 8,500 bases long and composed of a 5' non-coding region (NCR), an open single reading frame, and a short 3' non-coding region. It is polyadenylated, on the 3' end and has small virus encoded protein, Vpg, covalently attached to the 5' terminus. The major portion of the FMDV genome composed of a large single open reading frame of 6996 nucleotides encoding a polyprotein of the 2332 amino acids type O (Forss *et al.,* 1984). Four different areas are distinguished for the polyprotein namely; the L, P1, P2, and P3.Another feature, unique to FMDV, is that there are three species of Vpg encoded by protein 3B, termed 3B1, 3B2, and 3B3. All the encoded Vpg variants have been demonstrated to be attached to the 5' terminus of viral RNA (King *et al.,* 1982). The L protein denotes the leader protein, where 2 initiation sites (AUG codons) have been identified in FMD virus, namely; Lab and Lb (Sangar *et al.,* 1987). The P1 gene product is the precursor of the capsid proteins 1D, 1B, 1C, and 1A. Firstly, the intermediate P1 precursor is processed with the help of viral protease 3Cpro to produce VP0, VP1, and VP3 where the products combine to form empty capsid particles. The mature virion is produced after the encapsidation of the virion RNA

that is accompanied by the cleavage of VP0 to VP2 and VP4. The P2 (2A, 2B,2C) and P3 (3A, 3B, 3C, 3D) regions encode for non-structural proteins that are involved in viral RNA replication and protein processing (Belsham, 1993).

2.2.6. Genetic variation

The genetic variability observed in FMD viral genome is the consequent of a two-step process. First, the replication of viral RNA is normally error-prone as a result of the absence of proofreading capacity in the 3D-encoded RNA dependent RNA polymerase. Secondly, there is competitive selection, which is continuously acting on the genome. Thus, those FMD virus mutants with a selective advantage in the prevailing region will be better represented than FMD virus mutants with a selective disadvantage (Sahle *et al.,* 2004).

Mutation

FMD virus (FMDV) is prone to high rates of mutation during replication. This is usually as a result of a lack of replication mechanisms that checks for error during replication. RNA viruses that have such an incapability mutate at the rate of one nucleotide base change per 103 bases per replication cycle (Holland *et al.,* 1982). It is also estimated that a mutation rate of up to 10^8 to 10^9 nucleotide substitution per year during an epizootiological cycle of FMD viruses can occur. Consequently, new variants of FMD viruses are continuously arising after each replication cycle, which constitute an intratypic population of FMD viruses with different degrees of genetic relationships, previously described as the quasispecies phenomena (Diez *et al.,* 1990). This may lead to a generation of viral diversity. Antigenic or genetic variability of the virus is usually as a result of alterations in the nucleotide compositions of the capsid genes (Lewis-Rogers *et al.,* 2008; Meyer *et al.,* 1994). Thus, the novel generations of variants are regarded as one of the major problems in the FMD by vaccination.

Selection

The RNA virus employed profile mutant production as one of the evolutionary mechanisms. The immune system of an infected animal, which presumably provides a power full selective force, is another driving force in viral evolution (Diez *et al.,* 1990)

Recombination

Recombination is an important mechanism that drives viral evolution and biology. In RNA viruses, recombination involves the exchange of genetic material between two non-segmented RNA genomes resulting from polymerase 'jumping' during RNA synthesis. It has been shown that exchange of genetic material occurs between viruses of the same serotype as well as between serotypes. Intratypic recombination happens more frequently and it seems that recombination events in FMDV occur more readily in the 3' half of the genome, than in the capsid genome of the Foot and mouth disease virus. Mutations as a result of recombination could result in the exchange of genetic material which could result in the emergence of novel generation of antigenic variants that may escape immune pressure (King *et al.,* 1982).

2.2.7. Antigenic variation

New antigenic variants are constantly being evolved or generated as a result of genetic variability due to mutation, selection, and recombination. Consequently, this bring about a situation where there is not only cross protection between FMD virus serotypes, but vaccination with one antigenic variant of serotype does not necessarily will protect an animal when challenged with a different variant of the same serotype (Sangare, 2002). In an effort to characterize the extent of the variation in antigen within the FMDV serotypes, World Reference Laboratory for FMD, (WRLFMD) established a protocol for FMD viral subtype identification which initially, was able to identified over 60 different subtypes, however, it became apparent that there is a continous evolution of novel intratypic antigenic variants, which makes it very difficult to identify specific subtypes (Asseged, 2005). Alteration to the genes encoding the capsid proteins may result in antigenic variation and evolvement of new subtypes (Haydon *et al.,* 2001). This can produce an immunologically distinct variant that could re-infect individuals that have been previously infected by related viruses. The degree of cross protection among different subtypes of the same serotype thus varies. Since there is continual antigenic drift in enzootic situation this is an important factor to consider when selecting vaccine strains (Mason and Grubman, 2009).

2.2.8. Serotypes and sub types

Currently there are seven serotypes of FMDV namely; O, A, C, Southern African Territories SAT 1, 2 and 3, and Asia 1, that infect cloven-hoofed wild and domestic animals. Within these serotypes, over 60 subtypes have also been identified through the use of immunological and biochemical tests; and novel subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used (OIE, 2012). At present, FMDV sequencing is increasingly being employed to establish intratypic variations of FMD viruses and grouping viruses in to genotypes and lineages (Sahle *et al.,* 2004).

2.3. Epidemiology

2.3.1. Geographical distribution

FMD virus has a global distribution, except in Western Europe, Australia and North America. The status of FMD in any country or region can be described as endemic, sporadic, or free. FMD-free regions can be defined by national borders (e.g. Australia, Indonesia), by supranational borders (e.g. Europe, North America) or by FMD free zones within non-free areas, that are usually maintained by animal movement control (e.g. Zimbabwe). FMD sporadic regions are usually characterized by repeated incursions of FMD viruses into regions where FMD does not usually occur. The disease is either controlled or disappears naturally without any intervention until the virus is introduced again in the following next months or years (Samuel and Knowles, 2001).

FMD has been eradicated in some regions of the world, usually by applying mass annual strategic prophylactic vaccination campaigns and by the application of strict zoo sanitary measures following the outbreaks of FMD. European continent falls mostly into this category. Countries, such as the United Kingdom, have controlled FMD without resort to vaccination. However, FMD is epizootic in several parts of the world and enzootic in most of the developing world, including Africa, Asia, and part of South America, the Middle and

Far East. This situation prevails despite continued efforts to have FMD control, and the massive use of FMD vaccine in the affected areas of the world (Asseged, 2005). Western parts of Europe have had recent outbreaks of FMD, which was brought under controlled successfully. This includes the FMD outbreaks that happened in 2001 in the United Kingdom, which spread to the Netherlands, Ireland and France, and different outbreaks in Greece and Italy. Japan has also recently eradicated FMD in the country (Leforban and Gerbier, 2002).

2.3.2. Host range

FMD is very infectious and affects over 70 species of wild and domestic *Artiodactyl* (Grubman and Baxt, 2004). Of the domesticated species, sheep, cattle, pigs, goats, and buffalo are all susceptible to FMD (OIE, 2012). In addition, many cloven-hoofed wild life species, such as deer, wild pigs and antelope may become infected, although, aside from the African buffalo, their role in the epidemiology of FMD is not certain (OIE, 2012). The susceptibility of the affected animals can vary with animal breed and strain of virus. The indigenous breeds of sheep, goats and cattle, in Asia and Africa are known to show relative resistance to FMD viruses because of the endemicity of the disease, and these animals are believed to be the primary source of FMD infection for countries previously regarded as FMD disease-free (Kitching and Hughes, 2002; Kitching, 2002a).

2.3.3. The role of carrier animals

Carrier state, in FMD, is described as an animal from which FMDV can be isolated from the oesophageal-pharyngeal (OP) region, more than 28 days after infection. Although it is well known that FMD virus persists in buffalo (for up to 5 years), cattle (for up to 3 years), Sheep (up to 9 months), and goats (between 3-6 month), the mechanisms underlying the persistence and the immunological pathway that eventually leads to viral clearance are not well understood (Bastos *et al.*, 2000). This can provide a mechanism for the sustenance of the FMD virus in nature and the cause of acute FMD episodes and may contribute to the emergence of novel antigenically variant viruses (Domingo *et al.*, 1998; Kitching, 2002b).

2.3.4. FMD Serotypes in Africa

FMD is known to be enzootic in sub-Saharan African countries, with the exception of Madagascar. Six serotypes, namely; A, O, C, SAT-1, SAT-2 and SAT-3, are co-circulating in most sub-Saharan African countries with marked differences in the distribution and prevalence of serotypes (Vosloo *et al.,* 2002). FMDV serotypes A and O are widely distributed throughout Sub-Saharan Africa, whilst serotype C appears to have "disappeared" from the world as a whole, at the present (Kitching, 2002a).

Historically serotype C is the rarest of the FMDV type to have occurred in Sub-Saharan Africa, having restricted to only three countries, namely; Kenya, Angola and Ethiopia (Vosloo *et al.,* 2002). The last outbreaks of FMD due to type C were reported between 1996 and 2000 in Kenya, a country where FMDV serotypes A, O, C, SAT-1 and 2 have circulated. No other country has as wide a range of FMDV serotypes in the circulation like Kenya (Kitching, 2002a). Serotype O is enzootic in some northern Africa countries, such as Egypt and Libya, while in Tunisia, Algeria and Morocco had also reported the FMD outbreaks due to serotype O. In West Africa and central Africa countries, four FMDV serotypes A,O, SAT-1 and SAT-2 have been reported since 1958, while majority of the outbreaks were linked to serotypes A and SAT-2 (Vosloo *et al.*, 2002). The three SAT serotypes are the most prevalent in southern and eastern Africa, SAT2 and SAT1circulated in West Africa and are the only FMDV serotypes to have made their way into the Middle East with SAT-3 showing the most restricted (Vosloo *et al.*, 2002), (Table 2.1). Due to poor reporting of outbreaks from the African continent, FMD is considered enzootic in most of the African countries with only Botswana, Morocco, Zimbabwe, Swaziland, Namibia, Lesotho, and the Republic of South Africa (based on serological survey) being regarded free of the disease by the OIE in 1999 (Kitching, 1999).

2.3.5. The role of wild life

FMD has been recorded in several wild life animal species, such as the African buffalo (*Syncerus caffer*), Impala (*Aepycerosmelampus*), Kudu (*Tragelaphus strepsiceros*),Warthog (*Phacochoerus aethiopicus*), and elephants. It is believe that Buffaloes are the reservoir and source of FMD infection for livestock in southern Africa due to their ability to both

transmit and maintain the disease. FMDV can continue in an isolated buffalo herd for up to 24 years, whilst an individual buffalo can maintain the infection for up to five years. Furthermore, buffaloes have been demonstrated to be a source of FMD infection for cattle under both natural and experimental conditions (Sangare,2002). The mechanism facilitating the transmission of SAT serotypes virus from buffalo appears to occur readily whenever; there is close contact between the two species (buffalo and cattle) and during acute stage of the infection, buffalo sheds large amounts of virus. Impala (*Aepyceros melampus*) is the most frequent infected species and its ability to act as an intermediary in disease transmission has been identified. Although researches have shown that impala do not become FMDV carriers; it appears that the FMDV can continue in impala populations for between 6-13 months (Vosloo *et al.,* 2002). Kudu (*Tragelaphus strepsiceros)* were reported to be gradually infected; with the carrier state of between 5-7 months has been. Experimental infection of warthog (*Phacochoerusa ethiopicus*) with FMDV serotype SAT2 virus resulted in severe clinical lesions of infection, and transmission to animals in-contact. However, these animals do not excrete virus to the level of domestic pigs and are not believed to play an important role in the epidemiology of FMD in Africa. Rare cases of FMD have also been reported in Indian elephant (*Elephas maximus*) and in the African elephant (*Loxodo Africana*) (Thomson, 1995b).

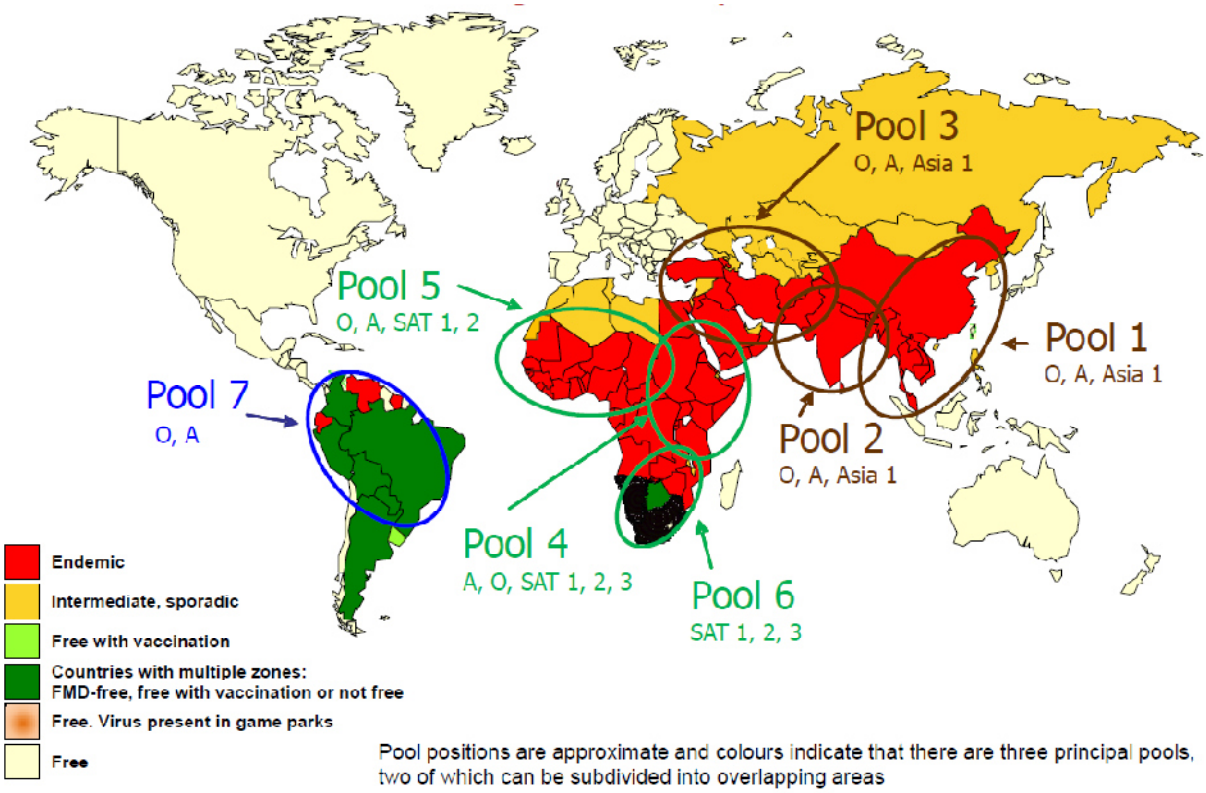

Figure 2.1: The conjectured status and distribution of FMD, showing regional virus pools). Serotype C has not been seen since 2004.
Source; (Hammond, 2012)

Table 2.1 Summary of topotype distribution of FMDV serotypes O, A, C, and SAT-1 -3 in Africa; in period 1990 to 2013 (earlier isolates are included when there is no representative of the genotype reports after 1990).

Serotype	Topotype	Genotype/ strain	Representative country / Countries
O	**EA-1 EA-2**		**Kenya (2010), Uganda (1996) Kenya (2011), Burundi (2003), DRC** (2011), Malawi (1998), Rwanda (2004), Tanzania (2009), Uganda (2007), Zambia (2010), Sudan (1999)
EA-3			Ethiopia (2011), Eritrea (2011), Niger (2007), Nigeria (2009), Somalia (2007), Sudan (2011), Kenya (1987), Libya (2011), Egypt (2012)
EA-4			Ethiopia (2013), Kenya (2010), Uganda(1999)
ME-SA			Sharquia-72 Egypt (2009)
ME-SA			PanAsia-2 Libya (2011), Egypt (2007)
ME-SA			PanAsia-1 South Africa (2000)
ME-SA			Algeria (1990), Egypt (1993), Ethiopia (1994),Eritrea (1996), Tunisia (1994), Tanzania (1998), Libya (1994)
WA			Algeria (1999), Cote d'lvore (1999), Burkina Faso (2002), Cameroon (2005), Ghana (1994), Guinea (1999), Gambia (1999), Mali (2007), Mauritania (2001), Niger (2005), Senegal (2006), Togo (2005), Tunisia (1999), Morocco (1999)
AFRICA	G-l		Kenya (2009), Tanzania (2012, 2013), Uganda (2002), Zambia (1990), Burundi (1990), DR. Congo (2011)
AFRICA	G-ll		Ethiopia (2005)
AFRICA	G-lll		Kenya (2005), Ethiopia (2005), Sudan(2007), Uganda (2002), Cameroon (2005), Egypt (2006)
AFRICA	G-IV		Egypt (2012), Eritrea (2009), Mali (2006), Nigeria (2009), Togo (2005), Cameroon (2005), Sudan (2006)
AFRICA	G-V		Ghana (1973)

Table 2.1 (Continue)

	AFRICA	G-VI	Mali (1997), 2006), Mauritania (2006) Gambia (1998), Senegal (1996), Burkina Faso (1994), Cote d'lvore (1996)
	AFRICA	G-VII	Egypt (2009), Ethiopia (2009), Kenya (2006)
	AFRICA	G-VIII	Kenya (1964)
	ASIA	Iran-05 BAR-08	Egypt (2011), Libya (2009)
C	AFRICA (I)	Ken-67	Kenya (2004)
	AFRICA (II)	Eth-71	Ethiopia (1983)
	AFRICA (III)		Angola (1973)
SAT-1	I (NWZ)		Kenya 2311), Tanzania (2010*, 2012), South Africa (2010), Zimbabwe (2003), Mozambique (2009), Zambia (2009), Malawi ,2001)
	II (SEZ)		Botswana (1998*), Namibia (2010), Zambia (2010), Zimbabwe (2004), Swaziland (2000), Mozambique (2010*)
	III (WZ)		Tanzania (1999), northern Zimbabwe (1997*). Botswana (2006*), Zambia (2012). Namibia (2011)
	IV (EA-1)		Uganda (2007*)
	V		Nigeria (1976), Niger (1976)
	VI		Nigeria (1981), Sudan (1976)
	VII (EA-2)		Uganda (1974)
	VII (EA-3)		Uganda (1997*)
	IX		Ethiopia (2007)
SAT-2	I		Botswana (2011), Malawi (2008), Mozambique (2010), Zimbabwe (2010), South Africa (2012), Burundi (1991), Kenya (1999), Zambia (1996*), Namibia (1998*)
	II		Botswana (2008), Zimbabwe (2010), Namibia (1998*), Malawi (2008), Ghana (1991

Table 2.1 (Continue)

	III	Botswana (2006*, 2012), Namibia (2008), Zambia (2009), Zimbabwe (2002), South Afrlcs (2011)
	IV	Ken/a 23C3). Tanzania (2012), Ethiopia (1991), Burundi (1991), Zambia (2012).

FMD virus transmission in Africa

	V	Ghana (1991), Rwanda (2000), Senegal (1975)
	VI	Gambia (1979), Senegal (1983)
	VII	Egypt (2012), Libya (2003, 2012), Cameroon (2005), Eritrea (1998), Niger (2005), Nigeria (2008), Senegal (2009), Sudan (2007, 2010)
	VIII	Rwanda (2001)
	IX	Kenya (1996), Uganda (1995)
	X	Uganda (2007*)
	XI	Angola (1974)
	XII	Uganda (1976)
	XIII	Sudan (2008), Ethiopia (2010)
	XIV	Ethiopia (1991)
SAT-3	I (SEZ)	Zimbabwe (1999), Kruger National Park (1997*), Mozambique (2010*), South Africa (2011)
	II (WZ)	Zimbabwe (1994*), Namibia (1998*), Botswana (1998*), South Africa (2011)
	III (NWZ)	Zimbabwe (1991*)
	IV	Zambia (1996*)
	V	Uganda (1970*), Uganda (1997*), South Africa (2011)

Foot-and-mouth disease (FMD) virus isolated from African buffalo.

Source: (Tekleghiorghis *et al.,* 2013)

Summary of topotype distribution of FMDV serotypes O, A, C, and SAT-1 -3 in Africa; in period 1990 to 2013.

2.3.6. Molecular epizootiology

Phylogenetic analysis of the virus protein 1 (VP1) region of FMDV has been used extensively to investigate the molecular epizootiology of FMD worldwide. These techniques have assisted in studies of the genetic relationships between different FMD virus isolates, geographical distribution of lineages, and genotypes. It is also used for the tracking of genetically and geographically linked topotypes and tracing the source and origin of virus during an outbreak (Knowles and Samuel, 2003a; Sangare *et al.*, 2003) Sequence differences of 30- 55% of the VP1 gene were recorded between seven serotypes of FMDV while different subgroups (genotypes, topotypes) were described by differences of 15 -20% (Knowles and Samuel, 2003a). Since 1987, the genetic distance analysis and phylogenetic resolution of the FMDV sequence of VP1 encoding gene have provided profound epizootiological information covering different levels of genetic relationships between FMDV field isolates (Sahle *et al.*, 2004; Samuel *et al.*, 1999). The evolutionary changes of virus are determined by comparing genomic material from more than one virus with each other. At present, DNA sequencing and phylogenetic trees are widely employed to illustrate the genetic relationship between viruses (Sahle, 2004).

2.3.7. Mode of transmission

FMD virus can be reproduced and be excreted from respiratory tract of susceptible animals leading to airborne transmission of virus during the acute phase of infection. FMD virus could be present in all the excretions and secretions of animals acutely infected, including the expired air. Therefore, the most important mode of spread in an infected animal is through respiratory aerosols (though proper humidity and temperature is required). When proper temperature and humidity are maintained, FMDV can be carried up to 250 km across the sea and up to 60 km across the land. The prior condition has been held responsible for the FMD outbreak that occurred in France and then spread to UK in 1981 (Ferris *et al.*, 1992), emphasizing the possibility of windborne spread of the virus under suitable environmental conditions. Computer models have been developed presently, that can predict the most probable wind-borne spread of the FMD virus from infected herds and permit for the examination of different control strategies (Sanson *et al*., 1991; Sahle, 2004). Other important avenues of spread are by direct contact between infected and susceptible

animals and indirectly by exposure of susceptible animals to the excretion and secretion of acutely infected animals. A person in contact with infected animals can have sufficient FMD virus in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals (Asseged, 2005).

2.4. Pathogenesis

The main route of virus entry in ruminants is via the inhalation of droplets, but intake of infected feed, inoculation with vaccines that has not been adequately inactivated, insemination with a contaminated semen, and contact with contaminating clothing and so on, can all cause infection. In animals infected through the respiratory tract, initial viral replication happens in the prepharyngeal region and the lungs area, this is subsequently followed by viremic spread to other tissues and organs of the body before the onset of clinical disease. FMD virus is thus, distributed throughout the body, to reach prefered sites of multiplication such as the epithelium of oral cavity, Oro-phrynx, feet, heart and the udder. Virus likely replicate in the mammary gland of a susceptible cow. Viral excretions start about 24 hours before the onset of clinical disease and persist for several days. The acute phase of the disease lasts about one week and viremia normally declines gradually coinciding with the onset of strong humeral responses (Murphy *et al.*, 1999). Recovered cattle develop neutralizing antibodies and may resist re-infection by the same subtype of FMD virus for up to one year. It was suggested that heat intolerance in most of the recovered cattle was a sequel to FMD and was caused by damage to the endocrine system (Radostits *et al.*, 2000)

2.5. Immune Response

The development of adequate neutralising antibodies level against FMD virus in a susceptible host correlates with protection. Infection with one-serotype produces complete protection against homologous virus, but little or no protection against heterologous viruses (Samina *et al.*, 1998). Immunity specific to serotype is based on the presence of neutralizing antibodies to one of the viral capsid protein, VP1, develops 7 to 21 days after exposure to the virus. The immunoglobulin M (IgM) is most prevalent in the early convalescent serum and is less specific to the different serotypes than Immunoglobulin G

(IgG). IgG is produced in the later stage during the FMD infection and the reaction between the serotype and the homologous antibodies is highly specific. It has been reported that healing of lesions and clinical recovery in infected animals would not occur until a few days after the IgG antibodies have developed. The localized antibody response, specific to anti-FMD IgM and IgA antibodies in the pharyngeal fluid of cattle develops 7 days after exposure to the virus, while IgG activity reaches pick in serum only 14-21 days after infection (Mulcahy *et al*., 1990).

The age of individuals has also been shown to influence the antibody response against FMD virus. Calves (age one week to six months) but deprived of maternal antibodies responded as well as, or better than 18 months old cattle to initial vaccination against FMD. Although serum antibody levels play an important role in host protection against FMD virus infection, the cellular responses mediated by T-helper and T- cytotoxic cells also play a role in the immune response to FMD virus infection (Sanzparra *et al.,* 1998).

2.6. Clinical signs

When susceptible animals come in contact with animals that are clinically infected, clinical signs normally develop in 3 to 5 days (Kitching, 2002a), even though in natural infection, the incubation period could range from 2-14 days. The severity of clinical signs of the disease varies with the strain of the virus, the exposure dose, the age, and breed of the animal, the host species, and its degree of immunity. The signs can range from a mild or in apparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2012).

In cattle, the initial signs are fever, anorexia, dullness and decrease in milk production. These signs are followed by smacking of the lips, excessive salivation, grinding of the teeth, serous nasal discharge, drooling, shaking, lameness, kicking of the feet and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the tongue, gums, dental pad, soft palate, muzzle, nostrils, interdigital space, teats and coronary band (Sahle, 2004; Woodbury, 1995). After vesicle formation, drooling may be more noticeable, and lameness or nasal discharge may increase. There may be abortion in pregnant cows, and young calves may die suddenly without developing any vesicle because of (Myocarditis) inflammation of the heart (Radostits *et al*., 2000). Morbidity can

reach100% in young animals, but mortality in adult animals is rare, although in young animals death can occur due to myocarditis and mortality can exceed 50% (Woodbury, 1995; Radostits *et al.,* 2000) . The course of FMD infection is between 2 to 3 weeks although the healing of mouth, feet and teat lesions may delay, resulting in low milk production, hoof deformation, breeding problems, mastitis, and failure to gain weight. A lactating animal may not recover to her production level before infection because of damage to the milk secretory tissue. A chronic panting syndrome characterized by dyspnoea, anaemia, hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (Burrow *et al.,* 1981).

In goats and sheep, if the clinical signs appear, it tends to be very mild, and these may include fever, dullness, and small vesicles or erosions on the gums, dental pad, lips, and tongue. Mild lameness may be the only sign. In game animals, there may be vesicles or erosions in the interdigital spaces or coronary bands. Infected pregnant animals may abort and nursing lambs may die without manifesting any clinical signs (Kitching and Hughes, 2002). In swine, the initial signs are fever, anorexia, reluctance to move, and squeal when forced to move. These signs are followed by the formation of vesicles on the heels, vesicles on the coronary band, vesicles in the interdigital spaces, and vesicles on the snout. Lesions in the mouth lesions are not too common and when they occur, they are smaller and of shorter duration than in cattle and tend to be a "dry" type lesion. There is no drooling; abortions may occur in pregnant sows and piglets can die without showing any clinical manifestation (Radostits *et al.,* 2000).

2.7. Pathology

In cattle, the lesions are single or multiple vesicles ranging between 2 mm to 10 cm. These may occur at all the predilection sites. Usually gross lesions on the tongue progress in the following manner; a small-blanched whitish area develops in the epithelium; fluid fills the area and a vesicle (blister) is formed; vesicle enlarges and may coalesce with adjacent ones and then rupture, leaving an eroded (red) area. Gray fibrinous coating forms over the eroded area that becomes yellow, brown or green till the epithelium is restored (Woodbury, 1995).

Usually, the vesicle in the interdigital space is large because of the stress on the epithelium caused by weight and movement. The lesion developed at the coronary band initially appears blanched; then there is the separation of the skin and horn. When healing takes place, new horn is formed, but a line is usually seen on the wall of the hoof resulting from the coronitis. Dead animals may have yellowish or grayish streaking in the myocardium indicating degeneration and necrosis. These features are known as "tiger heart" (Woodbury, 1995).

2.8. Economic importance

FMD is one of the most important diseases of livestock in the world in terms of economic impact. The economic impact of the disease is not only due to the potential of the disease to cause production losses, but also has to do with the reaction of veterinary services to the occurrence of the disease and to the imposition of restrictions on the trade of animals and animal products both locally and internationally (James and Rushton, 2002). FMD, therefore, poses a serious threat to the livelihoods of simple farmers, large intensive farming practices and the national and international livestock dependent economies of the countries (Asseged, 2005).

The direct production impacts in extensive production system include loss of milk due to the involvement of udder, and reduced animal draught power from lesions on the feet. FMD also causes reduction in rates of live-weight gain in growing animals due to inability to feed, and low reproductive capacity by increased abortion rates of up to 10% in infected animals during pregnancy; the disease also causes up to 6% calf mortality. Animal movement restrictions and international trade can cause profound losses (James and Rushton, 2002). The animal production and restriction of international trade imposed following FMD outbreak is of a major concern for livestock owners. The control of outbreak (slaughter of in-contact and infected animals, carcass disposal in disease-free zones) and the loss due to the ban on livestock and livestock products exports, costs several million US dollars for a single outbreak (Daggupaty and Sellers, 1990). A good example is the 2001 outbreak of serotype O (the Pan Asian strain) in Great Britain, a country that had been certified free of FMD since 1981. This devastating FMD epidemic of 2001 spread to France, Ireland and The Netherlands where the United kingdom alone were forced to

slaughter about 4 million in-contact and infected animals. The cost of this epidemic in the United Kingdom was estimated to be more than $29 billion (Samuel and Knowles, 2001).

2.9. Diagnosis

Clinical diagnosis based on physical lesion identification, in the early stage of infection, FMD virus or viral antigens can be detected using several techniques. However, different serological methods are used to detect antibody against FMD virus and is the main indication that infection has taken place.

2.9.1. Field Diagnosis

In cattle, FMD should be considered whenever salivation and lameness occur simultaneously and when a vesicular lesion is seen or suspected. Fever often precedes other clinical signs; therefore, febrile animals should be carefully examined. Early diagnostic lesions may be found before animals start to salivate, have a nasal discharge, or become lame. Clinical diagnosis can present many difficulties due to viral infections of the mucous membrane, which produce similar clinical signs. Differential diagnosis for FMD should include vesicular stomatitis, rinderpest, malignant catharal fever, the bovine herpes 1 infections, swine vesicular disease, vesicular exanthema of swine and bluetongue (Radostits *et al.*, 2000) .

2.9.2. Laboratory Diagnosis

Due to the highly contagious nature and economic impact of FMD, it is recommended that work on FMD virus laboratory diagnosis should be carried out in a virus-secure laboratory (OIE, 2012).

2.9.2.1 Specimens

Appropriate samples for laboratory diagnosis for FMD are; vesicular fluid which usually contains the huge quantity of virus. Epithelial tissues from vesicles and from recently ruptured vesicles are samples of choice for antigen detection, PCR and virus isolation (OIE, 2012). Oesophageal-pharyngeal of fluids (OP) can be collected when epithelium tissue is not available from ruminant animals especially in an advanced or convalescent cases and

infection is suspected in the absence of clinical sign, samples are collected by means of a probing cup and used for isolation of virus (Asseged, 2005). Other samples such as serum, and lymph nodes, blood with anticoagulant, thyroid gland, adrenal gland, kidney and heart are good sources of specimens to be collected from postmortem.

2.9.2.2 Agent detection tests and specimens for antigen detection

Vesicular fluid usually contains a huge quantity of viruses (OIE, 2012). Epithelium sample from vesicles and vesicle fluids are appropriate samples for virus isolation. Epithelial samples are kept in virus transport medium composed of an equal quantity of glycerol and 0.04 M phosphate buffer pH 7.2-7.6 and antibiotics. A suspension (10%) is prepared by grinding the sample using a sterile pestle and mortar with sterile sand with a small volume of PBS or tissue culture media and antibiotics. Culture media or PBS is added so that the final volume is ten times that of the epithelial tissue, producing a 10% suspension (OIE, 2012). The suspension is centrifuged at 13000 rpm for 10 minutes and the supernatant is used for virus isolation, antigen detection, PCR (etc) and related tests. When epithelium tissue is not available from ruminants, as usual in advanced or convalescent cases and infection is suspected in the absence of clinical signs, samples of esophago-pharyngeal (OP) fluid can be collected by means of a probang and used for virus isolation (OIE, 2012).

2.9.2.3 **FMD antigen detection (virus isolation)**

FMD virus will grow in wide varieties of primary and continuous cell cultures. Primary cells are cell cultures processed straight from tissue without any passage and therefore might contain a mixture of cell types, while continuous cell line are purified cell lines. The most sensitive cell culture for FMDV isolation is primary bovine thyroid (BTY) cells (House and Yedloutschnig, 1982). Continuous cell lines including foetal goat cell line (ZZ-R 127), baby hamster kidney cell (BHK), the pig kidney cell lines IB-RS-2, lamb kidney (LK) and MVPK-1 are also susceptible to FMDV infection. The foetal goat cell line (ZZ-R 127) is a rapid, sensitive, and convenient medium for FMDV isolation and is considered next to BTY in terms of sensitivity (Brehm *et al*., 2009). The sensitivity of virus isolation as a technique for the diagnosis of FMDV depends upon the quality and type of cells used

as well as the quality of the sample (Conlan *et al.*, 2008). Virus isolation is regarded as the "gold standard" method but it may take up to 1-4 days for results to come out and this will delay the confirmation of FMD virus.

2.9.2.4 **Complement Fixation Test**

Since it was first described by Traub and Mohlmann in 1946, the complement fixation (CF) test was commonly used in the diagnosis of FMD. It has been an important tool in the early studies for the comparison of the antigenic variation of two viruses. The CF test is based on the principle that complement (a series of serum proteins) serves as a mediator of many antigen-antibody reactions in which it is fixed in the formation of immune complexes. The presence of complement (usually provided by addition of guinea-pig serum) is revealed by its ability to mediate lysis of sensitized sheep red blood cells. Currently, the test is time consuming, less sensitive and no longer used to diagnose FMD (Sangare, 2005).

2.9.2.5 Enzyme-linked immunosorbent assays (ELISA)

Because the complement fixation (CF) test lacks sensitivity and cell culture isolation takes up to 2-7 days, a more sensitive, rapid and practical alternative to traditional assays was needed for an efficient diagnosis of FMD. The enzyme-linked immunosorbent assay (ELISA) was developed with a number of applications which include the detection of antigen, and antibody (Crowther& Abu-Elzein, 1979; Hamblin, *et al.* 1984). Later, the technique was improved by making a high-titer serotype- specific antisera, by the use of inactivated 146S virus antigens which give less cross-reaction and a higher sensitivity compared to the infective virus, and the establishment of a positive/negative threshold (Roeder &. Le Blanc Smith, 1986; Hamblin *et al.,* 1986). Currently, three procedures are use, the sandwich ELISA for antigen detection (Roeder & Le Blanc Smith. 1986), the liquid phase blocking ELISA for antibody detection (Hamblin *et al,* 1986, Esterhuysen *et al.,* 1985); (Esterhuysen *et al.,* 1985) and solid-phase FMD competitive ELISA (C-ELISA) are routinely used in the diagnosis of FMD.

2.9.2.5.1 Antibody detection by liquid phase blocking ELISA

The liquid phase blocking ELISA detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals (Hamblin *et al.,* 1987). The test is based upon specific blocking of the FMDV sample. Rabbit antigen-specific antisera for the different serotypes of FMDV are passively adsorbed to polystyrene micro wells. Serial dilution of test serum is allowed to mix with the specific FMDV antigen; the test serum-antigen mixture is then transferred to an ELISA plate coated with FMDV trapping antiserum (rabbit FMD antisera). The presence of antibodies to FMDV in the serum sample will result in the formation of immune complex and consequently reduce the amount of free antigen trapped by the immobilized rabbit antiserum. In turn, fewer guinea pigs anti FMDV detecting antibodies will react in the next incubation step after the addition of enzyme labeled (HRP) anti-guinea pig Ig conjugate. Following incubation, the substrate/chromogen solution, containing H_2O_2 is added to each well, before being stopped after 15 minutes by addition of sulfuric acid. A change in colour development is read with spectrophotometer at 492 nm filters, in comparison to antigen Control (Ca), containing free antigen only. The diagnostic threshold for this assay is set at 50% inhibition (50PI). If either or both replicate PI values of test serum fall above 50 PI, then that test serum is tentatively considered to be positive. If both replicate PI value of a test serum fall below 50 PI then the test serum is considered as negative (Ferris, 2008).

2.9.2.5.2 Antibody detection by 3 ABC ELISA

The detection of antibody to the polyprotein 3ABC proteins is useful indicator of FMD virus infection with any of the seven serotypes of the virus (Mackay *et al.*, 1998). Antibody to the 3ABC is only found in virus-infected animals but not in vaccinated animals (De Diego *et al.,* 1997). Briefly, the test is carried out as follows: Microtiter plates are supplied pre-coated with recombinant FMD virus 3ABC viral antigen; dilutions of the samples to be tested are incubated in the well of these plates. Any antibody specific for 3ABC binds to the antigen in the wells and forms antigen-antibody complex on the plate well surface. Unbound material is removed from the wells by washing. Peroxidase labeled anti-IgG conjugate is added, which binds to the antibodies of the sample complex with the 3ABC antigen. Unbound conjugate is removed by washing, and the Tetra-methylbenzidine (TMB) containing substrate is added to the wells. The degree of colour, which develops (optical

density measured at 450nm), is directly proportional to the amount of antibody specific to 3ABC present in the sample. The diagnostic relevance of the result is obtained by comparing the optical density (OD), which develops in wells containing the samples with the OD from the wells containing the positive control.

Compared to the liquid phase blocking ELISA, 3 ABC ELISA allows differentiation between samples from infected (3ABC positive) and vaccinated (3ABC negative) animals (Hamblin *et al., 1*987). The 3ABC ELISA is also rapid test for screening of large number of sera. In areas where more than one serotypes exist, the test is also cheaper compared to the conventional liquid Phase blocking ELISA, which has the disadvantage that each serum sample must be tested against all existing serotypes (Sangare, 2002).

2.9.2.5.3 FMD antibody detection (solid-phase ELISA)

A solid-phase FMD competitive ELISA (c-ELISA) has been developed that can be used for all seven serotypes of FMDV (Mackay *et al.,* 2001). The test is based on competition between serotype-specific guinea pig anti FMD antiserum and antibodies present in the test serum. The c-ELISA is more rapid than the LPBE and results can be obtained in the same day (4 – 5 hours). It was found to be more robust and 100% sensitive relative to the LBPE results. It has a specificity of >95% which is superior to the LPBE, and was used during the UK FMD outbreak to allow for rapid screening of serum samples for FMD antibodies (Mackay *et al*., 2001; Paiba, et al., 2004). Many other ELISAs have been developed and validated. These include commercially available kits e.g. Prionics types, O, A and Asia-1, others are used in FAO monitoring programmes (Brescia test).

2.9.2.6 Lateral flow device

A lateral flow device (LFD) employing monoclonal antibodies has been developed for the detection of FMDV (Ferris *et al.*, 2006). This test is based upon the principles of immuno-chromotograpy, in which soluble antigens (such as infected clinical materials) are allowed to flow through a porous strip. As the solution passes through the strip it first passes through a zone where it meets and solubilises dried labelled antibody conjugate and forms an immune complex. The antibody can be labelled with either colloidal gold or selenium. The fluid then flows through a detection zone containing immobilised antibody against the

antigen. The sensitivity for detection of FMDV serotype SAT 2 was enhanced from 65% to 90% when the monoclonal antibody (MAb) 1F10 in the devices was substituted with the MAb 2H6. With a specificity of 99.4% and comparable sensitivity of 88.2% for the detection of FMDV serotype SAT 2 antigens, this device is superior to the slower and more complicated antigen capture ELISA. The LFD procedure is also simple, rapid and easy to perform which means that it has the potential to be used as a pen-side test for diagnosis and serotyping (Ferris *et al.,* 2009).

2.9.2.7 Virus Neutralization Test (VNT)

The virus neutralization test is based on the principle that serum specific neutralizing antibodies bind to the virus and then block the virus from entering cells in cell cultures. Virus neutralization has been used to study the homologous and heterologous responses against FMD virus (Hyslop and Fagg, 1965; Hedger. 1968). The test identifies specific neutralizing antibodies whereas blocking ELISA detects FMD antibodies across all the serotypes. Serial dilution is performed on test serum after inactivation. The virus suspension (100 $TCTD_{50}$) is mixed with a serum sample incubated and inoculated onto the susceptible cell cultures. The growth of virus in cell cultures is manifested by cell destruction or cytopathic effect (CPE). No CPE confirms the presence of FMDV antibodies in the serum samples

2.9.2.8 Polymerase Chain Reaction (PCR)

Due to the rapid spread of FMD virus and the devastating economic consequences of the disease, it is essential to have a diagnostic test that is sensitive, accurate, rapid and easy to use. Reverse transcriptase PCR (RT-PCR) is used as a diagnostic tool to transcribe the RNA genome and make the first cDNA strand- Pairs of oligonucleotide primers arc chosen to flank the DNA region of interest that is amplified by a Taq DNA polymerase enzyme. Following cycles of.DNA denaturetion by heat, primer annealing by cooling, and strand extension with a thermostabile enzyme such as Taq polymerase; DNA is synthesized from a very small amount of template (Rasmussen *et al.,* 2003). Subsequent improvements in the PCR have reduced the time required for viral detection and for the accurate characterization of FMD viruses from diagnostic samples (Bastos, 1998; Locher *et al.,* 1995).

2.9.2.9 Nucleic acid recognition methods

The polymerase chain reaction (PCR) can be used to amplify the genome fragments of FMDV in diagnostic material. Specific primers have been designed to distinguish between each of the seven serotypes and in-situ hybridization techniques have been developed for investigating the presence of FMD virus RNA in tissue samples (Woodbury*et al.*, 1995).

Unlike many living organisms where the hereditary information is enclosed within a DNA genome, FMDV has an RNA genome that cannot be sequenced directly, but RNA is unstable and is usually first transcribed into cDNA prior to performing the nucleotide sequence. Revers-transcriptase (RT) when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes. In epidemiological studies of FMD virus, nucleotide sequencing of the VP1 gene has been used extensively to determine the relationships between the field isolates. The technique is also routinely used to investigate genetic variation, molecular evolution in carrier animals, and to identify the source of infection in outbreak conditions (Vosloo *et al.,* 2002). The molecular epidemiology of FMD is based on the comparison of genetic differences between virus isolates, and showing the genomic relationship between vaccine and field strains for all seven serotypes based on sequences derived from the 1D gene. Sequence differences of 30-55%of the VP1 gene are obtained among seven seroptypes while different subgroups (genotypes, topotypes) are defined by differences of 15-20% (Knowles and Samuel, 2003a). Reverestranscription PCR (RT-PCR) amplification of FMD virus RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform these comparisons (OIE, 2012).

2.9.2.9 Nucleotide sequencing

Unlike many living organisms where the hereditary information is encoded within a DNA genome, FMD virus has RNA genome that can be sequenced directly, but RNA is unstable and it usually first transcribed into cDNA prior to performing the nucleotide sequence. Reverse transcription (RT) when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes. In epidemiological studies of FMD virus, nucleotide sequencing of the VPI gene, has been used extensively to determine the

relationships between the field isolates. The technique is also routinely used to investigate genetic variation, molecular evolution in carrier animals, and to identify the source of an infection in outbreak conditions (Bastos, 1998; Beck and Strohmaier, 1987). The first genetic relationships of FMD virus type A. O. and were constructed using this approach (Beck and Strohmaier, 1987).

The nucleotide sequence of the major immunogenic protein, VPI was also used to subtype the European FMD viruses type A and O recovered from different outbreaks (Beck and Strohmaier, 1987), They reported that the use of nucleotide sequences is not only a rapid and accurate technique for sub-typing FMD virus but also differentiates variants of a given subtype. They also demonstrated that a single nucleotide change could be detected in the nucleotide sequencing of the isolate from Germany in 1984 (O Zusmarshausen) and strain 01 Kaufbeuren. Subsequent studies using this approach have provided crucial epidemiological insights which include among others, the use of nucleotide sequences for the identification of virus variants arising from laboratory cell passage (Saiz *et al.,* 1993). the identification of trans-boundary virus transmission (Saiz *et al*., 1993)(Samuel *et al.*, 1999) and evidence of prolonged persistence of a particular virus type in the field (Freiberg *et al.,* 1999). Sequence data has also been instrumental in identifying outbreaks resulting from inadequately inactivated vaccines (Krebs *et al.*, 1991)and for refuting vaccine involvement in outbreaks (Locher *et al.*, 1995).

2.10 Prevention and Control

The official attitude of a country regarding control of a disease depends on how seriously the disease affects the country, the financial and technical ability of the country, and what its neighbors are doing. The degree of control of FMD thus varies as follows (Paton *et al.,*2009**)**: Routine vaccination is used where the disease is endemic; in contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use strict movement controls and slaughter of infected and contract animals when outbreaks occur (OIE,2012).

2.10.1. Endemic Areas

Animal movement control and vaccination is usually used as control measures in endemic areas. Vaccination of susceptible animals against FMD could protect animals from developing clinical disease when administered by the parenteral route. Transudation of serum antibodies against FMDV into the mucosae has been use as one of the mechanisms to prevent virus attached to susceptible cells. FMD vaccination is achieved using inactivated vaccines that could provoke protective immunity against each type of FMD antigens incorporated in the vaccine. Therefore, when immunizing animals, it is important that the vaccine carries the same serotype and subtype of virus circulating in the area. This necessitates monitoring of the serotype and subtype during an outbreak because FMD virus mutates easily during natural passage through different species. Protection provoked by aqueous aluminum hydroxide based vaccine can protect for 4 to 6 months while a oil emulsion vaccine can protect for up to 1 year (Gonzalez *et al.,* 1991).

Intratypic differences of the field strains of FMD viruses should also be considered in the selection of master seed virus for vaccine production (Grubman and Mason, 2002.). Immunity to one serotype can only provide protection against the homologous viruses. In some cases, there is the need to used inactivated bi-, tri-, or polyvalent vaccine containing all the representative strains of the serotypes which are circulation in the area; therefore, active disease surveillance as well as sound laboratory facility should be in place to be able to identify and characterise the FMDV. Adequate vaccination coverage should be ensured to achieve herd immunity.

When protective levels of antibodies are attend in the majority of individuals in the population, the maintenance or establishment of the disease within the population is not likely to occur. For FMD, 80 to 85% is estimated as the protective levels of virus-neutralizing antibody to achieve herd immunity (Asseged, 2005).In calves that have maternal antibodies, vaccination should be delayed to allow the maternal antibodies to decline usually up to three months at that time a high proportion can be expected to respond effectively to vaccination whereas for calves born from non-vaccinated dams, the first vaccination may be at 1 week of age (Garland, 1999).

2.10.2. Disease-Free Areas

Stamping out

The stamping out involves the practice of slaughtering of all infected and in-contact susceptible animals. This is usually carried out after thorough epizootiological and laboratory investigations. Zoo-sanitory measures with, the enactment of animal movements restriction is usually employed. Stamping out measures could be extended to preemptively slaughtering of other herds in which there is no evidence of the clinical disease, but which have been epizootiologically linked with an outbreak. Furthermore, " stamping out policy" is usually done with full compensation paid to farmers affected (Sangare, 2002).

The more affluent FMD-free nations, those with an economically significant live animal and animals product export trade, and those whose livestock are highly susceptible to FMD, have contingency plans to deal rapidly with confirmed FMD outbreaks (Sangare, 2002).

Emergency vaccination

The emergency vaccination aims to achieve protective immunity as fast as possible to susceptible herds and also to reduce the virus load in circulation in order to limit the risk of spread of the disease (Asseged, 2005).

The vaccination is usually applied to animals not already exposed to Foot and Mouth disease virus. It is usually done outside the 3 km protection zone and outside any predicted aerosol spread of virus from an infected premise. All the vaccinated animals are naive to FMD antigen, and might usually require a minimum of 3-4 days in order to develop protective immunity. This would thus form a ring vaccination strategy around the infected area, consequently, preventing further disease spread, and allows the FMD outbreak to expire within the buffer zone or protection zone, where herds infected would quickly be identified and slaughtered (Asseged, 2005).

2.11 Foot and Mouth disease in Nigeria

FMD was first recorded in Nigeria in 1924 as an outbreak due to type O virus (Libeau, 1960). Subsequently, other serotypes like; A, SAT 1 and SAT 2 were recognized and each

new serotype was linked with trade cattle entering Nigeria from surrounding neighboring countries.

Over the years no serious efforts have been made to control FMD in Nigeria, probably because attention is diverted to the control of other economically important disease like Bovine Contagious Pneumonia, Rinderpest etc. (Abegunde *et al.,* 1988, Fasina *et al*., 2013). However, FMD will continue to become an important livestock disease in Nigeria especially as the livestock production system becomes more intensive and the pastoral system begin to settle in response to an increased animal protein. It will be necessary to develop FMD control strategies to reduce the impact of the disease in Nigeria. In outbreaks that occurred between 2007 and 2009, both serotype SAT 2, A, and O are known to have circulated in Nigeria. Sequence analyses indicated that serotype O, that circulated in Nigeria 2007/2008 is genetically closely related to FMD virus isolate that caused extensive outbreaks in Sudan in 2004 and 2005. Similarly, the SAT 2 viruses were closely related to isolates from Sudan 2007 and Niger Repulic, 2005 (Fasina *et al.*, 2013). In all those outbreaks, animal movement from within and without the country as well as sharing of water and grazing points were the risk factors associated with the infection (Fasina *et al.*, 2013).

CHAPTER THREE

DETERMINATION OF FOOT-AND-MOUTH DISEASE SEROPREVALENCE AND EXPOSURE FACTORS ASSOCIATED WITH SEROPOSITIVITY OF CATTLE HERDS IN NORTH-CENTRAL NIGERIA

3.1 Introduction

Foot and Mouth Disease (FMD) has been recognized as an important trans-boundary animal disease impacting negatively on the cattle industry since the sixteenth century (Mahy, 2005). Seven distinct serotypes namely; A, O, C, Asia-1, SAT-1, SAT-2, and SAT-3 have been identified. It is known that infection with one serotype does not confer immune protection against another serotype. Different subtypes can be identified within a serotype by biochemical and immunological tests (OIE, 2012). The disease is known to exhibit high fever, loss of appetite, salivation, and vesicular eruptions on the feet, mouth and teats of lactating cows (Thomson, 1995b). FMD has a broad host range, high degree of infectivity, rapid replication rate and multiple transmission routes, which makes it very difficult and expensive to control and eradicate (Alexandersen and Mowat, 2005).

The disease has a high morbidity although mortality is low in adult animals. However, myocarditis may occur in young animals resulting in death. The recovered animals may remain in poor physical condition over long periods of time leading to economic losses for livestock industries (Molla *et al*., 2010).

FMD is endemic in most of sub-Saharan Africa, except in a few countries in southern Africa, where efforts were made to control the disease by the separation of wildlife from

susceptible livestock using barrier veterinary cordon fencing in combination with prophylactic vaccination (Vosloo *et al.,* 2002). Furthermore, due to the endemicity of the disease, and the fact that FMD does not normally cause high mortality in adult animals, FMD outbreaks are not often perceived as important and are poorly reported or investigated further to determine the causative serotypes. However, this is now changing, a number of countries are now recognizing FMD as one of the most important trans-boundary animal diseases that should be controlled in order to access profitable international markets for livestock and livestock by-products as well as to maximize the full genetic potential of the animals (Ayelet *et al.,* 2009).

There the dearth of information about the actual situation of FMD in Nigeria and the neighbouring countries. There are regular outbreaks, no national control strategy, no enforcement of legislation for disease reporting to veterinary authorities, and animal movement control is poor. Since most of the cattle population in Nigeria are from the neighboring countries of West and Central Africa, the animals are at perpetual risk of infection from the enzootic strains as well as antigenic variants prevalent in neighboring countries.

Studies have shown that, FMD serotypes O, A, SAT 1 and SAT 2 have circulated in Nigeria between 1924 and 2009 (Lazarus, *et al*., 2012; Fasina *et al.,* 2013; Olabode *et al.*, 2013; Nawathe, & Goni, 1976; Owoludun, 1971). However, recent sampling conducted between 2007 and 2009 have indicated that despite the endemicity of FMD in Nigeria with its attendant production losses in livestock, very little is known about the epidemiology of FMD in Nigeria (Fasina *et al.*, 2013). The prevalence of FMD can be determined serologically by measuring the antibody level to the 3ABC non-structural protein (NSP) (De Diego *et al.,* 1997).

The objectives of this study were to determine the seroprevalence of FMD and identify the risk factors associated with seropositivity of FMD in cattle from North-Central Nigeria.

3.2 Materials and Methods

3.2.1. Study location

The specimens for this study were collected from two states in the north-central geo-political zone of Nigeria. The two states are, Plateau and Niger States.

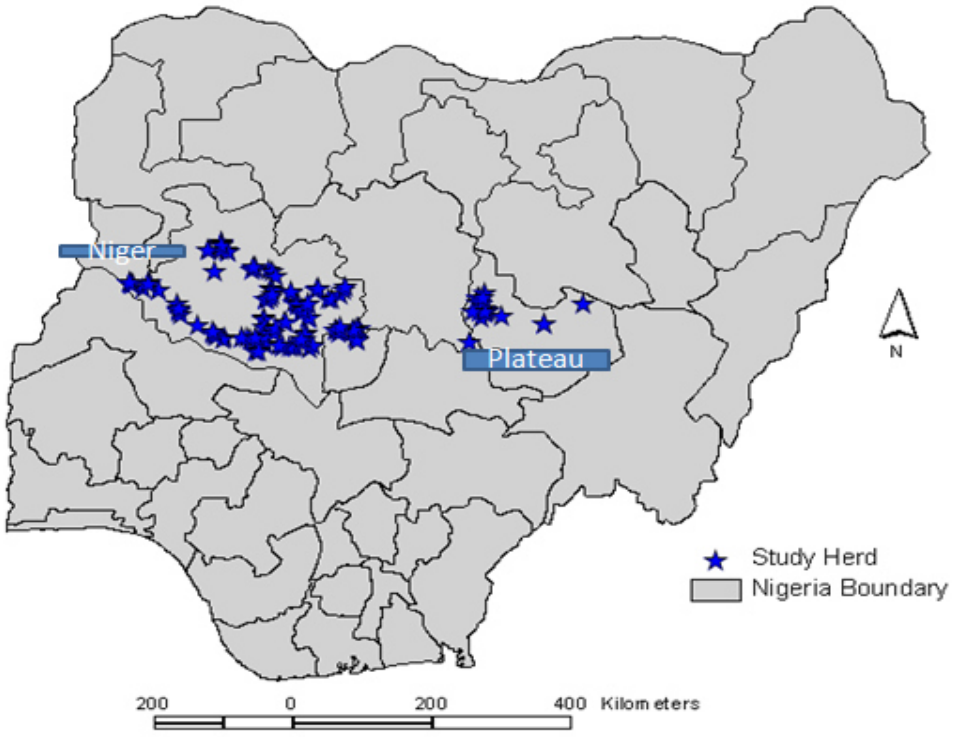

Figure 3.1: Map of Nigeria showing the distribution of the sampled herds in the North Central Nigeria.

3.2.2 Study animals and sampling technique for serum collection

Study animals were cattle selected from the animal population in Plateau and Niger states. These states were selected based on their geographical location, proximity to the livestock market, ruminant population density, movement pattern, as well as cattle trek route and international boundary. Individual animals were randomly selected so that about 10% of animals from each herd were sampled to represent the herd. A total of 150 cattle herds were sampled by multi-stage sampling method in the two selected states of the North-Central Nigeria.

The sample size for the seroprevalence study was determined by using a prevalence of 56.3% based on a previous study (Ishola *et al.,* 2011).The sample size was determined using a sample size determination method described by Thrusfield (2005) with 95.0% confidence interval and desired precision of 0.05. The calculated sample size was 378. However, to improve precision, the sample size was increased 3-fold and a total of 1250 cattle were sampled in this study.

3.2.2 Study Design

A cross-sectional study was undertaken from February 2013 to April 2014; using three-step multistage sampling, 1,206 sera were collected from 150 herds in Plateau (589) and Niger (617) States. One thousand, two hundred and six (1206) sera were used for laboratory analysis using 3ABC Non-structural protein ELISA. The potential risk factors for FMD in the study area were assessed by a pre-tested structured questionnaire in all the states. The questionnaire was designed to assess the most important factors that could be associated with FMD; such as animal location, management system, mixing at the watering point, animal movement pattern and international boundary crossing.

3.2.3 Serum sample collection

Whole blood was collected from the jugular vein using 10-ml sterile plain vacutainer tubes and stored overnight at room temperature for serum separation. Each serum was transferred

into a sterile cryovial, bearing the age and sex of sampled animal and was transported in an icebox to National Veterinary Research Institute, Vom, Nigeria (NVRI), and stored in the freeze at 20°C until analyses. The assay was conducted at the Foot and Mouth Research centre, National Veterinary Research Institute, Vom**,** Nigeria. FMD seroprevalence was estimated using 3ABC ELISA (Bronsvoort *et al.,* 2006).

3.2.4 Detection of antibodies against FMDV using Non-structural proteins (NSPs) ELISA

All the 1206 serum samples were subjected to FMD screening test using PRIOCHECK FMD-3ABC NS protein ELISA (NSP-ELISA). The PRIOCHECK FMD-3ABC NS protein ELISA kit is designed to detect FMDV specific antibodies in bovine serum. The test was useful because it was able to discriminate animals that had been infected (wild virus induced antibodies) from those that had been vaccinated with purified vaccine (vaccine induced antibodies)

The ELISA serology was performed according to the Manufacturer's instructions. Briefly described, 80 µl of the ELISA buffer and 20µl of the test sera were added to the 3ABC antigen coated test plates. Negative, weak positive and strong positive control sera were added to designated wells on each test plate, gently shook and incubated overnight (18 hours) at 22°C. The plates were then emptied and washed six times with 200µl of washing solution and 100µl of diluted conjugate were added to all wells. The test plates were sealed and incubated for 60 minutes at 22ºC. The plates were then washed six times with 200µl of the washing solution and 100µl of the chromogen (Tetra-Methyl Benzidine) substrate was dispensed to all wells of the plates and incubated for 20 minutes at 22ºC following which 100µl of stop solution was added to all the wells and mixed gently. Readings were taken on a spectrophotometer Multiskan® ELISA reader (Thermo Scientific, USA) at 450 nm and the OD450 values of all samples were expressed as Percentage Inhibition (PI) relative to the OD450 using the following formula PI = 100 – [OD450 test sample/OD450 max] × 100. Samples with PI of ≥ 50% were considered positive while those with PI < 50% were declared negative. Since the 3-ABC ELISA for FMD was 100% specific and > 99% sensitive, the percentage prevalence was taken as true prevalence (Sørensen *et al.,* 1998; Bronsvoort *et al.*, 2006;).

3.2.5 Data collection and analyses

The data (numerical) generated were stored in Microsoft Excel and coded for analysis. Seroprevalence was calculated on the basis of 3ABC ELISA test results. Serological data were subjected to statistical analysis using SPSS (version 13) and Open Epi (version 2). Chi-square (χ^2) was used to assess the existence of association with FMD seropositivity. The associations of individual categories of each exposure factor cattle movement, age, sex, mixing at watering points and management systems with seropositivity of FMD were analyzed using univariable logistic regression. This univariable analysis assumed all other factors were constant and one category was used as a reference. In all the statistical analyses, Confidence Interval was at 95%.

Geographical coordinates of cattle herds (points of blood samples collection) were recorded using hand-held global positioning systems (Garmin *eTrex* GPS™ receiver), sorted in Microsoft Excel 7 and stored; ArcGIS 10.1 software was used to construct thematic maps of spatial distribution of FMDV seroprevalence and serotypes in the study area.

3.3 Results

The overall seroprevalence of FMD in North Central Nigeria was found to be 70.98% (856/1206) (95%CI: 68.37-73.49). The seroprevalence of FMD was found to be higher in Niger State (85.4%; 95CI:83.46-88.03%) than in Plateau State (54.2%; 95CI:50.12-58.2) (Table 3.1). The difference in the prevalence between the two states was statistically associated with FMD seropositivity ($p<0.05$). Sex, management system, trans-boundary crossing and herd mixing at the watering points were found to be positively associated with FMD seropositivity ($p<0.05$).

Seroprevalence based on geographical zone

Niger North recorded the highest seroprevalence of 93.9% (171/182) (95CI:89.74-96.8), followed by Niger East, 85% (204/240) (95CI:80.06-89.1), Niger South 83.08% (162/195) (95CI:45.64-64.36), Plateau South, 62.17% (166/267) %(95CI:56.24-67.84), Plateau Central 55.14% (59/107) (95CI:45.64-64.36) and the lowest prevalence was recorded in

Plateau North 43.12% (94/215) (95%CI:37.2-50.4). The difference in seropositivity was statistically significant (Table 3.2)

Table 3.1 Seroprevalence of FMD based on State distribution by 3ABC ELISA

State	Number of Sera tested	Number of sera Positive	Prevalence %	(95%CI)
Niger	617	537	85.4	(83.46-88.03)
Plateau	589	319	54.2	(50.12-58.16)
Total	1206	856	70.98	

χ^2=156.4; p-value= < 0.05

Seroprevalence based on Local government Area

The Local Government areas were compared in terms of the prevalence of FMD. The highest prevalences were recorded in Bosso, Shiroro and Dangi Local Governments' Areas respectively (100%). However, the lowest prevalences were recorded in Jos South (16.5%) area and 0% prevalence was recorded in Bokkos (Table 3.3).

Seroprevalence based on age category

Age of animals sampled were analyzed in two categories < 2years (Young) and >2 years (Adult).The seroprevalence in Adult was higher (70.01%) than in the Young (67.7%). The difference in seroprevalence was, however, not statistically associated with the age of the cattle (P<0.4656). The odd of FMD seropositivity is more in adult (1.14) than in young animals (Table 3.4).

Seroprevalence based on sex category

Higher disease prevalence was observed in females 71.9% (207/529) than in Males 30.4% (112/210). The difference in prevalence between the two sex groups was found to be statistically significant ($\chi 2$ =129.1:P>0.05). The odd ratio of FMD was 4.78 (3.60-6.54) times in females than males. (Table 3.5)

Table 3.2 Seroprevalence of FMD based on geographical zones by 3ABC ELISA

Geographical zones	Number Sera tested	Seropositivity 3ABC ELISA	Prevalence %(95%CI)	
Plateau North	215	94	43.12	(37.2-50.4)
Plateau Central	107	59	55.14	(45.64-64.36)
Plateau South	267	166	62.17	(56.24-67.84)
Niger South	195	162	83.08	(77.35-87.86)
Niger East	240	204	85	(80.06-89.1)
Niger North	182	171	93.9	(89.74-96.8)
Total	1206	856		

χ^2=184; df=5; $p < 0.05$

Seroprevalence based on management system

The study revealed higher disease prevalence in nomadic management system (75.8%) followed by sedentary management system (66.3%) and a lower prevalence was observed in Intensive Management (1.8 %) system (1.8 %). The difference in prevalence between the nomadic and sedentary management system was not statistically significant χ^2=10.79: P>0.05). However, the difference in disease prevalence among the three management systems was statically associated with FMD seropositivity (χ^2=123:P<0.05).

The Odd of FMD in nomadic and sedentary husbandry systems was 171.9 (23.54-1256) times more than intensive management system (Table 3.6) Management system showed a positive association with FMD seropositivity as FMD risk factor.

Seroprevalence based on cattle movement

The study showed a higher prevalence of FMD seropositivity in cattle that cross national boundary (88.75%) than those that move within the country (57.7%) and the difference in FMD seropositivity was statistically significant (χ^2=99.35:P<0.05). The odd of FMD in cattle crossing the national boundary was 5.184 (3.75-7.43) times greater than those that move within the country. Table 3.7

Seroprevalence based on herd mixing at the watering point

The study revealed higher FMD seropositivity in herds that indicated mixing at the watering points (75.8%) than those that do not mix at watering points (1.8%). The difference in seropositivity was statistically significant (χ^2=143.9:P<0.05).The Odd of FMD was 171.8 (23.82-1253) times greater in herds that mixed at water points than those that do not.

Surveys to determine the seroprevalence of FMD in abattoir and cattle market as FMD hot spots were conducted. The findings revealed that Seroprevalence of FMD in these study areas was statistically not significant. The prevalence in Abattoir (65.1%) and cattle market (69.1%) were equally distributed.

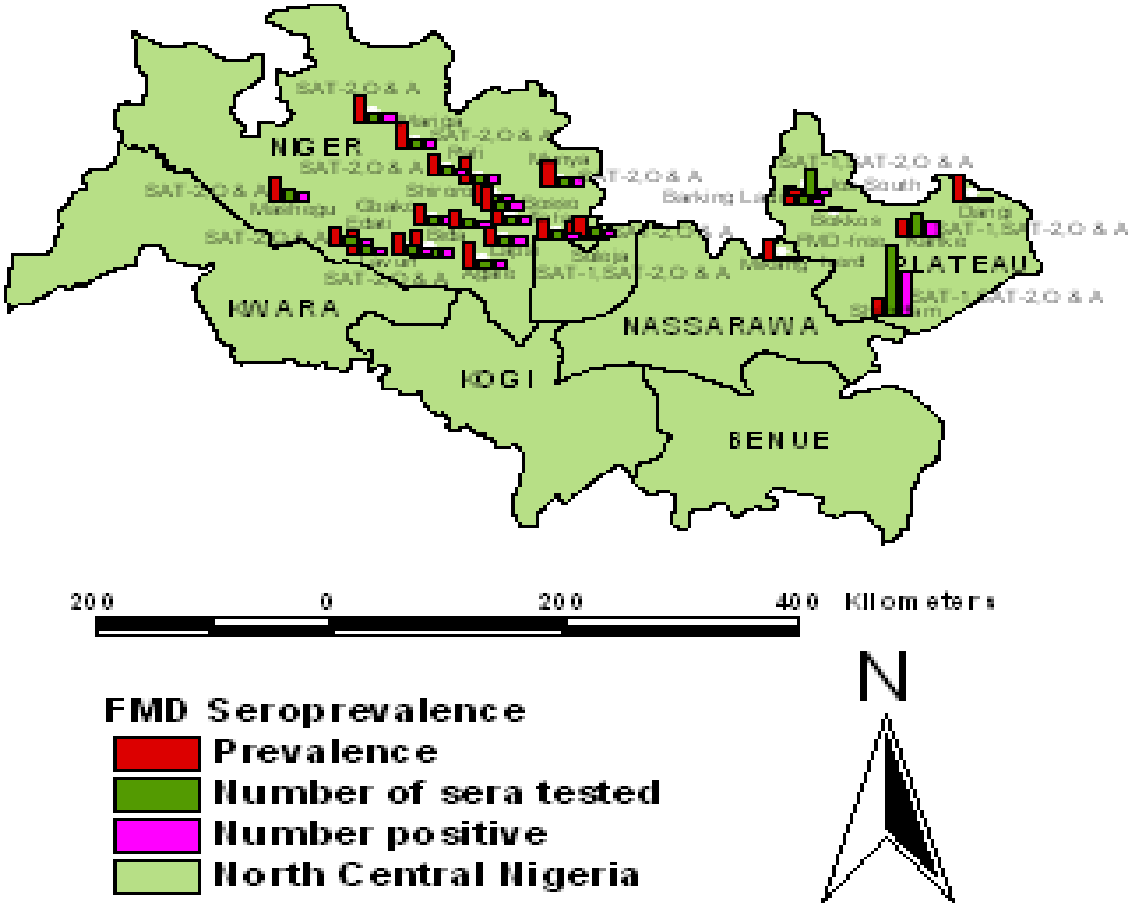

Fig 3.2: Spatial distribution pattern of Foot-and-Mouth Disease Virus seroprevalence and serotypes in North Central, Nigeria

Table 3.3 Seroprevalence of FMD based on Local Government Area of cattle herd location

Local Government	Number of Sera Tested	Number of sera Positive	Prevalence (%)
Shandam	261	162	62.1(56.1-67.8)
Kanke	87	49	56.3(45.8-66.5)
Mikang	6	4	66.7(26.2-93.9)
Barking Ladi	25	16	64 (44.1-80.8)
Dangi	10	10	100(74.1-0.0)
Jos South	97	16	16.5(10.08-24.88)
Bokkos	10	0	0 (0.0-25.89)
Agaie	28	25	89.3 (73.6-97)
Lapai	32	25	78.1 (61.5-89.9)
Bida	30	27	90 (75.2-89.6)
Gbako	31	24	77.4 (60.4-89.6)
Lavun	31	23	74.2(56.8-87.23)
Edati	29	24	82.8(65.8-93.4)
Katcha	29	18	62.1(43.7-78.23)
Mokwa	31	21	67.7(49.9-82.3)
Suleja	29	20	68.9(50.6-83.7)
Tafa	29	22	75.8(57.9-88.8)
Gurara	28	20	71.4(52.86-85.8)
Paikoro	29	25	86.2 (70-95.5)
Bosso	29	29	100(90.2-0.0)
Changanga	31	28	90(75.9-97.5)
Shiroro	30	30	100(90.5-00)
Munya	33	32	96.9(85.9-99.9)
Rafi	33	24	72.7(55.8-85.8)
Kontogora	35	34	97.1(86.7-99.9)
Mashegu	35	30	85.7(71.1-94.6)
Mariga	35	32	91.4(78.4-97.8)

Table 3.4 Seroprevalence of FMD based on Age category using PrioCheck 3ABC NSP-FMD ELISA

Age	Number	%	Serological status		Prevalence	
			+ve	-Ve	(%)	OR
Adult	767	72.5	537	230	70.01 (66.7-73.2)	1.14(0.83-1.48)
Young	291	27.5	197	94	67.7 (62.2-72.9)	1
Total	1058	100				

χ^2=0.5324 p-value >0.4656

Table 3.5 Seroprevalence of FMD based on Sex distribution using PrioCheck 3ABC NSP-FMD ELISA

Sex	Number	%	Serological status		Prevalence	
			+ve	-Ve	(%)	OR
Male	322	30.4	112	210	34.8(29.70-40.10)	1
Female	736	69.6	529	207	71.9 (66.50-75)	47.8(3.60-6.54)
Total	1058	100				

χ^2=129.1 p-value <0.05

Table 3.6.Seroprevalence of FMD based on Management system using PrioCheck 3ABC NSP-FMD ELISA

Management System	Number	%	Serological status +ve	-Ve	Prevalence (%)	OR
Sedentary	511	48.3	339	172	66.3(62.2-70.3)	
Nomadic	491	46.4	372	119	75.8(71.8-79.4)	9.3 (4.81-19.02)
Intensive	56	5.3	1	55	1.8 (0.089-8.50)	1
Total	1058	100				

χ^2=125.4 Df=2 p-value <0.05

Table 3.7 Seroprevalence of FMD based on Trans-boundary Border Crossing using PrioCheck 3ABC NSP-FMD ELISA

Trans-boundary Crossing	Number	%	Serological status +ve	-Ve	Prevalence (%)	OR
Yes	417	39.4	370	47	88.7 (85.4-91.5)	5.184(3.75-7.43)
No	641	60.6	370	244	57.7(53.8-61.5)	1
Total	1058	100				

$\chi^2 = 99.35$ $p < 0.05$

Table 3.8 Seroprevalence of FMD based on Cattle herd mixing a watering point using PrioCheck 3ABC NSP-FMD ELISA

Mixing at the Watering point	Number	%	Serological status +ve	-Ve	Prevalence (%)	OR
Yes	1001	94.7	759	242	75.8 (73.1-78.5)	171.8(23.8-1253)
No	56	5.3	1	55	1.8 (0.09-8.5)	
Total	1057	100				

χ^2=143.9 p-value <0.05

Table 3.9 Seroprevalence of FMD Hot spots using PrioCheck 3ABC NSP-ELISA

FMD Hot spots	Number	Serological status +ve	-Ve	Prevalence % (95%CI)
Cattle Market	81	43	11	69.1 (58.50-78.50)
Abattoir	67	44	13	65.7 (51.40-76.30)
Total	148			

$\chi^2 = 1.088$; p-value > 0.05

3.4 Discussion

The findings of this study showed that Foot-and-Mouth Disease is enzootic in North Central Nigeria which limits prospects in local livestock production, with outbreaks occurring throughout the year. In this study intensive, nomadic and sedentary cattle herds in North-Central Nigeria were investigated for antibodies against FMD-virus and risk factors for seroposivity. The overall seroprevalence of the disease was found to be 70.98% (95%, CI: 68.37-73.49). This is consistent with the results of previous surveys conducted in Nigeria, in which a seroprevalence of 75.11% was reported by Olabode *et al.*(2013) from a study conducted in Kwara State. In a study conducted at the borders states in Nigeria, Lazarus *et al*.(2012) reported seroprevalence of 64.7%. Ehizibolo *et al*.(2010) and Ishola *et al*. (2011) respectively reported seroprevalence of (64.3%) and (56.3%) from studies carried out in Plateau State. The result of this study confirmed that FMD is still an enzootic disease in the North-Central part of Nigeria and this is corroborated by the fact that vaccination programme is not being practiced among the sedentary as well as nomadic pastoralist system in the region. There is also unrestricted herd mobility, continuous contact and intermingling of different herds at watering points, communal grazing areas and porous borders. In addition, clinical diseases are usually underreported. This prevalence represents a higher prevalence than the 55% national prevalence reported by Abegunde *et al*.(1988).

Higher seroprevalence was recorded in Niger state (85.4%) than in Plateau State (54.2%). This could be attributed to the fact that many of the herds sampled indicated trans-boundary animal movement between Nigeria and the Republic of Benin. Niger State shares international boundaries with the Republic of Benin, consequently, the animal population move freely across the border in search of feed and drinking water. In most part of West and Central Africa, the role of wildlife in the epizootiology of FMD has not been fully studied (Hedger and Condy, 1985; Thomson, 1995b; Alexandersen *el al.,* 2002). However, the presence of wildlife population along the national park in Borgu Niger State, might be a probable exposure factor that may have contributed to high FMD seropositivity observed in this area. It has been established that countries like Nigeria with less developed livestock industries; the presence of many species of cloven-hoofed animals provides a possibility of

reservoirs of the infectious virus being established. It is believed that these free roaming species may normally come in contact with domesticated livestock, providing an opportunity for disease transmission. In comparison to the high seroprevalence observed in Niger north, Plateau north had the lowest seroprevalence which might be attributable to the fact that most of the cattle sampled in this area strictly practice intensive and sedentary management system contrary to the nomadism and extensive systems observed in most part of Niger north.

Age category seropositivity revealed a higher seroprevalence in cattle aged >2 years than in young cattle aged <2 years old. However, there was no association in seropositivity to age groups. The relative low seropositivity in young animals might be due to low exposure to risk factors. This is as a result of the practice of keeping young animals around the homestead and around areas separate from adult animals. Radostits *et al.*(2000), has indicated that young animals are relatively more susceptible than the adults, even though the present study showed that seroprevalence of FMD in adult cattle is slightly higher than that of the young cattle. This might be due to the fact that, adult cattle have repeated exposure and close contacts with other animals due to free animal movement. Generally, mortality is higher in young animals over 20% compared to 2% in adults. It has been observed that during outbreaks, the morbidity rate in cattle can be up to 100% while mortality in young animals could be up to 40% (Fiebre, 2015).

Furthermore, exposure factor to FMD seropositivity indicated both age groups had equal odds of FMD infection. Age association with FMD seropositivity was consistent with the study conducted by Olabode *et al*.(2013) and Ishola *et al*. (2011) which reported higher prevalence of FMD in adult cattle than in young ones.

The higher seropositivity observed in female cattle was consistent with the findings of Olabode *et al*. (2013), who reported a risk difference in association with sex in Kwara State, Nigeria. Also, Mazengia *et al.*(2010) had reported higher incidence of FMD in females in Northwest Ethiopia. However, more of the animals sampled were female as opposed to male cattle, therefore, the significant association in seropositivity in sex could be attributed to a small number of males sampled as both male and female animals are equally at risk.

Nomadic and sedentary management systems revealed a higher prevalence respectively, whereas, a lower seropositivity was recorded in the intensive management system. The higher seroprevalence recorded in nomadic and sedentary management systems might be as a result of unrestricted cattle movement, contact with the different herd and mixing at watering points, whereas the lowest prevalence recorded in the intensive management system could be attributed to restricted movement, less contacts with other herds and mixing at watering points. The study further revealed that the odd of FMD infection is 171.9% times more in nomadic and sedentary management than in intensive management system. This finding is in agreement with a study conducted in Southern Ethiopia by Megersa *et al*.(2009) where pastoral system was identified as one of the major risk factors for FMD transmission.

The seropositivity due to herd movement had indicated that the herds that reported movement across national borders recorded higher seropositivity relative to herds that reported movement within the country. This might be attributed to contacts with wildlife reservoirs which are continuous source of infection, as well as contact with different herds and different locations. All the herds that indicated national border crossing were in Niger State.

Cattle herd mixing at watering point had higher likelihood of being classified as FMD seropositive than those that do not mix at watering points, infection was observed to be 5.2 times higher in animals crossing national borders than those that do not cross national borders. This study is in agreement with other studies which reported that the movement of herds in search of pasture and water from one area to another is a significant risk factor for the occurrence of FMD *(*Habiela *et al.*, 2010a; Megersa *et al*., 2009; Molla *et al*., 2009).

Herds that reported mixing at the watering point with other herds recorded the highest seroprevalence relative to those that do not mix with other herds. Watering point was observed to be a common place where cattle of different herds meet in search of water, thereby serving as foci of FMD transmission. The odd of FMD infection was observed to be 17.8 times in herds mixing at the watering points than does that do not mix at all. A similar observation was made in Thailand by Cleland *et al.* (1996) where the odds of FMD increased by 1.6 for every additional village that shared a water source (and village equates

with the herd in our study). This correlation might be due to either an increase in potential for transmission or from higher virus survival in a more humid microclimate around water sources (Dawe *et al*., 1994; Donaldson and Ferris, 1975).

The equal distribution of prevalence in abattoir and cattle market samples from study area is insignificant, which could be attributable to the fact that most of the cattle population being slaughtered in Nigeria abattoirs are directly purchased from the local cattle markets.

In conclusion, identifying the risk factors of FMD is the first step toward progressive control pathway for FMD control. This study has established that FMD is enzootic in North central Nigeria, and it has also been able to identify some of the risk factors associated with FMD seropositivity in the study area. Further study to determine the possible role of wildlife and small ruminants in the epizootiology of FMD in the study area is strongly recommended. This will help in the implementation of an effective FMD control programme.

CHAPTER FOUR

SPATIAL PATTERN OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPES IN NORTH CENTRAL, NIGERIA

4.1. Introduction

Foot and mouth disease virus (FMDV) is a communicable disease of cloven-hoofed animals including wildlife (Anderson *et al.,* 1993). It is characterized by high morbidity, vesicle formation and erosion in the mucosa of the mouth, nose and interdigital space. FMD is usually associated with devastating economic losses, although mortality is low (about 5%) in adult animals. The economic losses arises from factors such as high calf mortality (50%), decreased calving rate due to infertility and abortion; severe reduction in production of milk and meat because of the characteristic wasting nature of the disease, loss of draught power resulting from lameness and loss of access to international market due to trade embargo imposed on importation and exportation of animal meat and animal products from FMD affected areas (Ezeokoli *et al.*, 1988). Therefore, to guarantee protection against outbreak situations, an appropriately matched vaccine to the field virus is required (Paton *et al.,* 2005).

The etiological agent of FMD, is classified within the genus *Aphthovirus* in the family *Picornaviridae* (Racaniello, 2011). Seven serotypes of the viruses have been identified as serotypes O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1. It is also known that infection with one serotype does not provoke immune protection to the other serotypes, many strains are

identified within serotypes through biochemical and immunological tests (OIE, 2012). Six of the seven serotypes of FMDV that exist worldwide have been known to circulate in sub-Saharan Africa, namely A, O, C, SAT 1, SAT 2, and SAT 3 (Vosloo *et al*., 2002). FMDV in endemic settings across the world have been categorized into six pools; each comprising a different geographic location with different predominant serotypes and West Africa belongs to pool 5 (O, A, SAT 1 and 2), (Paton *et al.,* 2009). Two cycles of FMD occur in sub-Saharan Africa, one where the virus circulates between wildlife and domestic animals and the other where the virus spreads among domestic animals (Vosloo *et al.,* 2002). In some parts of southern and eastern Africa, the cycle between wildlife and domestic animals occurs, while in West Africa, due to the perceived low numbers of wildlife population, the disease is maintained mainly in domestic animals. Four serotypes have been found to be circulating in West Africa (A, O, SAT-1, and SAT-2) (Wungak *et al.,* 2015; Ehizibolo *et al.,* 2014;Olabode *et al.,* 2014 ;Sangare *et al.,* 2004)

FMD remains endemic in Nigeria since the first documented case in 1924 which was attributed to outbreaks in cattle herds caused by serotype O virus (Libeau, 1960). Subsequently, other serotypes (A, SAT-1 and SAT-2) have been identified with trans-boundary animal movement of trade cattle associated with the outbreaks (Wungak *et al.,* 2015). Between 2007-2009, (FMD serotypes A, O and SAT 2 have been reported to be a major cause of outbreaks in Nigeria (Fasina *et al*., 2013). Even though, FMD outbreaks are a regular and extensive occurrence, clinical and laboratory investigation for identification and genotyping of the virus has never been exhaustive and complete, because of poor surveillance system and animal disease control policy.

The presence of multiple FMD serotypes and the occurrence of subclinical forms of the disease render FMD control very difficult, particularly in pastoral agriculture. This study was designed with the objective of identifying the FMDV serotypes circulating in the study area, the prevalence of FMDV serotypes, and the spatial distribution of FMDV. The information generated will provide knowledge to researchers, vaccine manufacturers and policy makers to effectively deploy resources to control FMD outbreaks.

4.2 Materials and Methods

4.2.1 Study design

A total of 155 of non-structural protein positive sera by 3ABC ELISA were selected from Niger and Plateau states of the study area, and were screened for FMDV serotype-specific FMD antibodies using Solid-Phase Competitive ELISA for antibodies specific to FMDV, serotypes A, O, SAT-1 and SAT-2.

4.2.2 Sample collection for antigen detection

Tongue epithelial specimens (40) from clinically sick animals were collected purposively between June 2011 and October 2014 from North-Central States (Plateau:26; Kogi:4; Nassarawa:6; Benue:4). Animals were clinically examined for the presence of FMD lesions on the mouth, teats, nostrils, and feet and samples were collected. Clinical specimens of epithelial tissue were collected from animals that showed typical clinical signs of FMD. Epithelial tissue was 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 (Penicillin, Streptomycin, Gentamycin and Amphrotericin B) within pH 7.2 to 7.6 (OIE, 2012), and samples were transported to the laboratory on cold-chain and stored at -20°C until processed. Both field FMD samples as well as the cell-culture supernatant were screened using antigen detection ELISA for serotypes A, O, SAT-1 and SAT-2 at the National Veterinary Research Institute, Vom Nigeria.

4.2.3 Tissue preparation for virus isolation and antigen detection

The samples were prepared as described previously (OIE, 2012). Briefly, the epithelial samples were taken from virus transport medium and were blotted dry on absorbent paper to reduce the glycerol content, which is toxic for cell cultures, and weighed out. A suspension was prepared by grinding the sample with sterile sand in a sterile mortar using pestle and with a small volume of tissue culture medium (Universal viral transport medium) Becton, Dickinson and Company USA. The further quantity of Universal viral transport medium was added until a final volume of nine times that of the epithelial sample had been

added, giving a 10% suspension. This was then clarified on a bench top centrifuge at 2000 ***g*** for 10 minutes. Clarified sample supernatants were stored at –80ºC, for antigen detection and virus isolation. Clarified sample supernatants for virus isolations were filtered through a millipore filter of 0.22 μm pore size.

4.2.4 Detection of serotype–specific antibodies against FMDV

The ELISA serology was performed according to the Manufacturer's instructions. A Solid-Phase Competitive ELISA (SPCE) from IZSLER, Biotechnology Laboratory (Brescia Italy) was used. The assay is a solid phase competitive ELISA (SPCE) using a selected neutralizing anti-FMDV monoclonal antibody (MAb), specific for FMDV serotype O, A, SAT-1 and SAT-2 respectively to measure antibodies against these serotypes. The test can be applied to measure antibodies in serum or plasma samples of FMDV Infected or vaccinated animals of any susceptible specie (Grazioli *et al*., 2008).

The FMDV antigen is captured by a serotype-specific MAb, for the serotypes (O, A, SAT 1& SAT 2) coated to the solid phase with the function of catching antibody. ELISA micro plates were supplied pre-coated with FMDV serotypes O, A SAT-1 and SAT-2 antigen captured by the homologous MAb respectively.

The samples were distributed at a single dilution 1/10, by distributing 45µl of ELISA diluents buffer and 5µl of each test serum. Briefly, 45µl of ELISA diluents buffer was added to all the wells excluding negative controls wells. A 50µl volume of negative control serum was added to all the four negative control wells. A 5µl volume of positive control serum was added to the two positive control wells. A 5µl volume of test sera was added to all the remaining wells. The plate was gently shaken and incubated for 1hour at room temperature (temperature range 18-22^{o}C).Without washing, 25µl of the appropriately diluted Horse reddish peroxidase (HRPO)-conjugate was added to all the wells. The plate was covered and incubated for 1hour at room temperature (temperature range 18-22^{o}C). After the 1hour incubation, the plate was emptied and 200µl of washing solution was added and incubated for 3 minutes at room temperature. The plate was emptied and 3 cycles of washing were repeated leaving the last one for 5 minutes at room temperature. A 50µl volume of substrate/chromogen solution was added to all the wells and incubated for 20

minutes in the dark. 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. Serum end-point titre was expressed as the highest dilution producing 50% inhibition, with serum having end point titre ≥50% being classified as positive.

Percentage inhibition produced by positive control and test sera was calculated as follows:

% inhibition=100-(serum OD/reference OD*)x 100

*Reference OD=mean OD of four wells processed with the Negative control.

Criteria for test validity

Spectrophotometric readings must be ≥ 1 OD in wells of the negative control.

The positive control serum is expected to give ≥ 90% inhibition at 1/10 dilution

Interpretation

Sera samples were considered:

- Positive when producing an inhibition ≥ 70% at the 1/10 dilution;
- Negative when producing an inhibition < 70% at the 1/10 dilution;

4.2.5 Detection of FMDV antigen using Ag-ELISA

The ELISA serology was performed according to the Manufacturer's instructions. A Solid-Phase Competitive ELISA (SPCE) from IZSLER, Biotechnology Laboratory (Brescia Italy) was used.

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 homogenates of epithelial and in vesicular fluids. Only in these clinical specimens, the FMD virus usually achieves the concentration required to provide a positive signal in ELISA assays. 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.

Briefly, samples were diluted 1/2 in diluents buffer. A 50µl volume of each sample was distributed in 12 wells of a row, two replicates for each type specific catching MAb and for the pan-FMDV-MAb. Fifty µl of the diluents buffer was added in all the wells of G and H rows. The plate was incubated at room temperature (temperature range 18-22^{o}C). After the 1hour incubation period, the plate was emptied to remove all remaining residual fluid. A 200µl volume of washing solution was added and, incubated for 3min at room temperature (18-22^{o}C). The plate was emptied and the circle of washing was repeated 3times. After the washing, A 50µl volume 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 1 hour incubation period, four cycles of washing was repeated as above leaving the last one for 5minutes. After that 50µl of the substrate-chromogen solution was added to all 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

4.2.6 Data analyses

The data were stored in Microsoft Excel spreadsheet. Descriptive statistics was carried out using Microsoft Excel spreadsheet and proportion was obtained using Open Epi. Version 2.3

Spatially referenced data were presented in ArcGIS 10.1 environment and used for construction of thematic maps of the spatial distribution of FMD serotypes that were identified.

Descriptive and categorical spatial distribution pattern was generated for each FMD serotype identified. Purely Spatial Scan statistics using Kulldorf (2002) method, assuming a Bernouli distribution pattern was computed on SatScanVersion 9.1. Significance was set at $p < 0.05$. Clustering analysis was used as a tool to study the spread of diseases in animal populations.

4.3 Result

The result based on states showed that 51 sera in Niger state were positive for serotype A, 71 were positive for serotype O, 58 for serotype SAT-2, and 13 were positive for SAT-1, while in Plateau state the results revealed that 50 were positive for serotype A, 49 were positive for serotype O, 82 were positive for SAT-2, and 39 were positive for SAT-1 (Table 4.1)

An overall serotype-specific prevalence of 79.4% (123/155, 95CI:72.4-85.18) was recorded for serotype O, 65.2% (95CI:57.41-72.3) was recorded for serotype A, 52.9% (95CI:45.03-60.67) was found SAT-2 and 33.55% (95CI:26.45-41.26) was recorded for SAT-1 Table4.2

Distribution of antibodies to FMDV serotypes revealed that 82.6% sera analyzed showed evidence for exposure to more than one FMDV serotypes while 17.4% tested positive to only one serotype. Fig 4.1

Percentage distribution of FMDV serotype specific antibodies showed that serotypes O had the highest percentage distribution of 34%, followed by serotype A 28%, SAT 2 23% and SAT-1 had the lowest percentage distribution of 15%, Figure 4.2.

Evidence of exposure to multiple serotypes of FMDV showed that 12.3% of the sera had evidence of the presence of antibodies against four serotypes circulating, 30.97% had evidence of presence of antibodies against three serotypes circulating, 22.6% had evidence for the presence of antibodies against two serotypes and 17% showed exposure to only one serotype of the virus, Table 4.3.

Distribution of the circulating FMDV serotypes showed that serotypes A, O, SAT-1 and SAT-2 were circulating in all the six geographical zones of the study area Table 4.4.

Based on the epithelial samples analysed, FMDV serotypes A, O, SAT-1 and SAT-2 were detected circulating in the area. Serotype O had the highest prevalence rate of 52.2%, followed by serotype A with 25% prevalence, SAT-2 had a prevalence of 20.8% and the

lowest is SAT-1 with a prevalence of 4.1%, (Table 4.5&4.7).The result revealed that in some outbreaks, the herds were exposed to multiple serotypes of FMDV.

The result of the analysis performed by FMDV serotype-specific antigen capture ELISA to determine the presence of FMDV antigen distribution in North Central Nigeria revealed the co-circulation of four serotypes namely; FMDV serotype A, O SAT-1 and SAT-2 during the period 2011-2014 (Table 4.5&4.7). These results coincided with the result obtained for antibodies serotypes specific.

FMDV serotypes identified in different states of the North central, Nigeria besed on outbreaks situation, revealed that serotypes A, O, SAT-1 and SAT-2 co-circulated in Plateau State during the period of this study, serotype A was detected in Nassarawa State and serotype O was detected in Kogi and Benue states. However, the detection of only one serotype of FMDV in Nassarawa, Kogi and Benue states is attributed to few samples collected from those states.

The distribution of FMDV serotypes based on outbreak samples showed that serotype O had the highest proportion, (54.2%) followed by serotype A, 25%, SAT 2 20.8% while the lowest was SAT 1 (Table 4.6 & figure 4.3).

Two FMDV serotypes A and O were detected in an outbreak that occurred in a cattle herd in Jos South LGA of Plateau State in 2014, and antigen to three different FMDV serotypes i.e A, SAT1 and SAT 2 were detected in a herd in Langtang LGA of Plateau State, in an outbreak that occurred in 2012.

Purely Spatial analysis for clusters with high rates using the Bernoulli model revealed clustering of FMD positivity in cattle herds within the coordinates (9.53N, 8.81E) at 3.10Km radius in Jos South LGA of Plateau State. However, the cluster was not statistically significant (p=0.83) (table: 4.8 and fig 4.5)

Table 4.1 Distribution of FMDV Serotypes Per State by Antibodies SP-ELISA

States	Serotype A	Serotype O	SAT-1	SAT-2
Niger	51	71	13	58
Plateau	50	49	39	82
Total	101	123	52	82

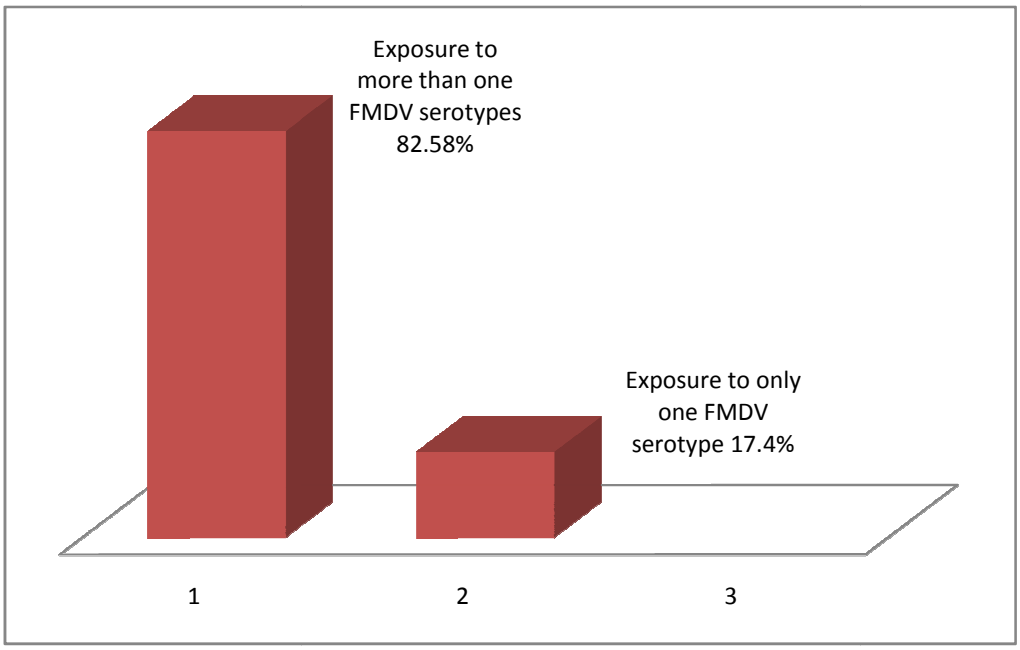

Figure: 4.1 Distribution of antibodies to FMD virus serotypes in North Central Nigeria

Legend

1 ☐ **Sera sample having FMDV antibodies to more than one FMDV serotypes**

2 ☐ **Sera sample having FMDV antibody to only one FMDV serotype**

Table 4.2 Prevalence of FMDV Serotypes in the study Area Antibodies SP ELISA

FMDV Serotypes	Frequency	Prevalence %	95% Confidence Limit
Serotype A	101	65.2	(57.41-72.3)
Serotype O	123	79.35	(72.4-85.18)
SAT-1	52	33.55	(26.45-41.26)
SAT-2	84	52.9	(45.03-60.67)

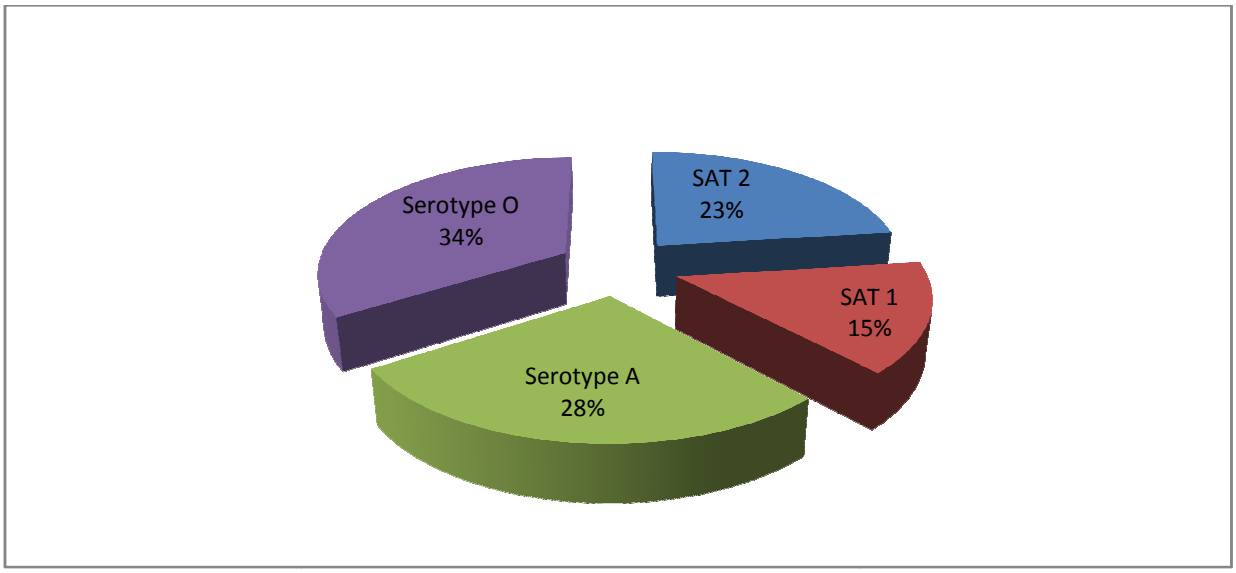

Figure 4.2: Proportions of FMDV serotypes among cattle populations in North-Central Nigeria

Legend

Serotype A

Serotype SAT 1

Serotype O

Serotype SAT 2

Table 4.3 Evidence of Exposure to multiple FMDV Serotypes Ab SP ELISA

FMDV Serotypes Exposure	Number of Sera	Proportion CL
Evidence of exposure to the four(4) serotypes circulating in North central Nigeria	19	12.26 (7.769-18.15)
Evidence of exposure to Three (3) Serotypes circulating in North Central Nigeria	48	30.97 (24.07-38.6)
Evidence of exposure to two (2)serotypes circulating in Nigeria	35	22.58 (16.52-29.66)
Evidence of exposure to only One (1) serotypes circulating in Nigeria	27	17 (12.05-24)

Table; 4.4 Distribution of the Circulating FMDV Serotypes amongst the Agro-Ecological zones of the study area

Agro-Ecological zones	FMDV Serotypes Circulating
Plateau North	Serotype A, O , SAT-1 and SAT-2
Plateau Central	Serotype A, O , SAT-1 and SAT-2
Plateau South	Serotype A, O , SAT-1 and SAT-2
Niger Zone A	Serotype A, O , SAT-1 and SAT-2
Niger Zone B	Serotype A, O , SAT-1 and SAT-2
Niger Zone C	Serotype A, O , SAT-1 and SAT-2

Table 4.5: FMD Antigen Detection using BDL FMD ELISA for the Seven Serotypes and IZLER ELISA For Four Serotypes A, O , SAT1 and SAT-2

SAMPLE ID Nigeria	WRL-FMD Ref. No-	DESCRIPTION OF SAMPLE Location	WRL-FMD BDLAg-ELISA/ WRL-FMD	NVRI, Vom IZLER ELISA
KG/OKE/BUKU/5	NIG 3/2011	Bovine, Epithelium collected 26/06/2011/ Kogi state	O	O
KG/M5	NIG 2/2011	Bovine, Epithelium 26/06/2011	A	A
MKD/FMD2011/04E	NIG 1/2011	Bovine, Epithelium collected 11/06/2011	O	O
PL/DN/001E	NIG 5/2011	Bovine, Epithelium collected 20/07/2011/ Plateau state	SAT-2	SAT-2
PL/DN/006/E	NIG 6/2011	Bovine, Epithelium 20/07/2011/Plateau state	SAT-2	SAT-2
NS/DM/008	NIG 11/2011	Bovine probing, 02/08/2011/ Nassarawa state	NVD	NVD
PL/BK/08185	NIG 16/2011	Bovine epithelium collected 03/11/2011 / Plateau state	SAT-2	SAT-2
PL/BK/08196	NIG 17/2011	Bovine epithelium collected 03/11/2011/ Plateau state	SAT-2	SAT 2
PL/SH/2012	NIG 6/2012	Bovine Epithelium 03/11/2012/ Plateau state	-	-
PL/KA/12M	NIG 7/2012	Bovine Epithelium 09/09/2012/ Plateau state	NVD	O
PL/BLD/02B	NIG 8/2012	Bovine Epithelium 06/11/2012/ Plateau state	A	A
PL/BLD/01A	NIG 10/2012	Bovine epithelium 06/11/2012/ Plateau state	A	A
NS/WAM/03	NIG 11/2012	Bovine, Epithelium 07/11/2012/Nassarawa	NVD	A
PL/JS/KA/1	NIG 1/2014	Bovine, Epithelium collected, 03/01/2014/Plateau state	O	O
PL/JS/KA 2	NIG 2/2014	Bovine Epithelium 03/01/2014/ Plateau state	O	O
PL/JS/KA03	NIG 3/2014	Bovine Epithelium collected 03/01/2014/ Plateau state	O	O
PL/KA/4/14	NIG 4/2014	Bovine, Epithelium collected 14/01/2014/ Plateau state	O	O
PL/KA/06/04/A-2		Bovine, Epithelium 14/06/2014/ Plateau state		O

PL/KA/06/04/B-2	Bovine, Epithelium 14/06/2014/ Plateau state	O
JS/BI/8/7/14/c	Bovine, epithelium collected 18/7/2014/ Plateau state	A and O
JS/BI/6/7/14	Bovine, epithelium collected 18/7/2014/ Plateau state	O
BL/GA/07/14/1	Bovine, epithelium collected 20/7/2014/ Plateau state	O
BL/GA/07/14/2	Bovine, epithelium collected 20/7/2014/ Plateau state	O
PL/Js/Vwang/14	Bovine, epithelium Collected 31-7-14/ Plateau state	O
PL/Lang/E	Bovine, swab collected 29/11/2012/ Plateau state	A, SAT-1, SAT-2

NVD No Virus antigen detected.

Table 4.6: Distribution of FMDV Serotypes circulating in North Central Nigeria, based on Antigen detection

Serotypes	Frequency of FMDV Serotypes	Proportion of FMDV Serotypes
O	13	54.2 (34.3-73.04)
A	6	25 (10.8-44.9)
SAT-1	1	4.1 (0.21-18.8)
SAT-2	5	20.8 (8.06-40.3)
Total	24	

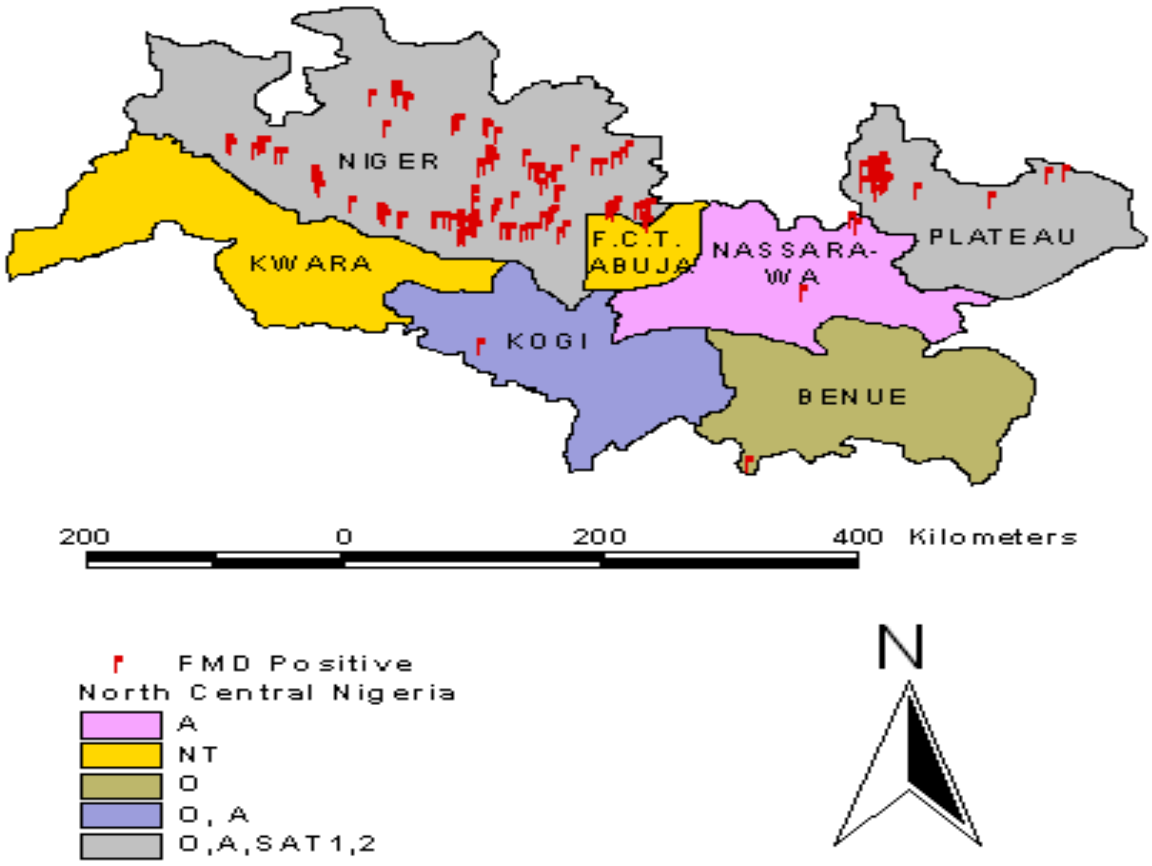

Figure: 4.3 Map of North Central Nigeria showing FMDV Serotype distribution in States of the study area: NT note tested

N T = States not tested for FMDV, FMDV Serotypes A, O, SAT 1 and SAT 2

Table 4.7: FMDV serotype identified in different origin of outbreaks using SPCE Antigen ELISA

Origin States of sample collection	Number of sample analysed	Serotypes Identified
Plateau state	19	A, O SAT1, and SAT2,
Nassarawa sate	2	A
Kogi state	2	O, A
Benue	1	O

Table:4.8 Purely Spatial analyses for clusters with high rates using the Bernoulli model

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SUMMARY OF DATA

Study period.....: 2000/1/1 to 2000/12/31
Number of locations.....: 154
Total population.....: 242
Total number of cases.....: 160

MOST LIKELY CLUSTER

1.Location IDs included.: 130, 131, 132, 134, 136
Coordinates / radius..: (9.530800 N, 8.818800 E) / 3.10 km
Population.....: 5
Number of cases.....: 5
Expected cases.....: 3.31
Observed / expected...: 1.51
Relative risk.....: 1.53
Log likelihood ratio..: 2.095759
P-value.....:0.83

NOTE: The sequential Monte Carlo procedure was used to terminate the calculations after 60 replications.

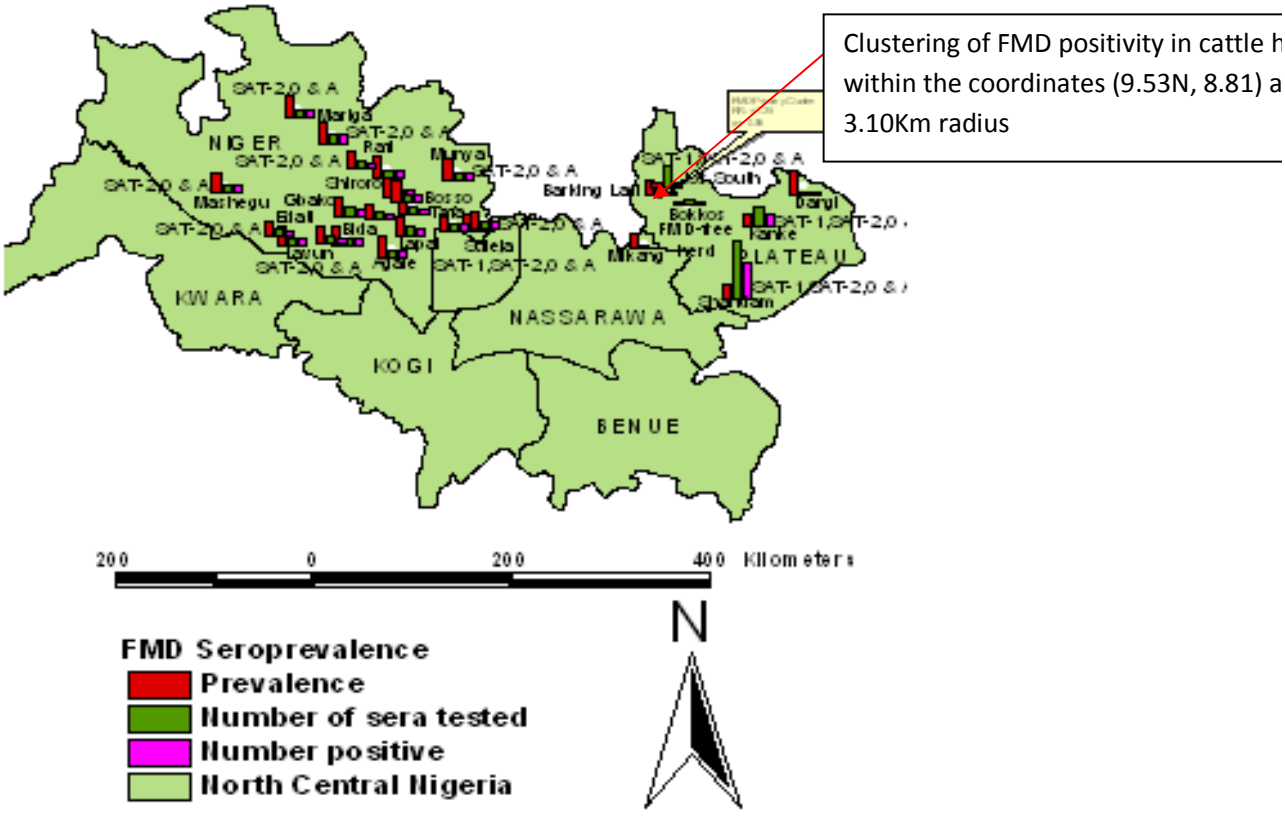


Figure 4.4 Map of the study area showing clustering of FMD positivity in cattle herds within the coordinates (9.53N, 8.81) at Jos South LGA Plateau State

4.5 Discussion

FMD is known to be enzootic to most countries in sub-Saharan Africa, including Nigeria. This study has demonstrated that FMDV remains enzootic among pastoral and sedentary husbandry systems within the study areas with multiple serotypes widely distributed. The four FMDV serotypes detected in the course of this study along with previous reports in Nigeria establishes the facts that these are the most prevalent serotypes circulating among cattle in the country (Fasina et al., 2013; Lazarus et al., 2015; Olatunde et al., 2014; Wungak et al., 2015). From available information, none of the cattle herds sampled ever practiced FMD vaccination and since routine prophylactic vaccination of cattle is not a common practice in the country, these results tend to present evidence of viral exposure. In this study, analyses of serotype-specific antibodies to FMDV indicates that FMD serotypes O, A, SAT-1 and SAT-2 are widely distributed and co-circulated within the study areas during the period of the study. The wide distribution of the FMDV serotypes in the region could be attributed to unrestricted movement of cattle within the zone, frequent contacts of different herds at watering and feeding points, lack of any meaningful control measure in place and husbandry management system that is being practiced by the nomadic pastoralists. This finding is also in consistent with the previous study conducted between 1960-1981, where FMD serotypes A, O SAT-1 and SAT-2 were detected (Owoludun, 1971; Nawathe & Goni, 1976; Ularamu*et al.*, 2015). Also, between 2007-2015, FMD serotypes A, O and SAT 2 were reported to cause disease outbreaks among pastoral and sedentary herds in Nigeria (Fasina *et al*., 2013b; Olatunde *et al.,* 2014; Wungak *et al*., 2015; Ularamu*et al*., 2015; Ehizibolo *et al*., 2016)

The findings also revealed that serotype O is the most prevalent serotype in the region. FMDV serotype O has been known to be the most dominant and most widely distributed serotype. It has the ability to be the most invasive serotype (Kitching *et al.,* 2005). Depa *et al.,*(2012) reported that serotype O was the most prevalent recorded in most of the FMD outbreaks worldwide.

Serotype A was the second in terms of prevalence, followed by SAT-2 while the SAT-1 was the lowest. This is consistent with a study conducted in Somali Eco-System in Kenya by Chepkwony *et al.* (2012) where they reported a higher prevalence of serotype O compared to the other serotypes.

Evidence of exposure to multiple FMDV serotypes showed that 12.3% of the sera samples had evidence of the presence of antibodies against four serotypes circulating, 30.9% had evidence of presence of antibodies against three serotypes circulating, 22.6% had evidence for the presence of antibodies against two serotypes and 17.0% show exposure to only one serotype of the virus. The detection of antibodies to multiple serotypes of FMDV in a serum sample could be attributed to recent infection by multiple serotypes or re-infection by different serotypes of FMDV. This finding is in agreement with a study conducted in Uganda where Mwiine *et al.,*(2010), reported a concurrent high antibody titres against serotypes O, SAT 2, SAT 1 and SAT 3 in the same serum samples in cattle herds and Namatovu et al.(2015) also reported multiple FMDV serotypes (A, O, SAT1and SAT 2) circulation in cattle herds in Ugandan. Doel (2005) reported that immune protection against one serotype does not confer immune protection against the other serotypes, therefore, the animal can be infected with a further rounds of infection with other FMDV serotypes. Another reason is the fact that, the presence of antibodies to different serotypes of FMDV is an indication of repeated infection or the development of carrier state in the animals. Alexanderson *et al.*(2005) reported that a number of cattle population exposed to FMDV become carriers, in which the animal continues to produce antibodies against the FMDV(s) without showing any clinical sign.

Spatial distribution of the circulating FMDV serotypes showed serotype A, O, SAT-1 and SAT-2 are co- circulating in all the six geographical zones of North Central Nigeria. This could be attributed to unrestricted movement of cattle within the zone, contacts of different herds at watering and grazing areas, lack of vaccination policy and husbandry system of fulani cattle owners as well as repeated exposures to FMDV.

82.6% (128/155) of the sera samples show evidence to exposure to more than one FMDV serotypes, while 17.4 % (27/155) of the sera samples show evidence to exposure of only one serotype. These findings revealed that there is co-circulation of multiple FMDV

serotypes per time in cattle herds in the study area. This has a great implication in vaccine formulation, as only a multivalent vaccine will be appropriate for the Nigerian situation.

The result of the analysis of FMDV serotype-specific antigen capture ELISA to determine the presence of FMDV antigen distribution in North Central Nigeria revealed the co-circulation of four serotypes i.e FMDV serotype A, O SAT-1 and SAT-2 during the period 2011-2014. This is an indication of the fact that FMD is endemic in Nigeria, with the field viruses co-circulating freely in the region. FMDV serotypes identified in different states of the North Central Nigeria based on an outbreaks situation revealed that serotypes A, O, SAT-1 and SAT-2 co-circulated in Plateau state during the period of this study, serotype A was detected in Nassarawa State and serotype O and A were detected in Kogi State while serotype O was detected in Benue State. However, the detection of only one serotype of FMDV in Nassarawa and Benue States and two serotypes in Kogi state could be attributed to the few samples collected in those states. Had more outbreaks samples been collected, more of other FMDV serotypes would have been detected as well. Two FMDV serotype antigen A and O were detected in outbreaks that occurred in a cattle herd in Jos South LGA of Plateau state in 2014 and antigen to three difference FMDV serotypes were detected in a herd in Langtang LGA of Plateau state, in an outbreak in 2012 (Table 5). This could be attributed to the exposure of the affected herds to co-circulation of multiple FMDV serotypes per time.

These findings also correlate the detection of FMDV serotype-specific antibodies to serotypes O, A SAT-1 and SAT-2 in sera samples investigated by using serotype-specific antibodies detection ELISA in the same North central Nigeria as seen above. With no cattle movement control, no FMD vaccination and pastoral agricultural system as well as the Trans-boundary movement of the cattle population in and out of Nigeria, the viruses will continue to be transmitted freely among the susceptible animal population including wildlife population. Di Nardo *et al.,* 2011 and Kivaria, 2003 reported that lack of animal movement control is an important mechanism for the spread of FMDV within and across international borders.

The result of antigen detection also confirms the prevalence obtained with FMDV serotype-specific antibodies, which also reveals that serotype O is the most dominant

serotypes (54.2%) followed by serotype A, 25%, and SAT 2 20.8% while the lowest is SAT 1 (Table 6& figure 3). This result is in consonance with a study conducted in Eritrea which reported serotypes O and A were the most predominant serotypes (Tekleghiorghis *et al.*, 2013).

Spatial analysis showed that FMD seropositivity and serotypes amongst cattle herds in the study area were diffuse (p=0.83).This is probably as a result of unrestricted movement of cattle within the zone.

In conclusion, this study revealed that four serotypes of FMDV are co-circulating in North-Central Nigeria. Consequently, used of multivalent vaccines comprising the four local circulating serotypes in addition to movement control are the best control options.

CHAPTER FIVE

ISOLATION, MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF FOOT-AND-MOUTH DISEASE VIRUSES IN NORTH-CENTRAL NIGERIA, 2011-2014

5.1 Introduction

Foot-and-Mouth disease virus (FMDV) belongs to the member of the viral family *Picornaviridae* which causes a highly contagious vesicular disease in cloven-hoofed mammals (Knowles and Samuel, 2003a). Even though mortality due to the disease is normally very low in adults, it is normally common in young animals. There is also considerable decrease in productivity and working ability of the animals which results in significant losses to the livestock industry and resource poor farmers. The disease has an important socio-economic impact in endemic countries especially in Sub-Saharan Africa and Asia (Rweyemamu *et al.,* 2002). It also brings about considerable economic consequences when outbreaks occur in disease free countries usually as a result of trade restrictions (Melo *et al.,* 2002), and is regarded as one of the most important barriers to the trade of livestock and livestock products worldwide. The virus possesses about 8,200 nucleotides of a single-stranded positive RNA molecule, within an icosahedral capsid consisting of 60 copies each of four structural proteins (VP1, VP2 , VP3, andVP4) (Jamal and Belsham, 2013). As is common with many other RNA viruses, FMDV is also known to be highly variable (Holland *et al.*, 1982; Mateu *et al.*, 1990). The virus is subdivided into

seven serotypes namely; O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1, of which, only serotypes O, A ,SAT 1 and SAT 2 are found to be circulating in Nigeria (Fasina *et al*., 2013). It has been revealed that VP1 is the most variable region among the polypeptides capsid and is regarded as the major immunogenic protein, because it contains a linear antigenic site able to elicit neutralizing antibodies enough to protect animals against FMDV (Bittle *et al.*, 1982; DiMarchi *et al.*, 1986). Beck and Strohmaier, (1987) were the first to employ the use of nucleotide sequencing for the study of the epizootiology of FMD during which they investigated the origin of outbreaks of types O and A in Europe over a period of 20 years. Since then the use of nucleotide sequencing became popular. Subsequently, VP1 region was used for the sequence analysis of serotype O (Samuel *et al*., 1990, 1988), serotype A (Marquardt and Adam, 1990; Samuel *et al*., 1988), serotype C (Samuel *et al.,* 1988), serotype Asia 1 (Ansell *et al.*, 1994; Woodbury *et al.*, 1994), and the study of SAT 2 (Bastos *et al.*, 2001; Vosloo *et al.*, 1992). Consequently, it is now possible to study the relationship between FMDV strains, and to group the different FMDV isolates according to their genetic relationships into phylogenetic trees. Recent data have demonstrated the usefulness of such phylogenetic studies in establishing the epizootiological links among FMDV isolates, following geographical movement of strains and facilitating identification of the source of virus strains. The molecular characterization of virus isolates is an important tool in control of FMD. Sequencing the FMDV region of the genome encoding the capsid proteins of the virus, provide the most detailed information about isolates as this region is variable between serotype and subtypes (Domingo *et al.,* 1980). Then FMDV genome hyper variable region is responsible for the vast antigenic diversity lies in the VP1 gene segment (Grubman and Baxt, 2004). Beck and Strohmaier, (1987) also observed that, the vast antigenic diversity of FMDV is a consequence of mutations in the VPI sequence.

Understanding the epizootiology of a disease is essential for the formulation of an effective and efficient FMD control strategies. Nucleotide sequencing represents an important component for tracking outbreak sources and epizootiological investigation.

The aim of this study therefore, was to carry out Isolation and molecular characterization of FMDV circulating in North Central, Nigeria and to determine the genetic relationships of these viruses with other FMD viruses from the neighboring countries.`

5.2 Material and Methods

5.2.1 Sample collection

Tongue epithelial specimens (40) from clinically sick animals were collected purposively between June 2011 and October 2014 from the North-Central States (Plateau:26; Kogi:4; Nassarawa:6; Benue:4). Animals were clinically examined for presence of FMD lesions on the mouth, teats, nostrils, feet, and samples were collected accordingly. Epithelial tissue samples were collected from animals that showed typical clinical signs of FMD. The samples were transported in the medium composed of equal amount of glycerol and 0.04 M of phosphate-buffered saline solution pH 7.2 to 7.6 with antibiotics (OIE, 2012), and samples were transported in cold-chain and stored at -80°C until processed at NVR, Vom. Both field FMD samples as well as the cell-culture supernatants were screened using Antigen detection ELISA for serotypes A, O, SAT-1 and SAT-2 at the Nigerian, National Veterinary Research Institute, Vom Nigeria and tissues samples were submitted to World Reference Laboratory for FMD (WRLFMD), Pirbright,UK, for further analyses.

5.2.2 Virus Isolation using ZZ-R 127 cell line (Foetal goat tongue)

It has been established that the most sensitive cell line for the isolation of FMDV is primary bovine thyroid (BTY) cells, but this cannot be passaged or frozen without impairing their sensitivity. Ensuring that there is always a fresh and suitable batch of primary BTY cells available for diagnostic purposes is quite laborious and expensive especially in disease endemic countries. Therefore, most diagnostic laboratories use other cell-lines that are more convenient to handle, either cells of bovine, ovine or porcine origin or continuous cell lines such as BHK-21 or IBRS-2. These protocols do not facilitate a reliable overnight detection of virus from clinical specimens (OIE, 2012). A foetal goat tongue cell line (ZZ-R 127) was used for the isolation of virus from clinical specimens.

This cell line has been demonstrated to be highly sensitive for the primary isolation and replication of FMDV. Its sensitivity is only slightly inferior to that of primary bovine thyroid (BTY) cells, most frequently used for FMDV isolation, but significantly higher than that of IBRS-2 cells (swine kidney cell line) (Brehm *et al.,* 2009) Confluent cell monolayers were washed with 10ml of PBS, pH 7.2-7.4, after which they were inoculated with filtered field epithelial sample supernatant and incubated for 30 minutes at 37. 8°C. Afterwards, fresh cell culture medium without FCS (foetal calf serum) was added and the cultures were incubated at 37 8°C and monitored for a cytopathic effect (CPE) for 48 h. When no CPE was observed, the cells were subjected to one freeze-thaw cycle, clarified and inoculated unto a fresh cell monolayer. The sample was considered negative if no CPE was observed after 48 hours of the second cell passage. If the CPE was observed, the viral suspension was stored at -80°C until used.

5.2.3 Isolation of FMDV using Bovine (Calf) Thyroid cells

The procedure was carried out according to the standard operating procedure for FMDV cell culture isolation at the World Reference Laboratory for FMD (WRLFMD), Pirbright, UK.

Briefly, sufficient growth media for virus Isolation was aliquoted and was allowed to warm. Confluent cell monolayer Tissue flasks (T25cm) were selected and the growth media discarded. The cell sheets were washed with 10 ml of PBS. The flasks were emptied and the media discarded. The flasks were inoculated with 0.5 ml of the virus as appropriate (0.1-0.5ml in general or as appropriate).The flasks were Incubated at 35-39°C for 20 – 30 minutes for the virus to adsorb. The required amount of growth media was added to the flasks. The lid of the flask was tightly closed. The surface of the flask was disinfected and placed inside an air/watertight container and was incubated at 35-39°C for 1-3 days with daily inspection. The flask was examined on the following day for cytopathic effect (CPE). The amount of CPE will determine the next step. If enough CPE was observed then the virus can be harvested. Where there was insufficient CPE, the flask was placed inside an air/watertight container, incubated and examined again on the following day.

5.2.4 Harvesting of isolates

The virus from the flask was decanted into an appropriate centrifuge tube and was centrifuged at 3000 rpm for 5 minutes. The clarified virus was put into a suitable container which was break resistant leak proof and suitable for the temperatures exposed to**,** 50% glycerol was added and mixed. The virus was stored at -50°C to -90°C until analysis.

5.2.5 Laboratory analyses

Virus isolation and antigen detection was conducted locally at the National Veterinary Research Institute, Vom Nigeria (NVRI) using antigen detection ELISA Solid-Phase Competitive ELISA and virus isolation using ZZ-R (foetal goat tongue cell line) according to (Brehm *et al.,*(2009) and RT-PCR. Samples submitted to the FAO World Reference Laboratory for FMD (WRLFMD) were prepared and subsequently tested. Virus isolation and antigen detection were carried out using primary bovine thyroid cultures (BTY) and a renal swine cell line: IB-RS-2: De Castro, 1964) and antigen detection ELISA (Ferris and

Dawson,1988).

5.2.6 One- step RT-PCR

Pan-FMD Oligonucleotide primers at a concentration of 10 pmol/µl were used. This include; Primer 1 sequence 5'-GCCTG-GTCTT-TCCAG-GTCT-3'(positive strand); Primer 2 sequence 5'-CCAGT-CCCCT-TCTCA-GATC-3' (negative strand). Also the following reagents were used. PCR water, 14.3, dNTPs Mix, 1.0, Primer F, 0.5, Primer R. 0.5, RNASE Inhibitor 0.2, 5x One-step RT-PCR buffer 5.0, One-step RT-Mix 1.0 (22.5) and RNA template 2.5 (25.0). The following; thermal profile was used: 50 ^{o}C for 30 min; 95 °C for15 min; 35 cycles of 95 °C for 15 s; 95°C for 30 s; 52°C for 30 s; 72 °C for 1 min, followed by a final extension of 72°C for 10 min. Total number of cycle was 35cycle. PCR products were analysed by electrophoresis on a 1.5% agarose-Tris-borate-EDTA gel containing 0.5 µg/mL ethidium bromide and DNA weight markers GeneRuler 100 bp DNA Ladder.

5.2.7 RNA Extraction and RT-PCR of Virus RNA for sequencing

Total RNA was extracted from 460µl of a 10% epithelial suspension or from cell culture supernatants using RNeasy kits (QIAGEN Ltd., Crawley, West Sussex, UK), according to the manufacturer's instructions, and re suspended in 50µl nuclease-free water. The RNA template (5 µl) was used in the one-step RT-PCR (illustra Ready-To-Go_ RT-PCR Beads;GE Healthcare UK Ltd. Little Chalfont, Buckinghamshire, UK). Oligonucleotide primers used for PCR amplification were selected based on serotype (Table 5.1).

The following thermal profile were used For FMDV serotype A: 42°C for 30 min; 94°C for 5 min; 35 cycles of 94 °C for 60 s; 55°C for 60 s; and 72°C for 90 s; followed by a final extension of 72°C for 5 min. Conditions were the same for the other serotypes, except for the annealing temperature of 50°C and 60 °C used for serotype O and SAT 2. PCR products were analysed by electrophoresis on a 1.5% agarose-Tris-borate-EDTA gel containing 0.5 µg/mL ethidium bromide. DNA weight markers (GeneRuler 100 bp DN51+.96 A Ladder Plus, Ready-To-Use; Fermentas Inc.,Hanover, MD, USA) were run alongside the samples to facilitate product identification and quantification. Post- PCR removal of deoxynucleoside triphosphates and primers was achieved using the QIAquick PCR Purification Kit (QIAGEN, UK) according to the Manufacturer's instructions, and re-suspended in 30µl nuclease-free water (Knowles *et al.*, 2009).

Two alternative primer combinations were used for RT-PCR: A-1C562F/EUR-2B52R, and A-1C612F/EUR-2B52R, which yield amplicon sizes of 866 and 814 bp, respectively

(Table1). Forward and reverse primer amounts were 20 and 40 pmol, respectively. Two to four sequencing primers were used to ensure coverage of the VP1 region on both cDNA strands (Table 1).

5.2.8 DNA sequencing

PCR amplicons were sequenced using the DTS Quick Start Kit (Beckman Coulter Inc., Fullerton, CA, USA) according to the Manufacturer's instructions and with the sequencing primers listed in Table 5.1. The sequencing reactions were run on a CEQ8000 Automated Sequencer (Beckman Coulter Inc., USA) according to the Manufacturer's instructions.

5.2.9 Phylogenetic analyses

Nucleotide sequences were aligned using BioEdit 7.0.5.3 (Hall,1999) and Clustal W 1.83 (Thompson *et al.,* 1994). These alignments were used to construct distance matrices using the Kimura-2-parameter nucleotide substitution model (Kimura, 1980) as implemented in the program MEGA 6.06 (Tamura *et al.,* 2013). Midpoint rooted neighbour-joining (NJ) trees were constructed using MEGA 6.06. To construct maximum likelihood (ML) phylogenies (Nei and Kumar, 2000), each data set was tested for 24 common nucleotide substitution models using MEGA 6.06. The model with the lowest Bayesian information criterion (BIC) score was chosen to construct each tree. All positions with less than 95% site coverage were eliminated. The robustness of each tree topology (NJ and ML) was assessed with 1000 bootstrap replicates. Individual FMD viruses were classified into geographically restricted clusters, also known as topotypes, as previously described (Knowles and Samuel, 2003b).

Sequence comparisons were made using BLAST sequence search that is also available from NCBI website (www.ncbi.nlm.nih.gov) using default search parameters.

Table 5.1: Oligonucleotide primers

Serotype	Primer name	Sequence (5′–3′)	Use for
A	A-1C562F/EUR-2B52R	TAC CAA ATT ACA CAC GGG AA	PCR
A	A-1C612F/EUR-2B52R	TAG CGC CGG CAA AGA CTT TGA	PCR & SEQ
O	O-1C244F/EUR-2B52R	GCA GCA AAA CAC ATG TCA AAC ACC TT	PCR
O	O-1C272F/EUR-2B52R	TBG CRG GNC TYG CCC AGT ACT AC	PCR& SEQ
SAT-2	SAT2-1C445F/SAT-2B208R	TGG GAC ACM GGI YTG AAC TC	PCR & SEQ
SAT-2	SAT2-P1-1223F/SAT-2B208R	TGA ACT ACC ACT TCA TGT ACA CAG	PCR
All	NK72	GAA GGG CCC AGG GTT GGA CTC	SEQ

Table 5.2: FMD Detection using PCR

SAMPLE ID Nigeria	WRL-FMD Ref. No-	DESCRIPTION OF SAMPLE	WRL-FMD RT-PCR	NVRI, Vom PCR
KG/OKE/BUKU/5	NIG 3/2011	Bovine, Epithelium collected 26/06/2011	FMDV GD	FMDV GD
PL/DN/001E	NIG 5/2011	Bovine, Epithelium collected 20/07/2011	FMDV GD	FMDV GD
PL/DN/006/E	NIG 6/2011	Bovine, Epithelium 20/07/2011	FMDV GD	FMDV GD
NS/DM/008	NIG 11/2011	Bovine probing, 02/08/2011	NGD	NGD
PL/BK/08185	NIG 16/2011	Bovine epithelium collected 03/11/2011	FMDV GD	FMDV GD
PL/BK/08196	NIG 17/2011	Bovine epithelium collected 03/11/2011	FMDV GD	FMDV GD
PL/KA/12M	NIG 7/2012	Bovine Epithelium 09/09/2012	FMDV GD	FMDV GD
PL/BLD/02B	NIG 8/2012	Bovine Epithelium 06/11/2012	FMDV GD	FMDV GD
PL/BLD/01A	NIG 9/2012	Bovine, Epithelium 06/11/2012	FMDV GD	FMDV GD
PL/BLD/01A	NIG 10/2012	Bovine epithelium 06/11/2012	FMDV GD	FMDV GD
NS/WAM/03	NIG 11/2012	Bovine, Epithelium 07/11/2012	FMDV GD	FMDV GD
PL/JS/KA/1	NIG 1/2014	Bovine, Epithelium collected, 03/01/2014	FMDV GD	FMDV GD
PL/JS/KA 2	NIG 2/2014	Bovine Epithelium 03/01/2014	FMDV GD	FMDV GD
PL/JS/KA03	NIG 3/2014	Bovine Epithelium collected 03/01/2014	FMDV GD	FMDV GD
PL/KA/4/14	NIG 4/2014	Bovine, Epithelium collected 14/01/2014	FMDV GD	FMDV GD
Pl/KA/7 B	NIG 5/2014	Bovine Epithelium collected 18/01/2014	NGD	FMDV GD
PL/KA/06/04/A-2		Bovine, Epithelium 14/06/2014		FMDV GD
PL/KA/06/04/B-2		Bovine, Epithelium		FMDV

	14/06/2014	GD
JS/BI/8/7/14/c	Bovine, epithelium collected 18/7/2014	FMDV GD
JS/BI/6/7/14	Bovine, epithelium collected 18/7/2014	FMDV GD
BL/GA/07/14/1	Bovine, epithelium collected 20/7/2014	FMDV GD
BL/GA/07/14/2	Bovine, epithelium collected 20/7/2014	FMDV GD
PL/Js/Vwang/14	Bovine, epithelium Collected 31-7-14	FMDV GD

NVD No Virus antigen detected.

Table5.3: FMDV Detection Using Cell culture Bovine thyroid (BTY) and ZZR (Goat tongue cell-line

SAMPLE ID Nigeria	WRL-FMD Ref. No-	DESCRIPTION OF SAMPLE	WRL-FMD BTY-Cell line	NVRI, Vom ZZR-127 Cell line
KG/OKE/BUKU/5	NIG 3/2011	Bovine, Epithelium collected 26/06/2011	Isolated	Isolated
KG/M5	NIG 2/2011	Bovine, Epithelium 26/06/2011	Isolated	Isolated
MKD/FMD2011/04E	NIG 1/2011	Bovine, Epithelium collected 11/06/2011	Isolated	Isolated
PL/DN/001E	NIG 5/2011	Bovine, Epithelium collected 20/07/2011	Isolated	Isolated
PL/DN/006/E	NIG 6/2011	Bovine, Epithelium 20/07/2011	Isolated	Isolated
NS/DM/008	NIG 11/2011	Bovine probing, 02/08/2011	NVD	NVD
PL/BK/08185	NIG 16/2011	Bovine epithelium collected 03/11/2011	Isolated	Isolated
PL/BK/08196	NIG 17/2011	Bovine epithelium collected 03/11/2011	Isolated	Isolated
PL/KA/12M	NIG 7/2012	Bovine Epithelium 09/09/2012	NDV	Isolated
PL/BLD/02B	NIG 8/2012	Bovine Epithelium 06/11/2012	Isolated	Isolated
PL/BLD/01A	NIG 9/2012	Bovine, Epithelium 06/11/2012	Isolated	Isolated
PL/BLD/01A	NIG 10/2012	Bovine epithelium 06/11/2012	Isolated	Isolated
NS/WAM/03	NIG 11/2012	Bovine, Epithelium 07/11/2012	NVD	NVD
PL/JS/KA/1	NIG 1/2014	Bovine, Epithelium collected, 03/01/2014	Isolated	Isolated
PL/JS/KA 2	NIG 2/2014	Bovine Epithelium 03/01/2014	Isolated	NVD
PL/JS/KA03	NIG 3/2014	Bovine Epithelium collected 03/01/2014	Isolated	Isolated
PL/KA/4/14	NIG 4/2014	Bovine, Epithelium collected 14/01/2014	Isolated	Isolated
PL/KA/06/04/A-2		Bovine, Epithelium 14/06/2014		Isolated

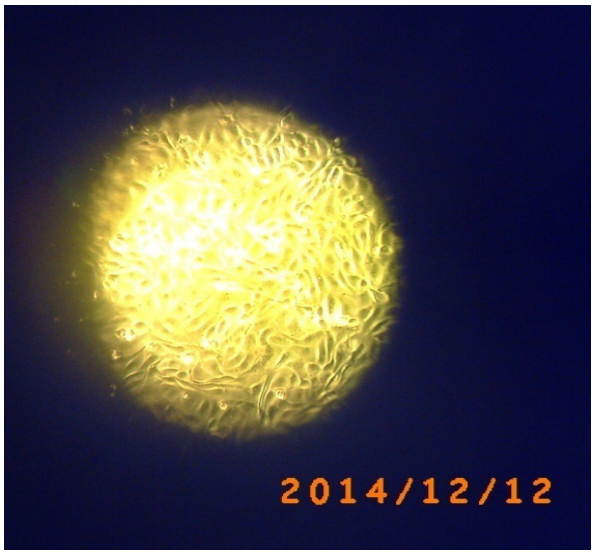
PL/KA/06/04/B-2	Bovine, Epithelium 14/06/2014	Isolated
JS/BI/8/7/14/c	Bovine, epithelium collected 18/7/2014	Isolated
JS/BI/6/7/14	Bovine, epithelium collected 18/7/2014	Isolated
BL/GA/07/14/1	Bovine, epithelium collected 20/7/2014	Isolated
BL/GA/07/14/2	Bovine, epithelium collected 20/7/2014	Isolated

NVD No Virus antigen detected.

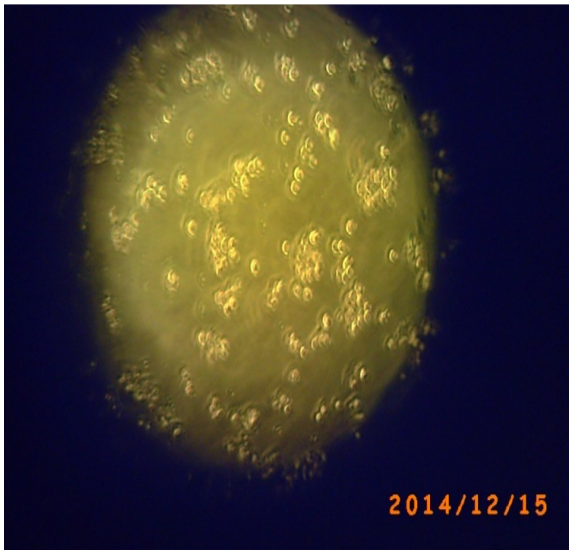
Table: 5. 4 FMDV isolated from four States of North-Central Nigeria

States	Number of Epithelial samples	Number of FMDV Isolates	FMDV Serotypes
Plateau	26	17	SAT 2, O, A
Kogi	4	2	A, O
Benue	4	1	O
Nassarawa	6	0	

FMDV isolates 20 out of which 12 were serotypes O, 4 were serotypes A and 4 were SAT 2



Normal cell



Cytopathic effect (CPE)

Fig 5.1 Showing ZZ-R 127 cell lines culture will intact and cytopathic effect (CPE)

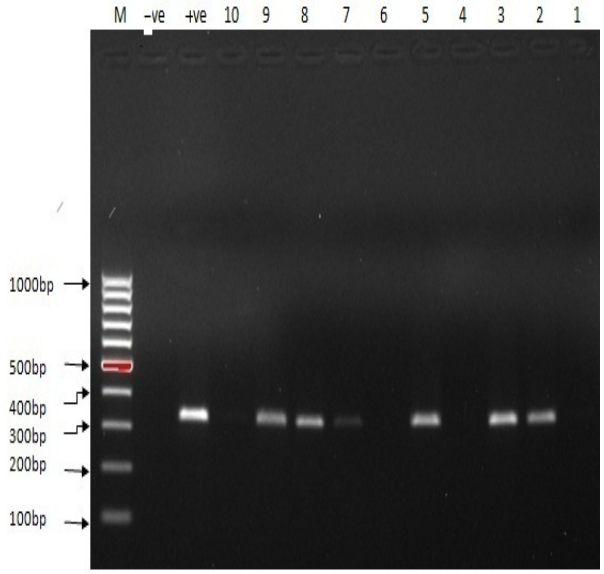


Fig. 5.2: Detection of FMDV genome by RT-PCR. Primers mixture PoF/PoR was used for targeting 3D coding region of the virus. M: 100 bp DNA ladder. Lanes 2, 3, 5,7 8 and 9 are positive FMDV isolates (322bp), while lane 1,4, 6 10 are negative

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5.3. Results

Phylogenetic analyses of VP1 nucleotide sequences provide evidence for the presence of topotype VII of serotype SAT 2, serotype O EAST AFRICA topotype (EA-3), O/WEST AFRICA (WA) topotype and a single monophyletic cluster of African topotype serotype A. These results demonstrate the close genetic relatedness of viruses in Nigeria to those from other African countries, including the detection of the spread of serotype O/EA-3 viruses into Nigeria.

5.3.1 Phylogenetic analysis of SAT-2

One distinct serotype SAT 2 evolutionary lineage topotype VII (Fig 1) was identified by sequencing the VP1 region of four (SAT2/NIG11-05, SAT 2/NIG11-06, SAT 2/NIG11-16 and SAT 2 NIG 11-17) FMDV SAT 2 serotypes. A unique West Africa lineage has also been reported for FMDV serotype O FMD viruses, all West African viruses were shown to constitute a single large evolutionary lineage covering a period of 26 years (Knowles *et al.,* 1998).

In this study, complete VP1 region of isolates from North-Central Nigeria were amplified and sequenced (fig.1). The sequences were compared with sequences obtained from Genbank (Fig 3). The analyses showed that the isolates were closely related to FMD virus SAT 2 sequences from Libya (2003), with 93.5% nucleotide sequence similarity, Niger (2000) with 92.1% sequences similarity, Senegal (2009), with 91.5% sequence similarity and Sudan (2007), with 91.1% similarity (Table 5.6).

5.3.2 Phylogenetic analysis of serotype O

Foot-and-Mouth Disease viruses of serotype O were recovered from cattle herds in Kogi state in 2011 (O/NIG/3/2011) and Kara cattle market, Plateau state in 2014 O/NIG/3/2014, O/NIG/1/2014, O/NIG/2/2014, O/NIG/4/2014 and O/NIG/5/2014 and Benue State O/NIG/1/2011.

Phylogenetic analysis showed that viruses isolated from Plateau and Kogi states were WEST AFRICAN (WA) topotypes, while the isolate from Benue State was EAST AFRICA 3(EA-3) topotype (Fig. 2).

The serotype O isolates from the same location in Plateau State were closely related to each other (99.84-100% identity). However, the virus from Kogi State is less similar when compared with isolates from Plateau state with 86.23%. Isolates from Plateau State revealed close genetic relationship with FMDV sequences from Adamawa State O/NIG/4/2012 (98.1%).

The VP1 sequences from North-Central Nigeria were compared with other VP1 sequences available in the Genbank. The result showed that the isolates from Plateau State revealed nucleotide sequence identity with serotypes sequences from Cameroon O/CAR/16/2000 87.0%, and O/CAR/28/2005 86.1 % id, while the isolates from Kogi State had sequence similarity with isolates from Togo, O/TOG/1/2004, 94.7% id, Ghana O/Lam/GHA/2012, 93.9%, Togo O/TOG/3/2005 93.6% and Benin O/BEN/28/2010 92.8%, (Table 5.7 Fig 5.2). FMDV isolates from Benue State belong to a different topotype (East African -3), and this cluster with the FMDV isolates that caused outbreaks in Sudan, 2005 O/SUD/3/2000 and 2004 O/SUD/3/2000 with percentage identity of 94.2 % and 92.8 % respectively (Table 5.8 & Fig 5.2).

5.3.3 Phylogenetic analysis of serotype A

The phylogenic analysis showed that FMD viruses recovered from cattle herd in Kogi and Plateau States fall into a single group or topotype (Africa topotype and belongs to genotype G-IV) (Fig 3). The African strains of serotype A has been classified as Africa topotype (Knowles and Samuel, 2003a).

FMDV of serotype A were recovered from a cattle herd in Barkin Ladi LGA of Plateau State and Kabba LGA of Kogi State. The 2012 FMDV isolates (ANIG/10/2012) from Barkin Ladi showed closest genetic relationship (< 3.91-4.07 nucleotite difference) with the isolates that caused outbreak in the same locality in 2009 (A/NIG/36/2009, A/NIG/38/2009). The isolate also showed close relationship with isolates from Bauchi State

(94.68 %) A/NIG/6/2013.The FMDV isolates serotypes A recovered in cattle herd in Kogi State in 2011 (A/NIG/2/2011) showed very close genetic relationship (< 4.54 % nucleotide difference) with the isolates recovered in cattle herds in Barkin Ladi in 2012.The isolates from this study were also compared with other serotype A sequences available in the Genbank. The result revealed that they were related to strains from Cameroon A/CAR/15/2000, 92-93% sequence similarity, Cameroon, 93.0% similarity (Vne/126/01/VP1), Togo A/TOG/5/2005 90.0% similarity, and Sudan A/SUD/3/77 87.6% (Table 5.5 & Fig 5.5).

Table 5.5: FMD Sequence similarities and differences from GenBank for Serotype A

Most Closely Related Viruses									
Pos.	Virus name	Filename	No. nt ntcomp.	No. match.	No. of ambig	% Id.	% Diff	Topotype	Strain
1	A/NIG/10/2012	NIG12-10	639	638	0	99.84	0.16	AFRICA	G-IV
2	A/N1G/36/2009	NIG09-36	639	614	0	96.09	3.91	AFRICA	G-IV
3	A/N1G/39/2009	NIG09-39	639	614	0	96.09	3.91	AFRICA	G4V
4	A/N1G/38/2009	NIG09-38	639	613	0	95.93	4.07	AFRICA	G-IV
5	A/NIG/2/2011	NIG11-02	639	609	0	95.31	4.69	AFRICA	G-IV
6	A/NIG/12/2012	N1G12-12	639	608	0	95.15	4.85	AFRICA	G-IV
7	A/NIG/8/2013	N!G 13-08	639	606	0	94.84	5.16	AFRICA	G-IV
8	A/NIG/6/2013	NIG 13-06	639	605	0	94.68	5.32	AFRICA	G-IV
9	A/NIG/3/2013	NIG 13-03	639	604	0	94.52	5.48	AFRICA	G-IV
10	A/NIG/7/2013	NIG 13-07	639	604	0	94.52	5.48	AFRICA	G-IV
Most Closely Related Reference Viruses									
Pos.	Virus name	Filename	No.nt comp	No. match.	No. ambig	% Id	o/0 Diff.	Topotype	Sfram
1	A/SUD/3/77 (GU566064)	SUD77-03	639	557	0	87.17	12.83	AFRICA	G-IV
2	A/KEN/42/66	KEN66-42	639	537	0	84.04	15.96	AFRICA	G-l
3	A/UGA/13/66(KF561705)	UGA66-13	639	537	0	84.04	15.96	AFRICA	G-Vll
4	A12/UK/119/32(M10975)	UKG32119	639	535 '	0	83.72	16.28	EURO-SA	Ai_2
5	A/GHA/16/73(KF561698)	GHA73-16	636	532	0	83.65	16.35	AFRICA	G-VI
6	A21/Lumbwa/KEN/64	KEN64-AA	639	531	0	83.10	16.90	AFRICA	G-l11
7	A/EGY/1/72(EF208756)	EGY72-01	639	529	0	82.79	17.21	AFRICA	G-ll
8	A/NGR/2/73(KF561704}	NGR73-02	639	526	0	82.32	17.68	AFRICA	G-V
9	A5/Allier/FRA/60	FRA60--F	636	522	0	82.08	17.92	EURO-SA	A_5
10	A/IRN/2/87 (EF208770)	IRN87-02	636	519	0	81.60	18.40	ASIA	lran-87

Table 5.6: FMD Sequence similarities and differences from GenBank for Serotype SAT

Most Closely			**Related Viruses**						
Pos.	Virus name	Filename	No, camp.	No. nt match.	No. of ambig.	% id.	% Diff.	Topotype	Strain
1	SAT2/NIG/6/2011	NIG11-06	648	648	0	100.00	0.00	VII	unnamed
2	SAT2/NIG/16/2011	NIG11-16	648	643	0	99.23	0.77	VII	unnamed
3	SAT2/NIG/17/2011	NIG11-17	648	643	0	99.23	0.77	VII	unnamed
4	SAT2/UB/1/2003 (JX570631)	LIB03-01	648	607	0	93.67	6.33	VII	Lib-03
5	SAT2/LIB/7/2003 (JX570632)	LIB03-07	648	607	0	93.67	6.33	VII	Lib-03
6	SAT2/NGR/1 5/2005 (KF112960)	NGR05-15	648	600	0	92.59	7.41	VII	Lib-03
7	SAT2/S EN/27/2009 (KF1 12967)	SEN09-27	648	594	0	91.67	8.33	VII	unnamed
8	SAT2/SUD/1/2007 (GU566071)	SUD07-01	648	593	0	91.51	8.49	VII	unnamed
9	SAT2/CAR/P1 2/2000 (VD44/1)(HM211082)	CAROO-AJ	648	592	0	91.36	8.64	VII	unnamed
10	SAT2/CAR/24/2005	CAR05-24	648	590	0	91.05	8.95	VII	unnamed
Most Closely Related Reference					**Virus**				
Pos.	Virus name	Filename	No. cornp.	No. nt match	No. of arnbig.	% Id.	%Diff.	Topotypt:	Strain
1	SAT2/CAR/8/2005 (JX570616)	CAR05-08	648	587	0	90.59	9.41	VII	unnamed'
2	SAT2/SAU/6/2000 (AF367135)	SAUOOA06	647	582	1	89.95	10.05	VII	unnamed
3	SAT2/KEN/2/84 (AY343941)	KEN84-AC	648	480	0	74.07	25.93	IX	unnamed
4	SAT2/KEN/3/57 (AJ251473)	KEN57G03	648	478	0	73.77	26.23	IX	unnamed
5	SAT2/ZAI/1/74 (DQ009737}	ZAI74-AA	642	473	0	73.68	26.32	VIII	unnamed
6	SAT2/ZAI/1/82(AF367100)	ZAI 82-01	647	474	1	73.26	26.74	X	unnamed
7	SAT2/UGA/51/75 (AY343963)	UGA75-AA	648	474	0	73.15	26.85	XII	unnamed
8	SAT2/ETH/2/91 (AY343938)	ETH91-AB	648	472	0	72.84	27.16	XIV	unnamed
9	SAT2/RW A/1/2000 (AF367134}	RWAOO-01	648	472	0	72.84	27.16	VIII	unnamed
10	SAT2/UGA/19/98 (AY343969}	UGA98-AA	648	472	0	72.84	27.16	X	unnamed

Table 5.7: FMD Sequence similarities and differences from GenBank for Serotype O

Most Closely Related Viruses									
Pos.	Virus name	Filename	No. nt comp.	No. nt match.	N	% Id.	% Diff	Topotype	Strain
1	O/NIG/2/2014	NIG14-02	639	639	0	100.00	0.00	WA	unnamed
2	O/NIG/3/2014	N1G14-03	639	638	0	99.84	0.16	WA	unnamed
3	O/NIG/4/2014	N1G14-04	639	638	0	99.84	0.16	WA	unnamed
4	O/NIG/4/2012	N1G12-04	639	627	0	98.12	1.88	WA	unnamed
5	O/NIG/5/2012	NIG12-05	639	627	0	98.12	1.88	WA	unnamed
6	O/CAR/1 6/2000 (HM211080)	CAROO-16	639	560	0	87.64	12.36	WA	unnamed
7	O/CAR/17/2000(HM211081)	CAROO-17	639	560	0	87.64	12.36	WA	unnamed
8	O/NIG/3/2011	N1G11-03	639	551	0	86.23	13.77	WA	unnamed
9	O/CAR/28/2005	CAR05-28	639	550	0	86.07	13.93	WA	unnamed
10	O/CAR/3/2005	CAR05-03	639	550	0	86.07	13.93	WA	unnamed
Most		**Closely**	**Relate**	**Reference Viruses**					
Pos.	Virus name	Filename	No. nt comp.	No. nt match.	No. ambig	% Id.	% Diff.	Topotype	Strain
1	O/GHA/5/93 (AJ303488)	GHA93-05	639	546	0	85.45	14.55	WA	unnamed
2	O/CIV/8/99 (AJ303485)	CIV99-08	639	544	0	85.13	14.87	WA	unnamed
3	O/SUD/2/86 (DQ1 65075)	SUD86-02	639	538	0	84.19	15.81	EA-3	unnamed
4	O/KEN/5/2002 (DQ165073)	KEN02-05	639	536	0	83.88	16.12	EA-2	Unnamed
5	O/PAK/1 6/2003 (DQ1 65068)	PAK03-16	639	536	0	83.88	16.12	ME-SA	Pak-98
6	O/UAE/4/2008	UAE08-04	636	532	0	83.65	16.35	ME-SA	lnd-2001c
7	O/KUW/3/97(DQ164904)	KUW97-03	639	534	0	83.57	16.43	ME-SA	lnd-2001a
8	O1/Manisa/TUR/69 (AY593823)	T	639	534	0	83.57	16.43	ME-SA	unnamed
9	O/IND/53/79(AF292107)	I	639	533	0	83.41	16:5	ME-SA	unnamed
10	O/BHU/3/2009	B	639	532	0	83.26	16.74	ME-SA	lnd-2001d

Table 5.8: FMD Sequence similarities and differences from GenBank for Serotype O

Most Closely Related Viruses									
Pos.	Virus name	Filename	No. nt comp.	No. nt match.	No. Of ambig.	% Id.	%Dif	Topotype	Strain
1	O/NIG/10/2011	NIG14-02	639	639	0	100.00	0.00	EA-3	unnamed
2	O/NIG/3/2007	N1G07-03	639	638	0	92.96	7.04	EA-3	unnamed
3	O/SUD/3/2005(GU566058)	SUD05-04	639	638	0	94.21	5.79	EA-3	unnamed
4	O/SUD/1/2005(GU566056)	SUD05-04	639	627	0	94.05	5.95	EA-3	unnamed
5	O/SUD/1/2004(GU566046)	SUD04-	639	627	0	92.80	7.20	EA-3	unnamed
6	O/ETH/1/2007(FJ7981370)	ETH07	639	560	0	87.17	12.83	EA-3	unnamed
7	O/GHA/5/93(AJ303488	GHA9	63	560	0	85.60	14.40	EA-3	unnamed

Table 5.9: Distribution of FMDV Sequences used in this study

REF.NO	Serotype	Geographical Origin	Date of collection	Accession	References
O/NIG11-03	O	Kogi, Nigeria	26/6/2011		This study
O/NIG114-01	O	Plateau, Nigeria	3/1/2014		This study
O/NIG114-02	O	Plateau, Nigeria	3/1/2014		This study
O/NIG114-04	O	Plateau, Nigeria	14/1/2014		This study
O/NIG/1/2011	O	Benue	11/6/2011		This study
O/KUW/3/97	O	Kuwai	1977	DQ164904	(Knowles et al., 2005)
O/SAU/3/2001	O	Saudi Arabia	2001	DQ164969	(Knowles et al., 2005)
O/IND59/2011	O	India	2011	KC506542	(Subramaniam et al., 2013)
O/IND103/2010	O	India	16/7/2009	KC506446	(Subramaniam et al., 2013)
O1/Manisa/	O	Turkey		AJ251477	(Knowles et al., 2005)
O/PAK/16/2003	O	Pakistan	2003	DQ165068	Knowles et al.,Unpubl
O1/N1451/	O			AJ004669	
UGA/10/98	O	Uganda	1998	HM211073	(Ayelet et al., 2009)
O/KEN/10/95	O	Kenya		EU919242	(Maree et al., 2014)
ETH/26/2007	O	Ethiopia	1/12/2007	FJ798138	(Ayelet et al., 2009)
ETH/54/2005	O	Ethopia	15/2/2005	FJ798118	(Ayelet et al., 2009)
SUD/5/89 VP1	O	Sudan	13/12/1987	GU566040	(Habiela et al., 2010b)
O/NEP/4/2003	O	Nepal		DQ165059	Knowles et al Unpublished
SUD/12/2004	O	Sudan	5/9/2004	GU566049	Knowles et al.,unpublished
O/SUD/1/99	O	Sudan		DQ165076	(Habiela et al., 2010a)
SUD/3/83	O	Sudan	1983	GU566036	(Habiela et al., 2010b)
CAR/16/2000	O	Cameroon	20/9/2000	HM211080	(Habiela et al., 2010b)
NGR_011/2001	O	Niger		JF749854	Xu et al.,unpublished
O/Lam/GHA/2012	O	Ghana	23/4/2012	KF305227	Romey et al,unpublished
TOG_4/2005	O	Togo		JF749858	Xu et al,unpulished
O/BEN/42/2010	O	Benin	30/7/2010	KC832987	(Romey et al., 2014)
O/BEN/28/2010	O	Benin	6/8/2010	KC832982	(Romey et al., 2014)
NIG 11-05	SAT2	Plateau, Nigeria	20/7/2011		This study
NIG 11-06	SAT2	Plateau, Nigeria	20/7/2011		This study
NIG 11-16	SAT2	Plateau, Nigeria	3/11/2011		This study
NIG 11-17	SAT2	Plateau, Nigeria	3/11/2011		This study
SEN/27/2009	SAT2	Senegal	9/10/2009	KF112967	(Hall et al., 2013)
NGR/15/2005	SAT2	Niger	31/12/2005	KF112960	(Hall et al., 2013)
LIB/1/2003	SAT2	Libya		JX570631	(Ahmed et al., 2012)
SAU/6/00	SAT2	Saudi Arabia		AF367135	(Bastos et al., 2003)
CAP/P12/2000	SAT2	Cameroon	2000	HM211082	(Ahmed et al., 2012)
CAR/1/2005	SAT2	Cameroon	2000	JX570615	(Ahmed et al., 2012)
EGY/13/2012	SAT2	Qalubia, Egy	1/3/2012	JX570625	(Ahmed et al., 2012)
NIG 2/2007	SAT2	Nigeria	1/9/2007	JX570636	(Ahmed et al., 2012)

LIB/29/2012	SAT2	Libya	16/2/2012	JX570633	(Ahmed et al., 2012)
SUD/1/2007	SAT2	Sudan	21/1/2007	GU566071	(Ahmed et al., 2012)
ERI/1/98	SAT2	Eritea	1998	AY343933	(Sahle et al., 2007)
A/NIG 12-10	A	Plateau, Nigeria	6/11/2012		This study
A/NIG 12-10	A	Kogi, Nigeria	26/06/2011		This study
A/NIG/1/2009	A	Nigeria	2009	JN680709	(Ehizibolo et al., 2014)
A/NIG/16/2009		Nigeria	2009	JN680724	(Ehizibolo et al., 2014)
A/SUD/3/77	A	Sudan	1977	GU566064	(Habiela et al., 2010a)
A/EGY/1/92	A	Kenya	13/5/1972	EF208756	(Kasanga et al., 2014)
21 KENYA iso7	A	Kenya	1964	AY593761	(Carrillo et al., 2005)
A/NGR/2/73	A	Niger	1973	KF112914	(Ludi et al., 2014)
A/BEN/36/2010	A	Benin	11/8/2010	KC832972	(Romey et al., 2014)
A/IRN/2/87	A	Iran	11/3/1987	EF208770	(Knowles and Samuel, 2003a)
A/IND/7/82	A	India	1980	FJ755014	(Khounsy et al., 2009)
A5ALLIER iso 45	A	France		AY593780	(Carrillo et al., 2005)
W Germany iso 42	A	Germany		AY593777	(Carrillo et al., 2005)
UGA/13/66	A	Uganda	1966	KF561705	(Kasanga et al., 2014)

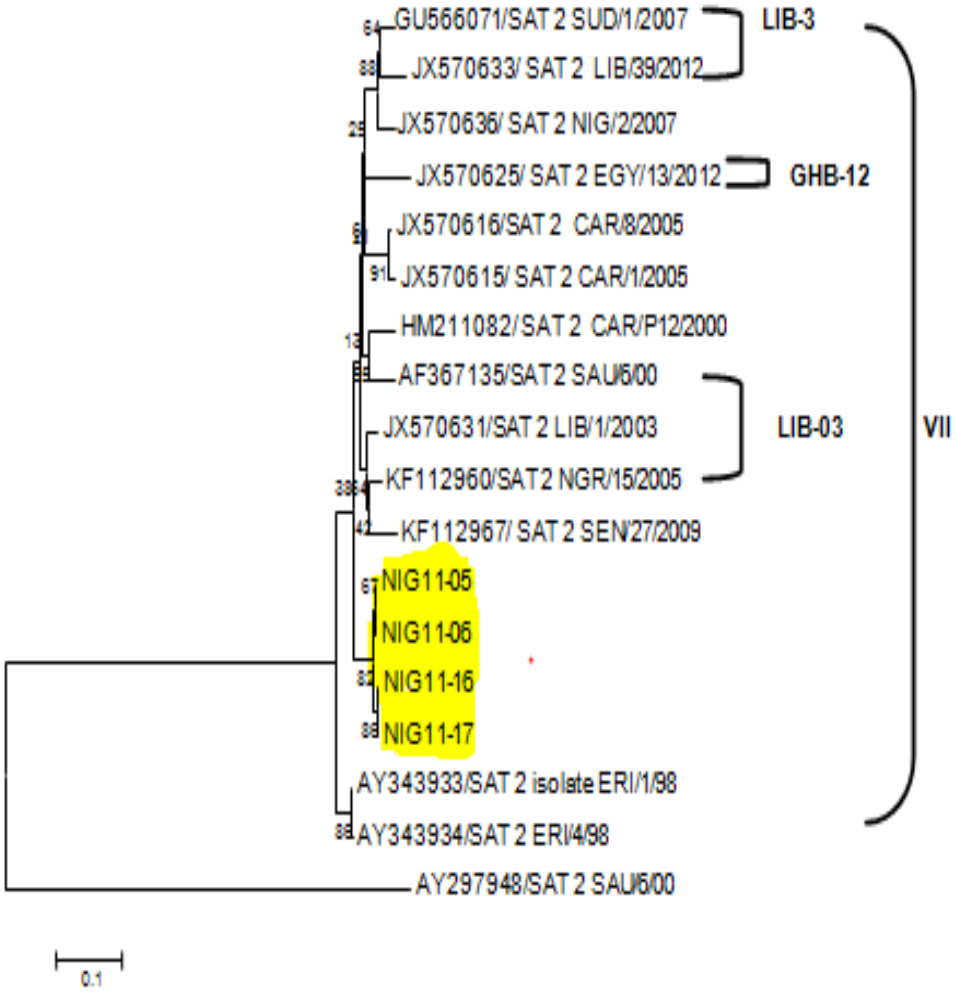

Fig.5.3. Midpoint-rooted maximum likelihood tree showing the relationships between the serotype SAT 2 viruses collected from Nigeria and other SAT 2 strains viruses from Africa countries.

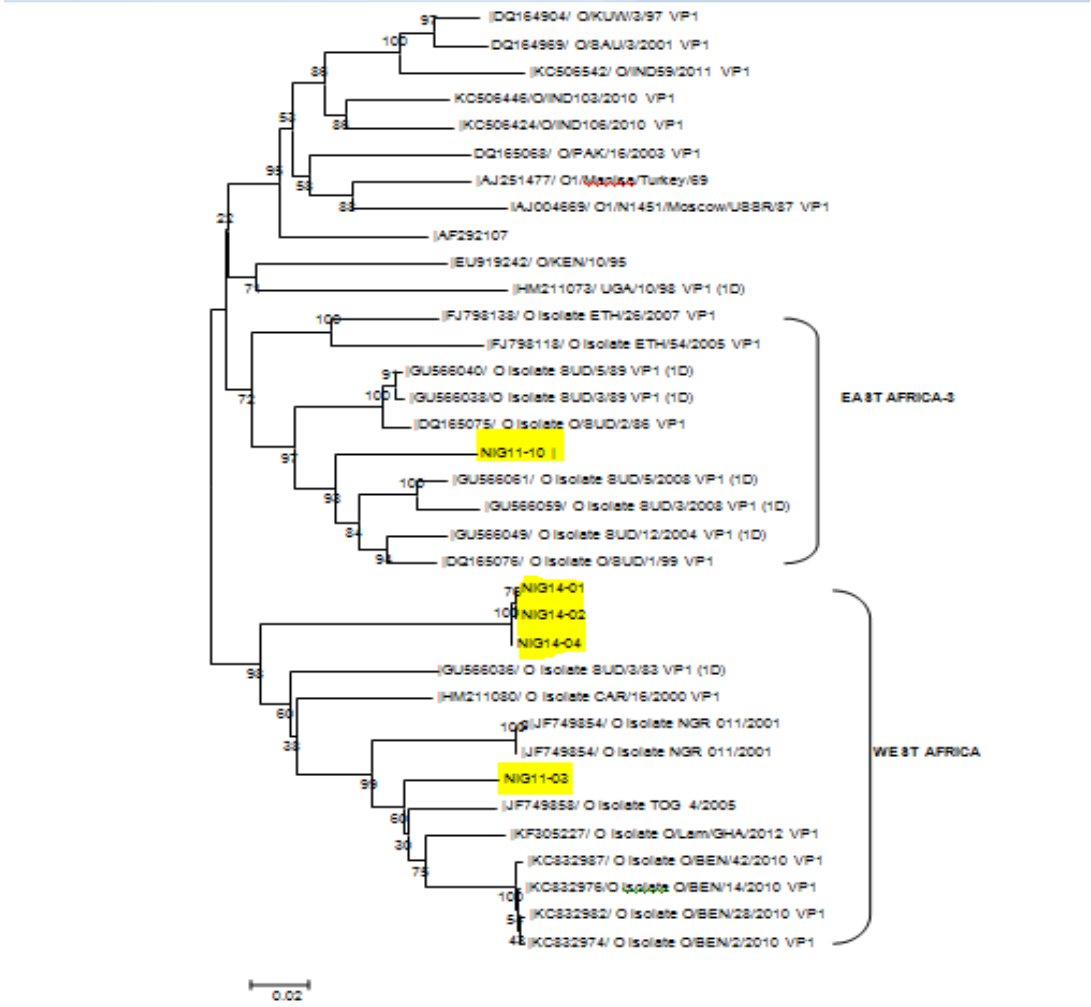

Fig.5.4. Midpoint-rooted maximum likelihood tree showing the relationships between the serotype O viruses collected from Nigeria and other serotype O strains viruses from Africa countries. Nt:639

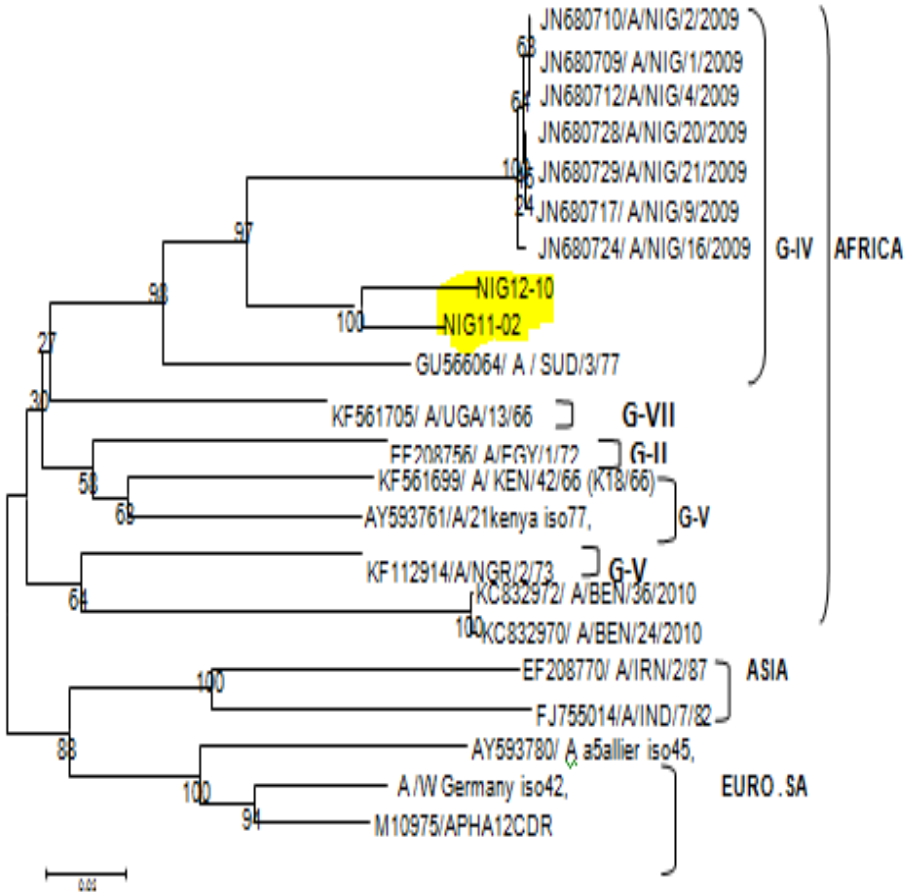

Fig 5.5 Midpoint-rooted neighbor-joining showing the relationship between the VP1 sequences of serotype A strains isolated in Nigeria and the closest VP1 sequences of type A from West, East African and Europe available in the GenBank database and the reference topotypes A. G-VII

5.4. Discussions

FMDV viruses are split into topotypes and lineages, within each serotype. Virus strains are placed into different topotypes based on geographical location and on having approximately 85% identity in the VPI protein (for SAT viruses 20% identity) (Ludi *et al.,* 2014).Topotypes are further classified into lineages based on sequencing and geographical region.

One distinct evolutionary lineage was identified by sequencing the VP1 gene of four FMDV SAT 2 serotype. A unique West Africa lineage has also been reported for FMDV serotype O FMD viruses, all West African viruses were shown to constitute a single large evolutionary lineage covering a period of 26 years (Knowles *et al.,* 1998).

Beck and Strohmaie.(1987) had used VP1 coding of FMD virus to investigate the relationship between the different isolates of the virus. It is assumed that viruses with < 5% of nucleotide differences in the VP1 sequences are considered as closely related, while those with >15% as unrelated (Knowles and Samuel, 2003).

VP1 analysis of the North-Central Nigerian SAT 2 FMDV serotypes isolates compared with other sequences in the Genbank database revealed that these viruses were related to other SAT 2 viruses previously isolated in West Africa. The SAT 2 isolates from the study area belong to SAT 2 topotype VII.

For comparison of the genetic relationships of SAT 2 FMD viruses, sequences from different geographical locations were selected for this study.

Virus diversity among SAT FMDV serotypes, especially SAT 2 is known to be high, consequently, 14 geographical distinct topotypes have been identified (Bastos *et al.,* 2003).

In this study, the complete VP1 region was amplified by RT-PCR and sequenced. The result showed that FMD virus serotype SAT 2 from North Central. Nigeria belongs to topotype VII of FMDV serotype SAT 2. The FMDV belonging to this topotype had been identified previously in African countries including Nigeria, Cameroon, Sudan, Liberia,

Uganda, Egypt, and Eritrea. The Nigerian viruses appeared to comprise only one topotype. Though Nigeria SAT 2 strains have not been named, it is believed that it is the same strain that has been circulating. It should be noted that isolates SAT2/NIG/16/2011 and SAT2/NIG/16/2011 were recovered from the same cattle herd in Bokkos Local Government Area (LGA) of Plateau State while isolates SAT 2/NIG/5/2011 and SAT 2/NIG/6/2011 were also recovered from the same cattle herd in Kanam Local government of Plateau State. The serotype SAT 2 viruses isolated from the same cattle herd i.e within herd diversity were 100% similar to each other while the kanam and Bokkos isolates were also closely related to each other (99.23-100% identity) indicating the circulation of the same strain. When compared with VP1 sequences available in the Genbank, all the four virus isolates clustered with virus isolates from Eritrea (1998), Libya (2003), Niger (2005) and Senegal (2009). This data suggest the possibility of new introduction of SAT 2 into Nigeria, as well as the persistence within the country and within West Africa. Comparison of these isolates with the SAT 2 virus that caused an outbreak in Nigeria in 1982 and 2007 showed 91.0% and 82.0% sequence identity respectively. The phylogenic distance between these two isolates <0.77%, indicated that the same virus was responsible for the outbreaks that occurred in the state in 2011. The spatial relationship between the two isolates from Bokkos and Kanam, which are about 200 km apart, can be explained by the uncontrolled movement of the pastoralist and their cattle especially during dry season in search of pasture and water. Fasina *et al*. (2013) stated that during cattle movement, herds share common watering points on trek routes permitting for direct and indirect contacts and for FMDV transmission to occur between cattle herds. This study revealed that they same FMDV serotype SAT 2 strain may have been circulating over the years in the study area and other parts of Nigeria and West Africa as a whole.

The complete VP1 sequence and phylogenetic analysis of FMDV serotype O isolates revealed that these viruses fall within West Africa (WA) topotypes and East Africa topotypes (EA-3).

The serotype O isolates from the same location in Plateau State were closely related to each other (99.84-100% identity) indicating the same virus circulating. The close genetic relationship between the isolates from Plateau state with sequences from Adamawa State

(98.1%) indicate that that same strain was circulating probably through uncontrolled cattle movement. However, the virus from Kogi State is less similar when compared with isolates from Plateau state (86.2%) sequence identity.

When these sequences were compared with other VP1 sequences available in the Genbank isolates from Plateau State clustered with sequences from Cameroon (2005) and isolate from Kogi clustered with sequences from Togo (2004), Ghana (2012) and Benin (2010). However, the virus isolate from Benue State clustered with EA-3 topotype from Sudan (2005) indicating that the viruses could have a common ancestor or origin. The isolates recovered from Kara cattle market in Plateau State showed a limited degree of variation in the VP1 gene, with values of greater than 99.3% genetic similarity or relatedness among them. Furthermore, the comparison with the nucleotide sequences recovered from a cattle herd in Kogi State in 2011 shows nucleotide relatedness with a nucleotide identity of less than 13.93%. The result shows that, it is the same topotype that has been circulating and it is responsible for causing many outbreaks in the past years in the study area. It has been stated that the differences in the genetic sequences of the FMDV of the same serotype do not necessarily reflect differences in antigenicity (Esterheysen, 1994). Despite the large divergence or nucleotide differences in genetic sequences between an isolate from Plateau and Kogi states, it may not have altered the antigenic specificity of the FMDV isolates (Mateu *et al.,* 1990). The observed difference in nucleotide sequences may have to do with the usual mutation of this virus during replication over the period of 3 years of circulation. The genetic relatedness between the isolates that caused an outbreak in Kogi State and the isolates that circulated in Togo 2004, Ghana 2012 and Benin 2010 with nucleotide difference of less than 5.32-7.20% identity explains a trans-boundary transmission of these viruses across West African sub-region. This also shows that this serotype O strain could be responsible for most of the outbreaks due to type O virus in the West African region in the past 7 years. The findings of this study have corresponded with a previous study (Fasina *et al*., 2013; Gorna *et al*., 2014) which confirmed the persistent circulation of this serotype O virus in the field. However, the isolate from Benue State belong to a different topotype (East African -3), and this cluster with the FMDV isolates that caused outbreaks in Sudan (2005 -2004). The detection of new topotypes in addition to the historic West African topotypes presents an additional challenge to the control of FMD by vaccination in Nigeria

as only vaccine formulated using the incorporation of these two topotypes will provide adequate protection against the serotype O virus in Nigeria. Most of the beef meat eaten in Nigeria came from the neighboring countries of Cameroon, Niger and Benin Republic and Bronsvoort *et al.(*2004) had noted that a well-known cattle trade exist between Sudan and Cameroon on the border with the Central Africa Republic. This probably explains the route for the introduction of the East African topotype-3 to Nigeria. This topotype was previously only known to exist in the East African countries.This data indicate the introduction of two separate serotype O/EA-3 and WA viruses into Nigeria, as well as the persistence of this topotypes within the country from 2009-2014.

This study has also provided relevant information in identifying the trans-border transmission of FMDV. The highlight here has further underscored the need to implement a collaborative control program between neighboring West Africa and Central African countries in order to control the disease.

The phylogenic analysis showed that FMD viruses serotype A recovered from cattle herd in Kogi and Plateau states fall into a single group or topotype (Africa typotype and belongs to genotype G-IV). The African strains of serotype A have been classified as Africa topotype (Knowles and Samuel, 2003a).

FMDV of serotype A were detected in Barkin Ladi LGA of Plateau state and Kabba LGA of Kogi State. The 2012 FMDV isolates (ANIG/10/2012) from Barkin Ladi showed closest genetic relationship (< 3.91-4.07 nucleotide difference) with the isolates that caused the outbreak in the same locality in 2009 (A/NIG/36/2009, A/NIG/38/2009) (Ehizibolo *et al.,* 2014). This is an indication of very little genetic variation in the nucleotide sequence of this virus despite 3 years of circulation in the same locality. This observed difference in the genetic sequence of less than 3.9-4.1 % is as a result of the normal biodiversity change or mutation with time that should be expected from viruses circulating in an endemic region.

The close genetic relatedness of isolates from Plateau State with sequences from Bauchi State (94.7 %) is probably due to free movement of cattle herds across the states. FMDV isolates serotypes A recovered from Kogi State in 2011 (A/NIG/2/2011) showed very close genetic relationship (< 4.54 % nucleotide difference) with the isolates recovered in cattle

herds in Barkin Ladi in 2012, and the virus isolates revealed even greater relationship (<3.13% difference) with the isolates recovered in Barkin Ladi in 2009.

Two Nigerian serotypes A isolates were compared with serotype A sequences available in the Genbank data base .The result revealed that they are related to strains from Cameroon A/CAR/15/2000, 92-93% sequence similarity, Cameroon, 93% similarity (Vne/126/01/VP1) and Togo A/TOG/5/2005 90% similarity, and Sudan A/SUD/3/77 with 87.64% similarity. This result indicated a trans-boundary movement of this virus across the region mostly through uncontrolled cattle movement and trade opportunities.

Overall, these results indicate a diverse range of viruses circulating among multiple states within Nigeria (Figure 1,2,3). There appears to be long-distance east-to-west movements of serotype O/EA-3, as well as the persistence of O/WA, A and SAT 2 circulating within Nigeria and West Africa. Long-distance movement of FMDV has been documented previously, including O/EA-4 in Ethiopia (Ayelet *et al.,* 2009) and SAT 2 in Egypt and the Middle East (Ahmed *et al*., 2012). Given the transhumance and pastoralism traditional practices in many parts of Africa, including Nigeria, and that animal movement across borders among many sub-Saharan countries are uncontrolled, there are no restrictions on human and animal movements and also due to the lack of animal movement records, it is difficult to determine the source of outbreaks or to trace the transmission of the disease over time.

This study has given an insight into the complex epidemiology of FMD in Nigeria, these findings can be used by veterinary authorities in Nigeria and the surrounding countries for vaccine candidate selection and FMD control programme initiatives.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

6.1 Conclusion

This study has demonstrated that FMD is an important socio-economic disease of cattle in North-Central, Nigeria with high prevalence of the disease reported. The study detected the co-circulation of serotypes A, O SAT 1 and SAT 2 in the study area. The detection of Serotypes O East African- 3 topotype in the study area reveals an evidence of trans-boundary virus transmission from East Africa probably through cattle movements or trade. Husbandry systems, cattle movement and cattle mixing at watering points were identified as some of the major risk factors associated with the transmission and establishment of FMD in North-Central, Nigeria.

Therefore, the control of FMD in Nigeria is very significant to ensure food security. However, control of FMD by vaccination seems the best option, as animal movement control will be difficult to achieve in view of the traditional pastoralist system and lifestyle. Use of broadly cross-protective vaccines comprising of all the circulating serotypes of Foot and Mouth disease viruses (circulating locally) is recommended as variant viruses could be introduced from neighbouring regions. Not only a good vaccine quality and matching antigens are needed, but also a good delivery system and effective vaccination coverage and frequency of vaccination. This would go a along with active participatory support from the farmers, governmental decision makers and manufacturers of vaccine to make FMD control in Nigeria a success.

This study has given an insight into the complex epidemiology of FMD in Nigeria; These findings can be used by veterinary authorities in Nigeria and the surrounding countries for current and future FMDV control and eradication initiatives.

6.2 Recommendations

- Project towards the production of polyvalent vaccine (containing the field isolates serotypes O, A SAT 2 and SAT 1) should be supported by the Government.
- Control and prevention policy for FMD should be formulated and implemented in Nigeria, as presently no such policy exits.
- An all inclusive nationwide FMDV control strategy along the OIE/FAO progressive control pathway for FMDV control should be initiated. This, when implemented would effectively reduce the occurrence and transmission of FMDV which in the long run, would improve national economies and food security and protect livelihoods.
- Study to determine the possible role of wildlife and small ruminants in the epidemiology of FMD in the study area is strongly recommended

6.3 Contribution to knowledge

- This study has confirmed the presence of four FMDV serotypes known co-circulating in Nigeria, namely; serotypes A, O SAT 1 and SAT 2.
- To the best of my knowledge, this study has provided the first comprehensive spatial distribution of FMDV serotypes in the North-Central Nigeria, indicating that only quadravalent vaccine which includes the local isolates containing serotypes A, O, SAT 1 and SAT 2 should be used for vaccination campaigns against FMD in Nigeria.
- The 71.0% sero-prevalence recorded in this study has provided an updated sero-prevalence status of FMD in the North-Central Nigeria.
- This present study has detected the encroachment of Serotype O East African-3 toptypes into Nigeria from the East Africa likely from Sudan through Cameroon.
- The study thus provided added information about the current epizootiological factors and events contributing to FMD outbreaks in Nigeria; these include:

    - Multiple serotypes of FMDV in circulation per time in a herd;

- Continued inter-herd transmission of serotypes, with country-wide implications;
- The study indicated an evidence of Trans-boundary transmission of FMDV across West Africa region.

- These isolates generated in this study can be used towards vaccine development.

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APPENDIX1

Questionnaire format for seroprevalence and associated risk factors determination

1. Background information:

State: L.G.A:

Geo. reference _____N:_____E :

Altitude_____

Owner's name_____

1. Age group

Age category <2 years: 2-4 years : >4 years:

2.2. Sex: Male: Female

2.3. Animal origin:

2.3.2. Introduced from other area yes _ No _

3. Herd composition

3.1 Herds of only cattle yes _ No _

3.2 Herds of cattle, Sheep and goats yes _ No _

4. Grazing habit of the livestock

4.2. Grazing all neighbors livestock together as one herd yes _ No _

4.3. Grazing house hold herd separately yes _ No _

4.4. Mixing at watering points yes _ No _

4.4. Herds not mixed at watering/watered at different site yes _ No _

5. Contact history to wild life

5.1. Have contact to wild animals usually yes _ No _

5.2. Have contact only rarely yes _ No _

5.3. Have no contact at all yes _ No _

6. Movement of animals:

6.1. Cross boundary border of Districts yes _ No _

6.2. Crosses boundary of Parks and sanctuary yes _ No _

6.3. Cross boundary of regional boundary yes _ No _

6.4. Cross boundaries of national boundary yes _ No _

7. Farming system of the area:

Nomadic _ Sedendary _ Intensive _

Investigated and completed by

Name _____

Date _____

(Day/month/year)

Signature _____

APPENDIX 2

ELISA RESULT

FMD 3ABC ELISA Results

Measurement count: 1 Filter: 450 Plate 1

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.601	0.602	1.059	1.326	0.116	0.620	0.058	0.744	1.391	0.095	0.731	1.697
B	1.639	0.104	1.299	1.460	1.166	0.768	0.863	0.805	0.644	0.592	1.179	1.665
C	0.516	0.839	0.805	1.068	1.021	1.172	1.208	0.464	0.126	0.191	0.433	1.644
D	0.476	1.443	1.086	0.186	0.457	0.565	1.469	0.251	0.996	0.625	0.131	1.666
E	0.168	0.584	0.681	0.517	0.081	0.398	0.729	1.366	1.313	0.337	0.663	1.690
F	0.180	0.789	0.306	0.150	0.179	0.619	0.418	0.283	0.510	0.207	0.934	1.686
G	0.521	1.306	0.740	0.532	0.112	1.303	1.571	1.329	1.418	0.506	1.197	1.709
H	1.464	1.376	1.041	1.004	1.142	0.458	0.787	0.124	0.291	1.052	1.647	1.653

Measurement count: 1 Filter: 450 Plate 2

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.571	1.254	0.649	0.116	1.523	1.130	0.343	1.391	1.411	0.571	0.778	1.079
B	1.610	0.122	0.199	1.169	0.260	1.325	0.408	0.199	0.114	1.459	0.214	1.434
C	0.574	0.281	0.852	0.243	0.935	0.296	1.307	1.233	1.253	1.177	0.543	1.381
D	0.578	1.238	0.364	0.332	1.343	1.192	1.218	0.177	0.923	1.285	1.335	0.836
E	0.217	0.298	0.101	0.086	0.842	0.367	0.353	1.269	0.100	0.054	0.078	0.054
F	0.207	0.410	0.438	1.265	0.081	1.225	1.124	0.135	0.110	0.889	0.786	1.222
G	1.327	0.507	0.763	0.579	0.907	0.863	0.689	0.113	1.291	0.558	1.129	0.087

H	0.769	0.087	0.110	0.104	1.093	1.218	1.427	0.969	1.557	1.024	0.172	0.313

Measurement count: 1 Filter: 450 Plate 3

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.636	0.222	0.331	0.437	0.746	0.910	1.049	0.778	0.449	0.093	1.622	0.599
B	1.636	1.123	0.628	0.446	1.582	0.172	0.281	0.058	0.077	0.351	0.074	0.647
C	0.503	1.303	0.148	1.370	1.040	0.082	1.387	0.890	0.207	1.168	0.889	0.312
D	0.488	0.417	0.080	0.054	0.226	1.119	0.769	0.981	0.811	0.863	0.929	0.731
E	0.272	1.048	1.464	1.417	0.335	0.081	0.644	1.383	1.114	0.941	1.443	1.365
F	0.195	0.616	0.761	0.386	1.437	0.283	0.252	0.094	0.646	0.080	0.061	0.347
G	0.343	0.672	0.150	0.493	1.071	0.274	1.316	0.136	1.057	0.126	0.104	1.172
H	0.318	0.363	0.177	0.482	0.156	1.207	0.610	0.665	0.404	0.062	0.159	0.290

Measurement count: 1 Filter: 450 Plate 4

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.811	0.794	1.201	1.274	0.103	0.608	0.073	0.957	0.077	0.188	0.282	0.467
B	1.783	0.731	0.223	0.857	0.125	0.402	0.872	0.581	0.069	0.177	0.543	0.114
C	0.543	0.080	1.255	0.207	0.684	0.745	0.106	1.116	0.060	0.334	0.066	0.065
D	0.586	0.946	0.541	0.353	1.066	0.091	0.077	0.497	0.974	0.133	0.601	0.136
E	0.190	0.239	0.753	0.063	0.301	0.337	0.062	0.449	0.059	0.675	0.843	0.220
F	0.218	0.101	1.005	0.195	1.541	1.027	0.077	0.122	0.811	0.053	1.266	0.702
G	0.059	1.322	0.075	0.099	0.062	0.125	0.112	0.134	0.080	0.601	0.050	0.139

H	1.531	0.150	0.322	0.453	0.526	0.906	0.121	0.083	0.120	0.154	0.189	0.058

Measurement count: 1 Filter: 450 Plate 5

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.713	0.118	0.099	1.132	0.173	0.186	0.114	1.133	0.322	0.112	1.484	0.311
B	1.733	0.062	0.058	1.508	0.062	0.069	0.051	1.342	0.067	1.302	1.168	0.164
C	0.599	0.280	0.115	0.902	0.291	0.071	1.105	0.842	0.096	0.211	1.052	0.893
D	0.583	0.105	0.991	1.196	0.098	0.094	1.032	1.289	0.073	1.228	0.079	0.065
E	0.210	0.065	0.900	1.160	0.171	0.227	0.132	0.664	0.893	1.051	0.094	1.636
F	0.213	0.128	1.146	0.935	1.412	0.688	0.939	0.111	1.379	0.121	0.974	0.060
G	1.123	0.052	1.348	1.638	0.150	0.093	0.099	0.078	1.166	1.226	1.322	1.524
H	0.064	1.239	1.258	0.082	0.947	0.499	0.198	0.111	1.339	1.304	1.168	0.120

Plate 6

Measurement count: 1 Filter: 450

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.743	1.266	1.599	0.637	0.964	0.323	0.233	0.930	0.143	0.261	1.111	0.958
B	1.703	0.389	0.191	1.667	0.601	0.904	0.874	0.100	0.880	0.901	1.537	0.688
C	0.532	0.114	0.891	1.508	1.367	0.694	0.827	0.834	1.140	1.492	0.779	0.733
D	0.529	0.174	1.261	0.135	0.070	0.288	0.179	1.052	0.635	0.201	0.246	1.473

E	0.206	0.193	0.050	1.198	0.177	0.372	1.533	0.079	0.074	0.200	0.812	0.678
F	0.213	0.139	0.922	0.459	0.117	0.991	0.625	0.854	1.208	1.689	1.431	0.692
G	0.582	0.084	1.156	0.699	0.356	0.989	0.589	0.880	0.960	0.791	0.109	0.160
H	1.284	0.400	0.464	1.218	0.841	0.838	1.162	0.456	0.135	1.369	1.319	0.147

Niger A Plate 1

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1.845	0.818	0.508	1.194	0.574	0.247	0.092	0.224	0.295	0.412	0.127	1.479
1.808	0.106	0.232	0.062	0.116	1.196	0.728	0.285	0.87	0.108	0.06	0.058
0.622	0.215	0.974	1.024	0.622	0.098	1.388	0.611	0.3	0.207	0.366	0.652
0.599	0.711	0.047	0.05	0.674	0.883	0.278	0.092	0.308	1.036	0.123	0.707
0.141	1.101	1.158	0.113	0.182	0.586	0.054	0.099	0.774	0.482	0.408	0.181
0.213	0.44	0.126	0.159	0.873	0.571	0.308	0.177	0.254	0.119	0.304	0.89
1.567	0.337	0.516	0.964	0.118	0.829	0.089	0.648	0.462	0.223	1.285	0.303
0.327	1.021	0.452	0.142	0.806	0.111	0.95	0.694	0.237	0.061	0.051	1.031

Plate 2

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

	1.61	0.177	0.544	0.651	0.17	0.27	0.234	0.806	0.574	0.206	0.996	0.056
	1.633	0.48	0.059	0.262	0.197	0.412	1.294	0.729	0.721	0.071	1.144	0.092
	0.526	0.137	0.047	0.42	0.058	1.083	0.123	0.312	0.474	0.564	0.943	0.108
	0.527	1.021	0.067	0.699	0.989	0.602	0.058	0.19	1.302	0.98	0.859	0.226
	0.174	0.704	0.923	0.789	0.094	0.498	0.545	0.643	0.522	0.152	0.624	0.873
	0.166	1.36	1.088	0.274	0.378	0.404	0.105	0.063	0.173	0.062	0.081	0.573
	0.888	0.997	0.055	0.391	0.324	0.055	0.245	1.092	0.51	0.049	0.096	0.95
	0.173	0.166	0.293	0.064	0.333	0.057	0.06	0.165	0.101	0.32	0.369	0.143

PL1 ZONE B

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.936	0.489	0.497	0.358	0.213	0.337	0.498	1.188	1.566	0.110	1.420	0.071
B	1.863	1.151	1.287	1.322	0.953	1.115	0.094	0.578	0.117	1.435	1.467	0.536
C	0.655	0.614	0.129	0.072	0.089	0.056	0.301	0.078	0.543	0.076	0.372	1.234
D	0.706	0.576	0.559	0.248	0.127	1.384	0.909	0.114	1.163	1.147	1.369	0.088
E	0.197	1.244	0.576	1.086	0.198	0.490	0.356	0.812	0.050	0.147	0.078	0.198
F	0.365	1.221	0.232	0.078	0.444	1.254	0.350	0.084	0.883	0.633	0.478	0.113
G	0.157	1.444	1.141	0.254	0.096	0.175	0.098	0.536	0.424	0.301	0.890	0.069
H	0.073	0.357	0.065	1.171	1.556	0.210	0.496	1.138	0.278	0.803	0.080	1.643

PL2

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.822	1.062	0.076	0.045	0.600	0.075	0.133	0.135	0.126	1.101	0.151	0.193

B	1.784	0.286	0.073	0.088	0.138	0.133	0.425	0.292	0.120	0.054	0.060	0.597
C	0.689	0.075	0.352	0.330	0.202	0.304	0.327	0.063	0.382	0.432	0.094	0.124
D	0.752	0.068	0.087	0.052	0.230	0.070	0.134	0.068	0.340	0.414	0.304	0.115
E	0.295	0.070	0.769	0.647	0.400	0.074	0.380	0.427	1.302	0.214	0.149	0.077
F	0.296	0.248	0.062	0.068	0.260	0.069	0.063	1.397	0.396	1.294	0.075	0.740
G	1.211	0.071	0.466	0.693	0.365	0.186	0.454	0.860	0.204	0.777	0.070	0.261
H	1.642	0.797	0.085	0.681	0.229	0.295	0.243	0.739	0.118	0.211	0.130	0.595

PL3

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.814	0.155	0.161	0.154	0.111	0.815	0.200	0.852	1.241	0.361	0.269	0.653
B	1.789	0.503	0.120	0.121	0.230	1.431	0.210	0.107	1.393	0.889	0.406	0.212
C	0.815	0.173	0.118	0.586	0.207	0.559	0.783	0.623	0.914	1.538	0.225	0.061
D	0.849	0.118	0.414	0.086	0.169	0.849	0.096	0.131	0.340	1.087	0.186	0.470
E	0.310	0.243	0.164	0.566	0.059	0.564	0.811	0.206	1.289	1.147	1.311	0.867
F	0.321	0.318	0.259	0.739	0.101	0.356	0.331	0.345	0.147	0.201	0.171	0.090
G	0.151	0.171	0.101	0.393	0.065	0.471	0.260	0.982	1.034	1.035	1.028	0.582
H	0.203	0.767	0.327	1.289	0.076	0.518	0.325	0.746	0.879	0.515	1.188	0.115

ZONE C

PL 1

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.954	0.838	0.243	0.071	0.123	0.077	0.332	1.436	1.247	0.234	0.099	0.925
B	1.904	0.215	0.259	0.250	0.122	1.045	0.153	0.156	0.047	0.284	0.551	0.089

C	0.534	0.127	0.257	0.094	1.111	0.441	0.091	0.369	0.315	0.149	0.122	0.229
D	0.597	0.078	0.111	0.076	0.090	0.173	0.144	0.222	0.110	0.901	0.314	0.153
E	0.182	0.127	0.170	0.198	0.203	0.195	0.679	0.219	0.395	0.071	0.067	0.123
F	0.140	0.064	0.066	0.125	0.068	0.276	0.139	0.907	0.063	1.203	0.097	0.078
G	0.897	1.089	0.109	0.105	0.176	0.148	0.806	0.202	0.624	1.134	0.341	0.249
H	0.402	0.867	0.683	0.165	0.168	0.361	0.169	0.803	0.151	0.712	0.426	0.158

PL 2

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.121	0.715	0.115	0.102	0.388	0.984	0.172	0.096	0.169	0.134	0.090	0.232
B	1.732	0.326	0.075	0.399	0.123	0.779	0.328	0.077	0.097	0.077	0.891	1.445
C	0.441	0.088	0.442	0.071	0.435	2.036	0.292	0.073	0.597	0.077	0.472	0.074
D	0.553	0.321	0.095	0.644	0.093	0.126	0.466	0.096	0.421	0.375	1.101	0.080
E	0.210	0.195	0.157	0.146	0.135	0.198	0.106	0.692	1.192	1.035	0.458	0.248
F	0.233	0.391	0.801	0.101	1.024	0.334	1.034	0.175	0.828	0.642	0.610	0.655
G	0.287	0.169	0.113	0.252	0.173	0.100	0.128	0.605	0.063	1.097	0.216	0.509
H	0.227	0.403	0.266	0.281	0.150	1.268	0.108	0.286	0.349	0.177	0.145	0.113

Plateau JS Plate 7

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.716	1.652	1.667	1.471	1.220	1.537	1.550	1.294	0.191	0.864	1.351	1.490
B	1.800	1.718	1.348	1.494	1.324	1.233	1.575	1.319	1.495	1.473	0.097	1.317
C	0.632	1.521	1.524	1.522	1.159	1.341	1.395	1.570	1.472	1.277	0.239	1.202
D	0.554	1.272	1.351	1.527	1.582	1.448	0.933	1.563	1.167	0.070	1.369	1.406
E	0.194	1.550	1.556	1.459	1.571	1.280	1.621	1.158	1.257	1.239	0.477	1.423
F	0.169	1.574	1.475	1.315	1.536	1.593	1.374	0.766	1.360	1.111	1.193	0.907

G	1.752	1.339	0.932	1.275	1.056	1.316	1.375	0.180	1.388	1.205	1.301	1.463
H	1.471	1.124	1.269	1.509	1.617	1.545	1.283	0.145	1.466	1.074	1.444	0.199

ELISA Result For FMDV Serotypes Detection

Measurement count: 1 Filter: 450 Type A Plateau

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.049	2.197	0.732	0.119	0.307	0.149	0.237	0.337	0.372	0.282	0.752	1.018
B	0.054	1.012	0.321	0.076	0.055	0.073	0.378	0.184	0.608	0.200	0.574	0.787
C	1.001	0.057	0.083	0.078	0.047	0.057	0.321	0.139	0.920	0.060	0.413	0.684
D	1.088	1.149	0.204	0.136	0.557	0.095	0.087	0.781	0.428	0.118	0.337	3.226
E	1.017	0.145	0.146	0.266	0.452	0.141	0.095	0.051	0.056	0.632	0.673	1.372
F	1.109	0.063	0.191	0.275	0.823	0.165	0.726	0.173	0.059	0.170	0.652	1.385
G	0.085	0.595	0.412	2.445	0.262	0.047	0.566	0.098	0.120	0.063	0.704	1.092
H	0.619	0.426	0.152	0.961	0.081	0.113	0.283	0.119	0.096	0.047	0.725	1.571

Measurement count: 1 Filter: 450 Type O Plateau

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.087	0.413	0.545	0.060	0.194	0.193	0.412	0.060	0.727	0.151	1.174	1.322
B	0.082	0.362	0.238	0.120	0.174	0.578	1.030	0.346	0.934	0.144	1.230	1.138
C	1.659	0.046	0.153	0.612	0.189	0.412	0.464	0.112	1.059	0.587	0.858	1.107
D	1.736	0.374	0.413	0.469	0.901	0.169	0.074	0.490	0.150	0.973	1.009	1.060
E	3.162	0.096	0.292	0.317	0.717	0.084	0.229	0.084	0.089	0.760	1.027	1.579
F	1.719	0.340	0.588	0.079	0.663	0.568	0.169	0.119	0.193	0.147	0.817	1.481
G	0.182	1.173	0.233	0.188	0.059	0.093	0.817	0.044	0.051	0.634	1.059	1.419
H	1.126	0.695	0.717	1.038	0.114	0.591	0.311	0.521	0.050	0.079	0.968	1.647

Measurement count: 1 Filter: 450 SAT-1 Plateau

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.159	1.965	1.069	0.839	2.125	1.222	0.933	0.136	2.327	0.191	2.574	2.176
B	0.142	0.483	2.348	0.439	0.930	1.204	2.254	0.706	2.221	0.079	1.443	1.771
C	2.092	0.554	1.784	0.073	2.410	0.270	0.162	0.874	1.998	1.159	0.963	1.992
D	2.056	1.644	2.092	1.615	1.027	1.013	0.738	1.082	0.631	2.034	0.115	2.062
E	2.173	0.676	0.395	2.069	1.697	0.602	0.417	0.323	0.070	0.700	1.996	2.282
F	2.114	1.299	1.897	0.646	0.781	0.234	0.789	0.196	1.302	0.094	1.701	2.518
G	0.398	2.637	1.533	0.443	0.304	0.549	1.671	0.072	0.247	0.554	1.755	2.426
H	1.362	0.797	1.805	2.371	0.536	0.802	2.347	0.654	0.664	1.017	1.892	2.281

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.186	0.845	0.908	0.261	0.300	0.647	0.719	0.168	1.070	0.181	0.287	0.225
B	0.231	0.325	1.087	0.201	0.184	0.374	0.999	0.485	0.458	0.097	0.263	0.161
C	1.068	0.286	0.271	0.863	0.731	0.584	0.275	0.138	0.245	0.195	0.366	0.159
D	0.971	0.594	0.280	0.753	0.808	0.638	0.541	0.536	0.411	0.196	0.386	0.153
E	1.118	0.810	0.957	0.516	0.245	0.594	0.909	0.277	0.258	0.938	0.295	0.510
F	1.095	1.209	0.599	0.757	0.529	0.745	0.233	0.955	0.193	1.422	0.206	0.315
G	0.292	0.423	0.460	0.279	0.614	0.306	1.100	0.322	0.147	0.147	0.118	0.278

H	0.590	0.951	1.139	0.723	0.590	0.407	0.354	0.243	0.320	0.601	0.635	0.238

Measurement count: 1 Filter: 450 SAT-2 Niger

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.613	0.408	0.483	0.789	0.386	0.365	0.176	0.087	0.739	0.079	0.459	0.372
B	1.791	0.261	0.514	0.174	0.118	0.779	0.490	0.209	0.095	0.326	0.774	0.183
C	1.621	1.108	0.604	0.238	0.344	0.132	0.705	0.248	0.325	0.264	0.400	0.762
D	1.703	0.155	0.295	0.500	0.343	1.212	0.268	1.534	0.512	0.307	0.556	1.285
E	0.208	0.238	0.122	0.149	0.301	0.849	0.165	0.099	0.845	0.768	0.576	0.233
F	0.234	0.247	0.257	0.385	0.347	0.737	0.479	0.187	0.542	0.548	0.303	0.212
G	0.277	0.379	0.338	0.520	0.515	1.173	0.074	1.466	0.614	0.235	0.510	0.760
H	0.219	0.811	0.211	0.190	1.044	0.979	0.166	0.172	0.333	0.539	0.351	0.059

Measurement count: 1 Filter: 450 SAT-1 Niger

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	2.675	2.519	1.753	0.850	0.641	1.046	1.761	2.120	1.376	2.285	0.774	0.123
B	2.715	1.589	2.375	1.695	0.810	1.208	0.487	1.947	1.029	0.795	1.137	0.116
C	2.679	1.461	1.211	1.318	0.180	0.909	0.863	1.585	1.656	1.387	0.691	1.664
D	2.674	2.513	1.583	2.369	0.053	0.858	1.495	0.782	1.552	0.085	1.623	1.410
E	2.731	2.443	1.001	1.242	2.099	0.356	1.733	0.591	1.110	1.237	0.098	1.547
F	2.782	2.444	0.461	1.253	0.821	2.116	1.571	0.083	0.070	0.248	1.548	1.483

G	2.699	2.097	0.419	1.087	0.080	1.155	2.617	1.485	0.867	0.945	0.589	0.390
H	2.709	0.551	1.188	1.651	0.444	0.786	2.186	1.263	0.635	0.296	1.459	0.066

Measurement count: 1 Filter: 450 Type A Niger

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1.140	1.190	1.354	0.804	0.077	0.098	0.130	0.586	0.195	0.049	0.173	0.273
B	1.124	0.076	0.676	0.293	0.464	0.257	0.137	0.246	0.196	0.213	0.068	0.186
C	1.089	0.457	0.206	0.433	0.069	1.262	0.099	0.303	0.187	0.160	0.077	0.704
D	1.122	0.082	0.458	0.200	2.363	0.684	0.286	0.472	0.825	0.102	2.186	0.156
E	0.050	0.420	0.688	0.447	0.096	0.054	0.231	0.077	0.637	0.536	1.387	1.000
F	0.071	0.055	0.051	0.164	0.359	0.090	0.098	1.450	0.565	0.669	0.257	0.898
G	0.289	0.230	0.242	1.043	0.495	0.592	0.131	0.534	0.314	0.146	0.260	0.803
H	0.098	0.434	0.628	0.080	0.459	0.156	0.397	1.019	0.218	0.320	0.321	0.627

Measurement count: 1 Filter: 450 Type O

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.815	0.167	0.054	0.345	0.050	0.066	0.043	0.063	0.306	0.045	0.120	0.110
B	0.801	0.054	0.121	0.065	0.366	0.157	0.101	0.057	0.092	0.054	0.068	0.318
C	0.809	0.317	0.083	0.058	0.053	0.344	0.106	0.057	0.212	0.048	0.052	0.182
D	1.815	0.091	0.072	0.238	0.068	0.439	0.068	0.375	0.166	0.068	0.086	0.217
E	0.062	0.087	0.045	0.500	0.352	0.122	0.150	0.757	0.047	0.229	0.254	0.337

F	0.061	0.047	0.053	0.075	0.345	0.075	0.135	0.048	0.249	0.049	0.108	0.516
G	0.079	0.084	0.083	0.095	0.330	0.108	0.386	0.183	0.192	0.047	0.149	0.546
H	0.065	0.112	0.781	0.117	0.266	0.359	0.275	0.119	0.057	0.046	0.158	1.013

Solid Phase Competitive ELISA for Antigen detection

Results	Type O Mab		Type A Mab 4D12		Type A Mab 5F6		Pan-FMDV Mab 1F10		SAT 1		SAT2	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative
B	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
C	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative
D	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	WeaK	Negative	Negative
E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative
G	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID
H	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	Invalid	VALID	VALID	VALID

Results	Type O Mab		Type A Mab 4D12		Type A Mab 5F6		Pan-FMDV Mab 1F10		SAT 1		SAT2	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
B	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
C	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative
D	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
G	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID
H	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID	VALID

APPENDIX 3

Scientific contribution emanating from the thesis

Journal Publication

Wungak Y. S., Olugasa B. O., Ishola O. O, Lazarus D. D., Ularamu G. H: *Foot-and-mouth disease (FMD) prevalence and exposure factors associated with seropositivity of cattle in north-central, Nigeria*. AFRICAN JOURNAL OF BIOTECHNOLOGY 07/2016; 15(24):1224-1232

Yiltawe S.Wungak, Olayinka O.Ishola, Babasola O.Olugasa, David D. Lazarus, David O.Ehizibolo and Hussaini G.Ularamu:Spatial pattern of Foot-and-Mouth Disease Virus serotypes in North Central, Nigeria. Manuscript submitted to ActaTropica for publication.

Conference Proceedings

Wungak Y S, Olayinka I. O, Lazarus D D, Ularamu G H, Olugasa B O: *Multiple serotypes of foot-and-mouth disease virus antibodies in an endemic setting: Implications for disease control*. Global Foot and Mouth Disease Research Alliance, Scientific Meeting, Hanoi, Vietnam; 10/2015

Wungak Y S, Olayinka I. O, Lazarus D D, Ularamu G H, Olugasa B O: *Foot-and-mouth disease control options in parts of West Africa*. Global Foot and Mouth Disease Research Alliance, Scientific Meeting, Hanoi, Vietnam; 10/2015

Wungak Y S, Olayinka IO, Lazarus D D, Ularamu G H, Olugasa B O: *Seroprevalence and distribution of foot-and-mouth disease in North-Central Nigeria*. Global Foot and Mouth Disease Research Alliance, Scientific Meeting, Hanoi, Vietnam; 10/2015