SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE VIRUS IN RUMINANTS IN KADUNA AND PLATEAU STATES, NIGERIA

BY

DAVID ODIONEHIZIBOLO

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

MARCH 2016

SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE VIRUS IN RUMINANTS IN KADUNA AND PLATEAU STATES, NIGERIA

BY

David Odion EHIZIBOLO DVM, M.Sc (ABU)
(Ph.D/VetMed/15986/2010-2011)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

MARCH 2016

DECLARATION

I declare that the work in this thesis titled **"Serological and Molecular Epidemiology of Foot and Mouth Disease Virus in Ruminants in Kaduna and Plateau States, Nigeria"** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria under the supervision of Professors J.U. Umoh, H.M. Kazeem and E.C Okolocha. The information derived from the literature has been duly acknowledged in the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

David Odion EHIZIBOLO	.....	.....
Name of student	Signature	Date

CERTIFICATION

This thesis entitled "**Serological and Molecular Epidemiology of Foot And Mouth Disease Virus in Ruminants in Kaduna and Plateau States, Nigeria"** by David Odion EHIZIBOLO meets the regulations governing the award of the degree of Doctor of Philosophyin Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. J.U. Umoh, ---------- ----------
Chairman, Supervisory Committee, Signature Date

Prof. H.M. Kazeem, ---------- ----------
Member, Supervisory Committee, Signature Date

Prof. E.C. Okolocha, ---------- ----------
Member, Supervisory Committee, Signature Date

Prof. E.C. Okolocha, ---------- ----------
Head, Department of Veterinary Signature Date
Public Health and Preventive Medicine,
Ahmadu Bello University, Zaria.

Prof. K. Bala, ---------- ----------
Dean, School of Postgraduate Studies, Signature Date
Ahmadu Bello University, Zaria

DEDICATION

To the Almighty God,
My lovely wife and Children,
And all members of the EHIZIBOLOsfamily

ACKNOWLEDGEMENT

I am forever grateful unto the Almighty GOD for the gift of life, special favour, strength and support to be able to successfully complete this PhD degree programme. May Your name be exalted in my life forever.

My sincere appreciation goes to the management of the National Veterinary Research Institute (NVRI), Vom for the opportunity to undertake the study. To members of my supervisory committee; Professors J.U Umoh, H.M. Kazeem and E.C Okolocha, I say thank you very much for your support, encouragement and guidance. Your contributions were wonderful and sincerely cherished. May God continue to protect and support you in all your endeavours. I like to also acknowledge the valuable contributions of a supervisor that passed on during the course of this study, late Professor I. Ajogi. May his gentle soul rest in peace.

Special appreciation to Dr. Andres M. Perez of the University of California, USA, Dr. Samia A. Metwally of the Plum Island Animal Disease Center, USA and Dr Kris De Clercq, Dr. Annebel De Vleeschauwer, Dr. Andy Hageman, Dr. Jean-Baptiste Hanon, Dr. David Lefebvre all of the Veterinary and Agrochemical Research Center (CODA-CERVA), Belgium. I am so grateful for your sincere support, encouragement and commitment to the success of this work. Even when I thought all hope was lost, you gave me assurance, solidly stood by me and ensured that I succeeded. I will never forget your kindness. Thank you, God will reward all your sacrifices and patience.

To my lovely wife, Blessing and children, Ehijiator, Onome, Oseme and Obehi, God bless you for your prayers, patience, understanding and love during the course of my study. May the merciful Lord continue to honor and uphold you. I love you all dearly.

My parents, Mr. and Mrs. Ehizibolo have been of immense support and encouragement. I thank you so much for your prayers, sound moral upbringing and lovely guidance. May the Lord continue to prosper and strengthen you by the day, and cause you to rejoice. Also, I appreciate my elder brother Dr. Emmanuel Ehizibolo and his family. You have been so wonderful, loving and supportive all through my study period, God will continue to exalt and enlarge your coast. To my twin brother Jonathan and his family, may the Lord bless you for all the prayers, best wishes and encouragement. Many thank to Eunice Ehizibolo, Dr. Peter Ehizibolo, Christopher Ehizibolo and Mrs. Christiana Aghedo my younger ones. May the Lord protect, prosper and honor you for your warm support and prayers. I love you all.

I am very grateful for the support and prayers of members of the Redeemed Christian Church of God, City of Glory parish. Your love and encouragement was instrumental tomy success.

To all my friends and colleagues, I say thank you so much for all the love and support you showed. I am very grateful to Dr. Grace Sabo Kia for the encouragement and kindness you showed during the course of this work. I appreciate the encouragement and kind support of Dr. (Mrs) M. Mohammad, Dr. Chukwu O. Chukwu, Dr. Reuben Ocholi, Dr. Anthony Chukwuedo and Dr. Chika Nwosu. I also cherish the sincere support of MallamAbdullahi of the Extension Department, NVRI, Vom. Thank you so much, and God bless you all in return.

Finally, I want to appreciate the entire Fulani pastoralists in Kaduna and PlateauStates for their cooperation and assistance in making this work a reality.

ABSTRACT

Foot and mouth disease virus (FMDV) has been known for centuries as one of the most fearful viral pathogens of cloven-hoofed domestic and wild animals, causing the foot and mouth disease (FMD), which exerts severe global economic impact on the livestock industry. FMD is endemic in Nigeria, with outbreaks which are mostly unreported occurring almost throughout the year in different parts of the country. The aim of this study was to investigate the epidemiology of FMD in Kaduna and Plateau States, Nigeria. Risk factors associated with FMD in the study areas were assessed via questionnaires administered to herdsmen. FMD prevalence was estimated using 3ABC enzyme-linked immunosorbent assay (3ABC ELISA) while antibodies against FMDV serotypes were detected by the solid phase competitive ELISA (SPCE) and the virus neutralization test (VNT). FMD viruses were isolated from ruptured epithelial tissue samples obtained from affected cattle in established cell layers and serotyped by antigen-capture ELISA. RNA was extracted from cell culture supernatant and the reverse transcription polymerase chain reaction (RT-PCR) was performed. The purified DNA amplicons were ligated into plasmid vector and pure clones were sequenced. The complete VP1 sequences were used for phylogenetic analysis. Farms with suspected FMD nearby, with contact with wildlife or with >100 animals, and animals of large ruminant species and in pastures other than nomadic grazing were significantly ($P<0.05$) associated with FMD. A 30.6% FMD sero-prevalence was estimated for Kaduna and Plateau States. Antibodies against five FMDV serotypes (O, A, SAT1, SAT2 and SAT3) were detected in Kaduna and Plateau States. Sixty FMDV isolates containing representative of serotypes O, A, SAT1 and SAT2 were recovered in Kaduna and Plateau States. The phylogenetic finding suggests these serotypes were genetically related to serotypes and topo types occurring in

West, Central, East and North Africa. It also indicates the identification of a new genotype of SAT1 in Nigeria. The epidemiology of FMD in Kaduna and Plateau States is complex because of the occurrence of multiple serotypes/subtypes of the virus detected and the levels of variation observed has significant implications for disease control in Nigeria. Farmer education is needful and vaccination of domestic livestock with appropriate vaccine strains is recommended to mitigate virus spread.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

.

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AgELISA	Antigen Enzyme-Linked Immunosorbent Assay
AGID	Agar Gel Immunodiffusion
ATPase	Adenosine triphosphatase
BIC	Bayesian Information Criteria
BTY	Bovine thyroid
CD	Cluster of Differentiation
CFT	Complement Fixation Test
CODA-CERVA	Veterinary and Agrochemical Research Centre
CPE	Cytopathic Effect
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of Congo
EA	East Africa
ELISA	Enzyme-Linked Immunosorbent Assay
ELTB	Electro Ttransfer Blot
EuFMD	European Union for the control of Foot and Mouth Disease
Euro-SA	Europe-South America
EUVB	European Union Vaccine Bank
FADDL	Foreign Animal Disease Diagnostic Laboratory
FAO	Food and Agriculture Organization
FLK	Fetal Lamb Kidney
FMD	Food and Mouth Disease
FMDV	Food and Mouth Disease Virus
HS	Heparin Sulphate
IBRS	Instituto Biologico Rim Suino

ICAM	Intercellular Adhesion Molecule
IL	Interleukin
INTA	National Institute of Agricultural Technology
ISA	Indonesia
IVB	International Vaccine Bank
KDSG	Kaduna State Government
KGR	Kachia Grazing Reserve
LAMP	Loop Mediated Amplification
LB	Luria Berteni
LGA	Local Government Area
LLR	Likelihood Ratio
LPBE	Liquid-phase Blocking ELISA
Mab	Monoclonal antibody
ME-SA	Middle East-South Asia
NAVB	North American Vaccine Bank
NSP	Non Structural Proteins
OIE	Organization International des Epizootics
OPD	O-phenylene-diamine dihydrochloride
ORF	Open Reading Frame
PABP	Poly (A) Binding Protein
PANAFTOSA	Pan-American Foot and Mouth Disease Centre
PBS	Phosphate Buffered Saline
PCP-FMD	Progressive Control Pathway for Foot and Mouth Disease
PIADC	Plum Island Animal Diagnostic Laboratory
PTBP	Polypyrimidine Tract Binding Protein

RF	Replicative Form
RNA	Ribonucleic Acid
rRT-PCR	Real-time Reverse Transcription-Polymerase Chain Reaction
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SAT	South African Territories
SEA	South-East Asia
SENASA	Servicio National de Sanidad Calidad Agroalimentoria
SP	Structural Proteins
SPCE	Solid Phase Competitive ELISA
SPSS	Statistical Package for Social Science
TAD	Transboundary Animal Disease
TAE	Tris acetate EDTA
TCID50	Tissue Culture Infective Dose 50
USA	United States of America
USD	United States Dollar
USDA	United States Department of Agriculture
UTR	Untranslated Region
VI	Virus Isolation
VIAA	Virus Infection-Associated Antigen
VN	Virus Neutralization
VNT	Virus Neutralization Test
VP	Virus Protein
WA	West Africa
WRL	World Reference Laboratory
WRLFMD	World Reference Laboratory for Foot and Mouth Disease

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Foot and mouth disease (FMD) is an acute, highly contagious viral infection of cloven-hoofed animals caused by foot-and-mouth disease virus (FMDV) typically characterized by a rapid febrile illness with development of massive vesicular lesion in the mouth and on the feet, causing considerable production losses in farm animals (Grubman and Baxt, 2004).Foot and mouth disease virus is a small, non-enveloped RNA virus that is classified as a member of the *Aphthovirus* genus within the *Picornaviridae* (Hedger, 1981; Carrillo *et al.*, 2005). Foot and mouth disease virus has been known for centuries as one of the most fearful viral pathogens of animals. The virus is distributed worldwide and can be transmitted by direct and indirect contacts via multiple routes including wind-borne transmission, exerting a severe global economic impact on the livestock industry (Alexandersen *et al.*, 2000; Sakamoto *et al.*,2002). Morbidity due tothe disease is nearly 100% among non-vaccinated animals. Although the disease is rarely fatal in adult animals (<5%); case-fatality rate in young animals may reach 50%, due to myocardial collapse (Sard, 1978).

Foot and mouth disease virus (FMDV) particles are naked icosahedrons comprising 60 copies each of four structural proteins VP1-4, encapsidating the viral genome which consists of an infectious molecule of single-stranded, positive-sense ribonucleic acid (RNA). The protein coding region is a continuous open reading frame of 6915 or 6999 nucleotides in length depending on which of two functional in-frame start codons is utilized. A polyprotein is synthesized from genomic RNA and processed by viral proteinases into four primary cleavage products, non-structural proteins (NSP) leader, Lab and Lb; structural proteins (SP) P1: P1A, 1B,

1C and 1D equivalent to VP4, VP2, VP3and VP1, respectively; NSP P2: (P2A, 2B and 2C) and NSP P3: P3A, P3B or VPg, P3C, and P3D (Lewis *et al.,* 1991; Knipe *et al.,* 1997). There are seven serotypes of FMDV and no cross-protection between the serotypes following infection or vaccination. Besides, there are considerable antigenic and genetic diversities within each serotype resulting in numerous subtypes (Carrillo *et al.,* 2005). Thus, an animal that has recovered from infection with one serotype becomes resistant to challenge by the same serotype but remains susceptible to infection by any other serotype (Belsham, 1993).

Foot and mouth disease virus serotypes are not uniformly distributed in regions of the world where the disease occurs. Six of the seven serotypes (O, A, C, South African Territories [SAT] 1, SAT 2, SAT 3) have occurred in Africa, four serotypes (O, A, C, Asia 1) in Asia and three (O, A, C) in South America and Europe (Rweyemamu *et al.,* 2008). FMD cannot be easily distinguished from other vesicular diseases by clinical findings only due to similarity of the clinical signs (Alexandersen *et al.,* 2000). Detection of FMDV with identification of the viral serotype must be rapid, sensitive and specific for effective disease control and proper selection of vaccines (Reid *et al.* 1999). Conventional laboratory diagnosis of FMD is achieved by concurrent cell culture virus isolation together with the enzyme-linked immunosorbent assay (ELISA) which indicates the type of viral antigen involved (Roeder and Le Blanc Smith, 1987; Ferris *et al.,* 1988; Mackay *et al.,* 2001) or by virus neutralization test (Golding *et al.,* 1976). An appropriate and reliable test has to be able to detect all types of FMDV for rapid and precise diagnosis (Clavijo *et al.,* 2003). The reverse transcription-polymerase-chain reaction (RT-PCR) assay has been proved rapid, specific and sensitive for detection and typing of the FMDV as well as for differentiating it from other vesicular diseases (Marquart *et al.,* 1995; Callens and De Clercq, 1997; Callens *et al.,* 1998). Several reports described the use of a wide range of primers

in RT-PCR assays, utilizing specific viral genomic sequences as targets, for detection of FMDV with high sensitivity within 24 h in a wide range of specimens (Reid *et al.,* 1999.)

Foot and mouth disease is considered endemic in much of Sub-Saharan Africa, with the exception of disease free zones in Southern Africa (Bronsvoort *et al.*, 2006).However, there are only sporadic submissions to the World Reference Laboratory (WRL), Pirbright, United Kingdom (Vosloo *et al.,* 2002). Review of reports submitted to the WRL for the whole of Africa between 1958 and 1992 (Ferris and Donaldson, 1992) and from 1948 to 2002 (Vosloo *et al.,* 2002) show that at least four of the six serotypes found in Africa, which include SAT 1, SAT 2, O, and A have occurred in Western and Central Africa at some stage. In West Africa, FMD outbreaks occur regularly in the absence of any known wildlife maintenance hosts. It is therefore possible that the disease is maintained in small ruminants and even cattle during inter-epidemic periods (Sangare *et al.,* 2003). The presence of large numbers of migrating nomads with their livestock may play a major role in spreading infection across borders. In Nigeria, FMD was first reported in 1924 as sporadic outbreaks attributed to serotype O (Libeau, 1960). Other outbreaks that resulted in the identification of serotypes A, SAT 1 and SAT 2 have also been reported (Nawathe and Goni, 1976; Owolodun, 1971; Durojaiye, 1981; Knowles *et al.,* 2008). Serological studies of FMDV in Nigerian livestock indicate a prevalence ranging from 13-36.5% (Dawe and Durojaiye, 1986; Abegunde, 1987; Obi and Newman, 1988; Ehizibolo, 2010). Trade cattle entering Nigeria from neighboring countries (Chad, Niger, Cameroon)and trade cattle markets are important risk factors of FMD transmission (Ehizibolo, 2010).

1.2 Statement of ResearchProblem

Foot and mouth disease is a highly contagious viral disease with a potential to cause economic losses from high calf mortality, decreasing calving rate due to infertility and abortion, severe reduction in milk and meat production, loss of draught power due to lameness and also psychological trauma for the livestock farmers (Perry *et al.*, 1999; Bayissa *et al.,* 2011).It is one of the major diseases that negatively influence trades in livestock and livestock products globally **(**Gall and Leboucq, 2004**).**Foot and mouth disease comes at huge costs and has delimited the effort of the herdsmen and resource poor farmers (including women and children) to benefit from the genetic potentials of their livestock (Barasa *et al.,* 2008; Senturk and Yalcin, 2008). FMD is an endemic transboundary animal disease (TAD) in Nigeriathat was first reported in 1924 as sporadic outbreaks attributable to serotype O virus (Libeau, 1960). Other serotypes (A, SAT1 and SAT2) have also been known to circulate (Owolodun, 1971; Nawathe and Goni, 1976; Durojaiye, 1981). Serological evidence of FMDV has been documented in domestic ruminant animals in Oyo State (Dawe and Durojaiye, 1986; Obi and Newman, 1988) and in Plateau State (Abegunde, 1987; Ehizibolo, 2010). Whereas the circulation of serotypes O, A and SAT2 have been reported in recent past in Nigeria, no report on the isolation of SAT1 virus from domestic or wild animals in Central and West Africa for the last three decades (Tekleghiorghis *et al.,* 2014). The current epidemiologic situation of FMD in domestic ruminant animals in Nigeria is uncertain and information on the disease situation is based on historic rather than up to date typing of FMDV in circulation. There is no effective national FMD surveillance or reporting system amidst the yearly outbreaks occurring in different parts of the country. Nigeria currently has a cattle population of over 16million (OIE, 2013) predominantly on transhumance production management system as well as a large and porous international and inter-state borders which serve as entry points for countless migrating nomads into the country and within the States

in search of feed and water resources without recourse to quarantine and control measures, and serve as a great limitation to the effective implementation of movements restrictions, national veterinary quarantine services and control posts. Furthermore, the weak and often under-funded veterinary services in Nigeria pose a great limitation to disease prevention and control. A knowledge of the current circulating serotypres/strains of FMDV in Kaduna and Plateau States, and in Nigeria as a whole is not certain, and requires our understanding for the progressive control of FMD in the country.

1.3 Justification of the Study

Since the first report of FMD in Nigeria in 1924 (Libeau, 1960), followed by other reports in 1970s and 1980s, there is dearth of information on the current status of FMDV in Nigeria. In an increasingly globalize world in which people as well as animals are moving, or are being moved across borders and between continents with greater frequency and speed than ever before, new implications are emerging for animal health problems and animal disease control system.

Foot and mouth disease is endemic in Nigeria, presently, FMD surveillance/control programmes are non-existent/inadequate in the face of continued outbreaks that are responsible for morbidity and economic loss, undermining the livelihood of the livestock farmers. Thus, FMD has a potential impact (economic and social) on Nigeria's livestock sector. The epidemiological situation of FMD in Nigeria is quite uncertain. A clear knowledge of the local situation (epidemiological factors, circulating virus strains) and the preparation and implementation of control programmes to reduce the impact of the disease are needed to enhance efficient livestock production necessary to ensure food safety. Since vaccination is important in the control of FMD in Nigeria, the best vaccination programme should target the topotypes and serotypes circulating within the country. Effective surveillance that will lead to vaccine production is required to

identify the circulating strains and serotypes in Nigeria. Therefore, this study has the potential of providinginformation required in the progressive control pathway for FMD specifically in Plateau and Kaduna States, and Nigeria in general, to progressively reduce the level of FMDV circulation and to mitigate the impact of FMD.The study would also provide an opportunity to confirm the serotypes/strains of FMDV that are currently existing in Nigeria.

1.4 Aim of the Study

The aim of the study was to investigate the epidemiology of Foot and mouth disease in ruminant animals in Plateau and Kaduna States, Nigeria

1.5 Objectives of the Study

The specific objectives were to;

i. Assess the epidemiological factors involved in FMD occurrence in Plateau and Kaduna States
ii. Determine the presence of FMDV antibody/antigen in the sera/epithelial tissuesof ruminant animals in Plateau and Kaduna States
iii. To isolate and characterize FMDV from clinically ill ruminant animals in Plateau and Kaduna States

1.6Research Questions

i. What is/are the epidemiological factors responsible for FMD transmission/spread in Plateau and Kaduna States
ii. What is the level of FMD antibody in Plateau and Kaduna States

iii. What is/are the FMDV serotype(s) circulating in Plateau and Kaduna States?

iv. What are the molecular characteristics and sequences of FMDV isolates obtained from Kaduna and Plateau States?

v. What is the genetic relationship of isolates obtained with circulating isolates from the neighbouring countries?

vi. Is/are there new FMDV serotype(s) involved in FMD outbreak in Nigeria?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Affected Species

Foot and mouth disease virus can infect most or all members of the order Artiodactyla (cloven-hooved mammals), as well as a few species in other orders. Each species varies in its susceptibility to infection and clinical disease (Kitching, 2002b; Kitching and Alexandersen, 2002; Kitching and Hughes, 2002).

Livestock susceptible to FMD include cattle, sheep and goats, pigs, water buffalo and reindeer (FAO, 1984). Others like Llamas, alpacas and camels can be infected experimentally, but do not appear to be very susceptible (Wernery and Kaaden, 2004). The virus can also infect at least 70 species of wild animals including African buffalo (*Syncerus caffer*), bison (*Bison spp*), elk, moose, chamois, giraffes, wildebeest, blackbuck, warthogs, kudu, impala, and several species of deer, antelopes and gazelles (Anderson *et al.,* 1975; Thomson *et al.,* 2003; Letshwenyo *et al.,* 2006). Susceptible non cloven-hooved species include hedgehogs, armadillos, kangaroos, nutrias, capybaras, guinea pigs, rats and mice. Infections have been reported in African and Asian elephants in zoos; however, African elephants are not considered susceptible to FMD under natural conditions in Southern Africa (Bhattacharya *et al.,* 2003).

On most continents, cattle are usually the most important maintenance hosts of FMDV, but some virus are primarily found in pigs, sheep or goats (Sutmoller and Casa Olascoaga, 2002). Cattle and African buffalo are currently the usual maintenance hosts for FMDV in Africa; African buffalo are currently thought to carry only SAT serotype (Vosloo *et al.,* 1996, OIE, 2012). Aside this, wildlife hosts do not seem to be able to maintain FMD viruses, and are usually infected by contact with domesticated livestock (Anderson *et al.,* 1975).

2.2 Geographical Distribution of Foot and Mouth Disease

Outbreaks of FMD have occurred in every livestock-containing region of the world with the exception of New Zealand, and the disease is currently enzootic in all continents except Australia and North America (Grubman and Baxt, 2004).

The serotypes of FMDV are not distributed uniformly around the world. The serotype O, A and C viruses have had the widest distribution and have been responsible for outbreaks in Europe, America, Asia and Africa. However, the last reported outbreak due to serotype C FMDV was in

Ethiopia during 2005 (Rweyemamu *et al.*, 2008) and so serotype C viruses may no longer exist outside of laboratories. The SAT 1-3 viruses are normally restricted to sub-Saharan Africa. However, there have been some limited outbreaks due to SAT1 viruses in the Middle East between 1962-1965 and 1969-1970 and then in Greece in 1962 (Knowles and Samuel, 2003). Similarly, there have been reports of minor incursions of the serotype SAT2 in Yemen in 1990 and in Kuwait and Saudi Arabia in 2000 (Grubman and Baxt, 2004). More recently, FMD outbreaks due to serotype SAT2 spread from sub-Saharan Africa through northern African countries (Egypt and Libya) and into Palestine (Valdazo-Gonzalez*et al.*, 2012). This serotype was also detected in Bahrain. The serotype Asia-1 FMDVs are generally confined to Asia, except for two incursions into Greece, one in 1984 and a second in 2000 (Jamal and Belsham, 2013).

Although considerable information is available on the virus, the disease and vaccines, FMD still affects extensive areas of the world (Jamal and Belsham, 2013). Foot and mouth disease free countries have introduced a number of measures to retain their status because of the heavyeconomic losses resulting from this disease. The USA has experienced FMD nine times since 1870 andeach time the disease was eradicated with strictslaughter and quarantine control procedures; the last FMD outbreak in the USA occurred near Montebello, California, in 1929. Infected hogs contracted the disease after being fed swill with meat scraps from a tourist steamship coming from Argentina. Since then, the USA has had restrictions on importation of susceptible animals and livestock products from countries where FMD is present. Canada has been free of FMD since 1952 and Mexico since 1953 (Jamal and Belsham, 2013).

Historically, FMD in Australia was documented in the early 1800s and early 1870s (Fisher, 1984). In 1871-1872, at least five episodes of FMD were documented in cattle that originated

from the UK, either bound for Australia, in quarantine or in a bull which had landed two months earlier (Fisher, 1984). The last outbreak of FMD in Australia was reported in 1872.

The pandemic serotype O virus (designated as the PanAsia strain) belongs to the Middle East-South Asia (ME-SA) topotype which has spread rapidly and vigorously (Knowles *et al.,* 2005). This lineage replaced the other lineages of FMDV previously circulating in the Middle East (Samuel *et al.,* 1997). This lineage has been responsible for disease outbreaks everywhere in the world where FMD is endemic or sporadic except for South America and been responsible for incursions into previously disease-free countries. The PanAsia lineage was first detected in India in 1982 (Hemadri *et al.,* 2002) but wasconfined to India until 1996. Its predominance in field outbreaks in India was, however, noticed from 1996 onwards (Hemadri *et al.,* 2002). It spread northwards to Nepal in 1990 and again in 1997-1999 and to Bhutan in 1998 and also toward the west, into Bahrain, Kuwait, Saudi Arabia, Syria, Yemen, Iran and Lebanon in 1998 and to UAE, Israel and Turkey in 1999 (Knowles *et al.,* 2005). The lineage spread further to China in 1999and into South East Asian countries causing outbreaks in Thailand in 1999, Malaysia and Laos (PDR) in 2000 and Vietnam in 2002. The virus also caused disease outbreaks in South Korea (Shin *et al.,* 2003) and Japan in 2000 (Sakamoto *et al.,* 2000). These two countries were previously free of FMD since 1934 and 1908, respectively. South Korea faced outbreaks again in 2002 and then in 2010 (Yoon *et al.,* 2011).

In 2000, the O-PanAsia virus was detected in Uzbekistan, Mongolia, Armenia, Georgia and Russia and then in 2001 in Kyrgyzstan and during 2001-2003 in Tajikistan (Scherbakov *et al.,* 2008). Also in 2000, the virus spread to KwaZulu-Natal Province in South Africa. This was the first recorded outbreak in that country due to serotype O and the first since 1957 in this region of Southern Africa (Sangare *et al.,* 2001; Bruckner *et al.,* 2002). The outbreak was attributed to the

feeding of swill to pigs from a ship which had originated from Asia. More recently, outbreaks due to SAT1 FMDV were reported in 2011 and further FMD outbreaks due to SAT2 have occurred in South Africa in 2012 while outbreaks due to SAT1 have recently been reported in 2013 (OIE, 2013).

In 2001, the PanAsia virus was introduced into Europe where it caused disease outbreaks in the UK and later spread to Ireland, France and the Netherlands. The outbreaks ofFMD in United Kingdom in 2001 were the first since a single case in 1981 (Knowles *et al.,* 2001b). Before the 1981 case, the UK was hit by a major outbreak of FMD in 1967 and 442 000 animals were slaughtered to achieve eradication of the disease (Segarra and Rawson, 2001). The 2001 outbreaks were controlled using a stamping out policy in which 6.5 million infected and in-contact animals were killed. The total economic losses due to the 2001 outbreak were estimated at between USD 12.3-13.8 billion (Thompson *et al.,* 2002). Another outbreak hit the UK in 2007,and this was traced to either the escape of virus from the vaccine production facility or the Institute for Animal Health, both sited in Pirbright (Cottam *et al.,* 2008).

Currently, the O-PanAsia-IIANT-10 strain is being incriminated in the extensive outbreaks in the whole Middle East and South Asian region. This strain was responsible for FMD in Pakistan in 2008, which spread westwards to Turkey, Israel, Libya and Bulgaria (Cottam *et al.,* 2008). Bulgaria experienced an outbreak of FMD at the end of 2010; the first case of the disease was detected in wild boar. Foot and mouth disease virus serotype O virus strain O-PanAsia-IIANT-10 was found to be responsible for the outbreaks at 11 separate sites which occurred in two separate phases (Cottam *et al.,* 2007; Valdazo-Gonzalez*et al,* 2011). The country had remained free of FMD for 14 years since its previous outbreak in 1996. The disease also affected cattle, buffalo, sheep and goats. A stamping out policy was used and a total of 1372 animals were destroyed.

Seropositive wildlife were detected near the outbreaks but the disease has not been maintained by them since no new cases of FMD were detected after April, 2011 and the country was declared free of FMD in July, 2011 (OIE, 2013).

2.3 Foot and Mouth DiseaseVirus Pools

Comparison of the virus protein 1 (VP1)coding nucleotide sequences reveals a tendency for similar viruses to reoccur in the same geographical area. This tendency reflects some degree of ecological isolation, likely reflecting pattern of animal movement and trade or specific wildlife reservoirs (e.g. African buffalo) within a region. Foot and mouth disease virus serotypes and strains vary within each geographic region and the concept of regional virus pools' provides an organizing principle for coordinating laboratory and epidemiology activities. Most common circulating types of FMDV are the basis of clustering countries in regional roadmaps. Based on genetic and antigenic analyses, FMDVs throughout the world have been sub-divided into seven regional pools (Di Nardo*et al.*, 2011; Sumption *et al.*, 2012). Virus circulation and evolution within these regional virus pools result in changing needs for appropriate vaccine selection.

The seven regional virus pools of FMD include: pools 1 (East and South-East Asia), 2 (South Asia), 3 (Central Asia and Middle-East), 4 (West Africa), 5 (West and Central Africa), 6 (Central and South Africa), and 7 (South America) (OIE/FAO, 2009).

Serotype O FMDV is the most widely distributed serotype of the virus (in 6 of the seven virus pools) whereas, in contrast; SAT3 is only present in pool 6 (within Southern Africa). The Asia-1, SAT1 and SAT2 serotypes also have quite limited geographical distribution. However, individual countries can have multiple serotypes in circulation at the same time and hence it is necessary to be able to determine which serotype is responsiblefor an outbreak if vaccination is to be used. Countries which are normally free of the disease can still suffer incursions of the virus which can have high economic costs.

2.4 Description ofthe Agent

2.4.1 Genomeorganization

Foot and mouth disease virus is a member of the family *Picornaviridae*, which is a non-enveloped virus consisting of four genera: the enteroviruses (polio virus), rhinoviruses (human common cold virus), cardioviruses (mengovirus) and the aphthoviruses (Grubman and Baxt, 2004). The FMDV genomehas a basic organization similar to those of other members of the *Picornaviridae*, and Rueckert and Wimmer (1984) established the nomenclature for the viral proteins. Within the virion, there are small amounts of a cleavage precursor of VP2 and VP4, called VP0 (1AB) (Rueckert, 1996), and one copy of a 23-to 24-amino-acid genome linked protein 3B (VPg), covalently bound to the 5' terminus of the RNA (Sangar *et al.*, 1977; Grubman, 1980). The RNA is translated as a single long open reading frame (ORF) into a polyprotein, this is followed by series of post-translational proteolytic cleavages to give both the intermediate and mature structure and NS viral proteins (Grubman *et al.*, 1984; Robertson *et al.*,1985). The genome ORF is divided into four regions based on the initial cleavage products. The 5' end, the L region, which encodes the N-terminal component of the polyprotein, contains two in-frame AUG initiation codons that results in the generation of two L proteins, Lab and Lb (Robertson *et al.*, 1985; Sangar *et al.*, 1987). The L protein, a papain-like protease ($L^{pro)}$ (Kleina and Grubman, 1992; Piccone *et al.*, 1995; Skern and Guarne, 1998), is autocatalytically cleaved from the polyprotein at its C-terminus.

Directly down the L region is the P1 region of the genome encoding the four structural proteins VP4, VP2, VP3 and VP1. Following is the P2, encoding three viral NS proteins 2A, 2B and 2C, and the P3 region, encoding NS proteins 3A, three copies of VPg, $3C^{pro}$ and $3D^{pol}$ (Mason *et al.*, 2003). The 2A region was historically considered part of the P2 region; however, genetic and biochemical evidence has shown that FMDV 2A peptide is cleaved as a P1-2A precursor

(Vakharia *et al.*, 1987). $3C^{pro}$ was identified as a viral protease (Klump *et al.*, 1984) and is involved in processing the viral polyprotein, while $3D^{pol}$ is the viral RNA-dependent RNA polymerase (Cowan and Graves, 1966; Polatnick, 1980; Robertson *et al.*, 1983). Each of these proteins playsa role in viral replication. The FMDV genome also contains untranslated RNA found upstream (5'untranslated region [5'UTR] and downstream (3'UTR) of the ORF (Mason *et al.*, 2003). The 5' UTR of the FMDV contains about 1300 bases and can be divided into five functional elements which play roles in virus translation and RNA replication (Forss*et al*, 1984; Grubman *et al.*, 1984; Robertson *et al.*, 1985). The most 5' segment, S fragment, encompasses about 360 bases and folds into a long stem-loop (Newton *et al.*, 1985; Bunch *et al.*, 1994). The function of the S fragment is not known, but it was suggested that it may play a role in maintaining genome stability in infected cells (Barton*et al.*, 2001) and may also be involved in the binding of proteins involved in genome replication (Andino *et al.*, 1993; Barton *et al.*, 2001). Following the S fragment is a poly(C) tract which comprises over 90% C residues with a small number of U and A residues. This segment is over 100 bases in length; however, the length of the poly(C) tract can be extremely variable (Coata Giomi *et al.*, 1984). The exact role that the poly(C) tract plays in FMDV replication is unknown. There is a series of RNA pseudoknotstructures of known function just after the poly (C) tract (Rieder *et al.*, 1993; Escarmis *et al.*, 1995). Down the pseudo-knots is a short hairpin loop structure, the cis-acting replicative element (cre) (Mason *et al.*, 2002) which is essential for RNA genome replication. The region between the cre and ORF contains a series of highly conserved stem-loop structures which together constitute the internal ribosome entry site (IRES) (Belsham and Brangwyn, 1990; Kuhn *et al.*, 1990). Ribosomes enter the genome internally at the IRES (Jang *et al.*, 1988; Pelletier and Sonenberg, 1988).

The 3´ UTR, which follows the ORF termination codon, contains short stretch of RNA which folds into a specific stem-loop structure (Pilipenko *et al.,* 1992) followed by a poly (A) tract of variable length carried on the genome (Dorsch-Hasler *et al.,* 1975). The 3' UTR appears to be important for genome replication (Rohll *et al.,* 1995; Melchers *et al.,* 1997), and the poly (A) tract probably functions in FMDV translation (Lopez de Quinto *et al.,* 2002) and may also play a role in Picornavirus RNA replication (Barton *et al.,* 2001; Herold and Andino, 2001).

2.4.2 Virus structure

Electron microscopy study shows that FMD virion appears to be around particle with a smooth surface and a diameter of about 25nm (Bachrach, 1968). The virion is a 140S particle consisting of a single-stranded RNA genome and 60 copies each of four structural proteins: VP1 (1D), VP3 (1B), VP3 (1C) AND VP4 (1A) (Grubman and Baxt, 2004). These four proteins assemble to form a protein sub-unit or protomer. Five protomers join to form a pentamer. Twelve pentamers join up to enclose a strand of RNA and so create a virus particle. The structure thus created has five, three and two fold axes of symmetry. The structural proteins VP1 to VP3,fold into an eight-stranded wedge-shaped β-barrel which fit together to form the majority of the capsid structure (Acharya *et al.,* 1989).The VP4 protein is buried within the capsid and has a myristyl group covalently attached to its N terminus (Chow *et al.,* 1987; Belsham *et al.,* 1991). The strands of the β-barrels of VP1 to VP3 are connected by loops which form the outer surface of the virion (Jackson *et al.,* 2003). Foot and mouth disease virus is distinguished from other picornavirusesby the lack of a surface canyon or pit, which has been shown to be the receptorbinding site for the entero-and cardioviruses (Belnap *et al.,* 2000; He *et al.,* 2000). Another feature of the virion is the presence of a channel at the five-fold axis which permits the entry of small molecules, such as CsC1, into the capsid, resulting in FMDV having the highest buoyant density of the

picornaviruses (Agol*et al.*, 1999; Jackson *et al.*, 2003). FMDV capsid can be dissociated at pHs of below 6.5 into 12S pentameric subunits, unlike those of other picornaviruses (Brown and Cartwright, 1961). This is thought to be due to a cluster of His residues at the interface between VP2 and VP3 which become protonated at low pH, weakening the capsid through electrostatic repulsion (Curry *et al.*, 1995; Ellard *et al.*, 1999). This low-pH induced instability of FMDV leads to differences in the mechanism of its uncoating upon infection of cells compared to that for other picornaviruses, and also probably plays a role in the targeting of the virus to specific tissues and organs in susceptible hosts.

2.5 Infectious Cycle

The infectious cycle of FMDV is relatively short in cultured cells. Depending on multiplicity of infection, newly formed infectious virions begin to appear at 4 and 6 hours after infection (Grubman and Baxt, 2004). The virus is cytocidal, and infected cells exhibit morphological alterations, commonly called cytopathic effects, which include cell rounding and alteration and redistribution of internal cellular membranes. The virus also causes biochemical alterations, including inhibition of host translation and transcription (Rueckert, 1996)**.**

2.5.1 Early interactions: adsorption, penetration, and uncoating

Generally, it is accepted that FMDV receptors play a role in tissue and organ tropism which leads to disease pathogenesis (Evans and Almond, 1998; Schneider-Schaulies, 2000) Foot and mouth disease virus binds rapidly to cells in culture at both $4^{o}C$ and $37^{o}C$ by using a limited number of receptor sites (Baxt and Bechrach, 1980). It was also suggested that while six of the seven serotypes bind to a single class of receptor site, some of the serotypes bind to a second class of receptors which were present at a high copy number (Baxt and Bechrach, 1980;

Sekiguchi *et al.,* 1982). A number of receptors have been shown to mediate FMDV infection in vitro. Antibody-complexed virus can infect cells via Fc receptor-mediated adsorption (Baxt and Mason, 1995; Mason *et al.,* 1993). In addition, an artificial receptor which consists of a single-chain anti-FMDV monoclonal antibody fused to intercellular adhesion molecule 1 (ICAM-1) has been engineered, and this receptor was also able to mediate infection (Rieder *et al.,* 1996). Jackson *et al.* (1996) reported that a type O_1 virus was able to utilize the glycosaminoglycan heparin sulphate (HS) as a co-receptor.

The penetration and uncoating of FMDV have not been studied in a great detail (Grubman and Baxt, 2004), however, some observation suggest that after adsorption to the cell surface, the 140S virion breaks down into 12S pentameric subunits, releasing the RNA (Baxt and Bachrach, 1980; Baxt, 1987). The breakdown does not occur at the cell surface, since particles eluted from the cell after absorption are fully infectious and still sediment at 140S (Baxt and Bachrach, 1980), it has been demonstrated (Carrillo *et al.,* 1984; Carrillo *et al.,* 1985; Baxt, 1987) that the virus probably breaks down upon entering an acidic endosome.

2.5.2 Viral translation

The RNA is released into the cytoplasm following uncoating by a-yet-unknown mechanism and begins a round of viral translation. The genome-linked protein VPg is cleaved by a cellular enzyme prior to translation of the incoming RNA (Ambros *et al.,* 1978; Ambros and Baltimore, 1980); however, mRNA containing VPg can form protein synthesis initiation complexes (Golini *et al.,* 1980). The FMDV IRES interacts with a number of cellular proteins, including initiation factors important for normal cellular mRNA translation. A host factor of 57kDa, identified as the nuclear polypyrimidine tract binding protein (PTBP) (Hellen, *et al.,* 1993; Kaminski *et al.,* 1995; Niepmann *et al.,* 1997), was shown to interact with at least two regions of the IRES (Luz and Beck, 1990; Pilipenko *et al.,* 2000). More recently, a second host factor, a 45-kDa protein, IRES-

specific trans-acting factor ($ITAF_{45}$), which is required for FMDV IRES-driven translation was identified. This protein, along with PTBP, is required for the formation of the 48S translation-initiation complex (Pilipenko *et al.*, 2000; Martinez-Salas *et al.*, 2001). Also, the 3' end of the genome may also be required for FMDV translation (Lopez de Quito *et al.*, 2002). Following initiation, translation results in the production of a single polypeptide which undergoes a series of cleavages leading to the production of both structural and NS proteins. The primary cleavage reactions are performed by three different proteases-the L^{pro}, 2A and $3C^{pro}$ (Vakharia *et al.*, 1987; Ryan *et al.*, 1991; Bablanian and Grubman, 1993; Grubman *et al.*, 1995).

2.5.3 Viral transcription and genome replication

The first step in picornavirus RNA replication is the synthesis of a minus-strand RNA molecule. This system has not been studied in FMDV; however, the models of RNA replication developed for poliovirus are probably quite similar (Paul, 2002). It is thought that translation of the plus-strand RNA must cease before minus-strand synthesis begins (Gamarnik and Ardino, 1998). Following the initiation reaction, elongation of the minus-strand begins, catalyzed by $3D^{pol}$. For this to occur, the initiation complex must translocate to the 3' end of the plus-strand template. The mechanism by which this occurs is unknown, but one hypothesis suggests that binding of poly (A) binding protein (PABP) to the poly (A) tract positions this region of the plus strands near the *cre* (Paul, 2002). The elongation of the nascent strands results in the formation of a double-stranded molecule, the replicative form (RF) (Wimmer *et al*., 1993; Agol *et al*., 1999). After formation of the RF, new plus-strand synthesis can begin. For plus-strand synthesis to proceed, the RF must be unwound. The mechanism for this is unclear. The picornavirus 2C protein both has ATPase activity and contains helicase motifs (Dmitrieva *et al*., 1991; Pfister *et al*., 2000), but helicase activity has not been demonstrated (Pfister and Wimmer, 1999). It has been shown that 2C and a cellular protein (P38) bind to the minus-strand 3' stem-loops, and this

may act to destabilize the RF molecule (Rohll *et al.*, 1995; Roehl and Semier, 1995; Banerjee *et al.,* 1997). RNA synthesis occurs within a membranous replication complex, which is derived from membranes of the endoplasmic reticulum and Golgi and contains viral NS proteins encoded by both the P2 (2B, 2BC, and 2C) and P3 (3A and its precursors, $3C^{pro}$, and $3D^{pol}$) regions (Bolten *et al*., 1998; Egger *et al.,* 2002). Infection with picornaviruses results in a rapid inhibition of host cell transcription, which does not appear to be related to the inhibition of host cell translation (Kaarianen and Ranki, 1984).

2.5.4 Encapsidation and maturation

Encapsidation of the plus-strand viral RNA and maturation cleavage of VP0 and VP2 and VP4 to form the mature virion are the final steps in the replication cycle. The $3C^{pro}$ cleavage products of the P1 region are assembled into a protomer structure containing one copy of each of the proteins VP0, VP1, and VP3. Five protomers can assemble into a pentamer, and 12 pentamers assemble into the final capsid structure (Grubman and Baxt, 2004). Following encapsidation of the RNA, the maturation cleavage (VP0 to VP2 and VP4) takes place. A number of intermediate particles have been identified in picornavirus-infected cells, including protomers, pentamers, a particle containing RNA with an uncleaved VP0 (provirion) (Lee *et al.,* 1993), and a particle containing VP0 lacking RNA (empty capsid) (Grubman *et al*., 1985). Picornaviruses encapsidate only plus-strand RNA, linked to VPg, to the exclusion of all other viral and cellular RNAs (Novak and Kirkegaard, 1991). Also, only newly synthesized plus-strand RNAs are encapsidated, indicating that there is a link between active RNA replication and encapsidation (Harber *et al*., 1991; Nugent *et al*., 1999).

There are two models of picornavirus assembly. One proposes that pentamers assemble into empty capsids, followed by insertion of the RNA, and the second proposes that pentamers directly interacts with the RNA to form the provirion. In either case, it is known that

myristylation of the N terminus of VP0 is necessary for capsid formation (Marc *et al*., 1991; Moscufo *et al*., 1991; Ansardi *et al*., 1992). The maturation cleavage of VP0 into VP4 and VP2 is the final step in virion assembly, and this requires the presence of viral RNA (Arnold *et al*., 1987). Maturation cleavage is required for the generation of infectious virus (Lee *et al*., 1993; Knipe *et al*., 1997).

2.6 Antigenic Variation

The FMDV is antigenically heterogeneous. The presence of the seven serotypes and multiple subtypes and variants has added to the difficulty of laboratory diagnosis and control. Each serotype of FMDV is antigenically distinct from the other six serotypes. Furthermore, within each serotype, there is considerable antigenic diversity (Grubman and Baxt, 2004). The rise of new variants is inevitably caused by continued circulation of the virus in the field and the quasispecies nature of the RNA genome (Haydon *et al*., 2001 Domingo *et al*., 2003). RNA viruses in general and FMDV in particular, have very high mutation rates, in the range of 10^{-3} to 10^{-5} per nucleotide site per genome replication, due to lack or error correction mechanisms during RNA replication (Drake and Holland, 1999).

The high error rate leads to differences of FMDV replicated genomes from the original parental genome of 0.1 to 10 bases positions (Haydon *et al*., 2001), and the quasi-species concept was developed to explain the effects of errors in replication on the evolution of replicating RNA molecules (Eigen, 1971). The Concept envisions that with any population of virus, all genome sequences are not identical, and that selection occurs at the population level rather than at the individual level (Domingo *et al.,* 2003). Thus, there is not a "wild type" as such but rather an observed "average" phenotype which has adapted to and replicates "best" within any given environment (Grubman and Baxt, 2004). The environment can be either tissue culture or a

particular host species, and in either situation, immunologic pressure or physical conditions such as temperature or pH are influential. Any change in the environment can lead to the emergence of a new "average" phenotypic trait (Grubman and Baxt, 2004).

The quasi-species nature of the FMDV genome has been described (Domingo *et al.*, 1980), and whiles the concept is studied at the nucleotide level, the variability of FMDV populations is manifested when mutations lead to codon changes resulting in a region of the genome (P1 region) leading to antigenic variation. While mutations also occur within the NS protein-coding regions of the genome, they are probably less tolerated, since proteins encoded by these regions are necessary for viral replication and changes are likely to be lethal (Grubman and Baxt, 2004). In addition to changes due to mutations, FMDV has been shown to undergo RNA recombination in tissue culture (King *et al*., 1985; Wilson *et al*., 1988). These studies shows that recombination was more likely to occur within the regions of the genome coding for the NS proteins; however, a more recent report indicate that RNA recombination within the capsid-coding P1 region of the genome may contribute to genetic diversity in FMDVs isolated from the field (Tosh *et al.*, 2006b).

Antigenic variation in the field increases with time and most probably results from immunologic pressure placed on the virus by either the infected or vaccinated host species (Domingo *et al.*, 2003). This makes control extremely difficult, since even the best vaccine may induce immunologic pressure within the population that results in the emergence of a new variant. In addition, antigenic variation in FMDV has also been observed in tissue culture in the absence of immunologic pressure (Gonzalez *et al*., 1991; Domingo *et al.*, 1993; Fares *et al*., 2001), indicating that antigenic sites on the virion may also be involved in other virus functions. This observation has implications for vaccine production, since a number of tissue culture passages

are required to produce vaccine for a new variant, leading to the possibility that the virus eventually utilized as antigen may not provide the antigenic coverage needed (Grubman and Baxt, 2004). Antigenic sites on the surface of the FMDV virion have been identified for five of the seven serotypes of the virus (SAT1 and SAT3 being the only exceptions) (Haydon *et al*., 1998). At least four antigenic sites have been identified, involving one or more of the capsid proteins, VP1, VP2 and VP3; however, each serotype may not contain all four sites. Also, three of the sites have elements located within the flexible loops which connect the β-sheets of the viral proteins, and at least two sites include the C terminus of VP1 (Domingo *et al.*, 2003). While all of the sites appear to be necessary for a complete immunologic response to either infection or vaccination, the major antigenic site, to which most of the immune response is directed and which is common to all of the serotypes, is located within the G-H loop of VP1 (Borrego *et al*., 1995; Mateu *et al*., 1995) while this site is clearly the major antigenic site, antigenic variation in the FMDV is associated with mutations leading to amino acid replacements within all of the known extensive antigenic variation within FMDV, the changes are limited to very specific regions of the viral surface. This may be because changes within other regions of the capsid would compromise either viral structural integrity or virus identity (Domingo *et al*., 2003).

2.7 Foot and Mouth Disease VirusSerotypes

Food and mouth disease is caused by strains of seven immunologically distinct virus serotypes recognized globally and known as serotypes O, A, C, Asia l and the South African Territory (SAT) types; SAT1, SAT2, SAT3 (Domingo *et al*., 2003). Serotypes O and A were initially discovered by Vallee and Carre (1922). They showed that cattle that had recovered from clinical disease due to an FMD virus which originated in France became re-infected almost immediately when mixed with animals infected with FMD virus that originated in Germany. They named these serotypes after their place of origin; O for the department of Oise in France and A for

Allemagne (the French word for Germany). Their work was extended by Waldmann and Trautwein (1926) with the discovery of a third serotype which was named serotype C. Types A, O and C are the classical European serotypes which have spread to other continents such as South America probably due to importation of European cattle (Rueckert, 1985). Three additional serotypes were identified in samples originating from South Africa and they were named as Southern African Territories 1, 2 and 3 (SAT1, SAT2, SAT3) (Brooksby, 1957). The seventh serotype, Asia-1, was initially detected in a sample collected from a water buffalo at Okara, Punjab, Pakistan in 1954 (Brooksby and Rogers, 1957). Asia 1 is restricted to middle East and Asia and has not spread into Africa or Europe (Ansell *et al*., 1994), serotype O is widespread across the world (Knowles *et al*., 2001b), and SAT3 rarely occurs in species other than the Cape buffalo in Southern Africa (Thomson, 1994). All these serotypes are clinically indistinguishable, and there may be considerable variation in the disease presentation depending on the strain within a serotype, the species affected, and previous exposure (Kitching 2002b; Kitching and Alexandersen, 2002; Kitching and Hughes, 2002).

In many of the FMD endemic regions, more than one serotype may circulate, causing waves of infection, and recovery from infection with a strain of one serotype does not provide protection against strains of other serotypes (Kitching, 2002b). Also, one serotype may persist in a region and rarely appear clinically, producing only mild infection in the young stock as they lose their maternal antibody. However, clinical disease may be seen when a new serotype is introduced in that region (Kitching, 2002b).

2.7.1 Foot and mouth disease virus serotype O

Foot and mouth disease type O viruses were classically divided into 10 or 11 antigenic subtypes (Davie, 1964), although it is now recognized that antigenic variation with this serotype is not as extensive as once thought and relatively few vaccine strains are able to protect against most field

outbreaks. However, genetic diversity is much greater, allowing the classification of many distinct lineages (Samuel and Knowles, 2001a; 2001b). Beck and Strohmaier (1987) demonstrated that many FMDV type O viruses isolated from European outbreaks in the 1970's and 1980's were closely related to the O_1 vaccine viruses in use at that time. Interestingly, they also found viruses which were not closely related to the vaccine strains, and they suggested that these had been introduced from outside Europe. This was certainly the case in Thalheim, Austria in 1981 and Wuppertal, West Germany in 1982 when an atypical strain of type O FMDV caused outbreaks of FMD in pigs.

In serological tests the isolates showed marked differences to the European reference strains, but it was not until many years later, with the advent of molecular epidemiology, that these viruses were shown to be closely related to viruses that were usually limited to China and Hong Kong (the Cathay topotype). FMDV isolates belonging to serotype O have recently been shown to exhibit geographical and genetical distinct evolutionary lineages called topotypes (Samuel and Knowles, 2001b), eight topotypes of FMDV type O have been described (Samuel and Knowles, 2001a; 2001b). they were named Cathay, Middle East-South Asia (ME-SA), South-East Asia (SEA), Europe-South America (Euro-SA), Indonesia-1 (ISA-1), Indonesia-2 (ISA-2), East Africa (EA) and West Africa (WA;). Two of these topotypes are thought to be extinct (ISA-1 and ISA-2) and have not, so far, been detected outside Indonesia. The PanAsia strain is a member of the Middle East-South Asia (ME-SA) topotype (Samuel and Knowles, 2001b). Serotype O is the most common type of FMDV isolated from positive clinical field specimens submitted to the World Reference Laboratory for FMD (Ferris and Donaldson, 1992) and many of the current virus isolates belong to the ME-SA topotype (Samuel and Knowles, 2001a, b). Some lineages of

the type O are restricted in their host range to *suidea* (Cheng *et al*., 2006), whereas others, like the Pan-Asia strain are not restricted to a specific host (Knowles *et al.,* 2005).

Serotype O is widespread in countries of Africa, north of the equator. Recent evidence indicates that the presence of serotype O in countries of Southern Africa is the result of introductions from other continents, and is therefore probably exotic to this region (Sangare *et al.,* 2001). These exotic topotypes (I and V) have been recorded in South Africa and Angola. Topotype I was due to the introduction of the globally dominant Pan-Asian virus, whilst topotype V appears to have been an historical introduction from Europe/South America (Sangare *et al*., 2001). The remaining three topotypes (II-IV) occur in Africa alone, in three discrete regions, namely East Africa, North-West Africa and North-East Africa (Samuel and Knowles, 2001a; Sangare *et al*., 2001).

2.7.2 Foot and mouth disease virus serotype A

Foot-and-mouth disease type A viruses have always been considered to be antigenically the most diverse of the Eurasian serotypes and up to 32 subtypes had been defined by the early 1970s (Davie, 1964; Pereira, 1977). Some of these subtypes have been shown to be genetically distinct (e.g A22, A24), although many have not been examined. Although this system of subtyping was discontinued, type A viruses are still considered to be extremely antigenically and genetically diverse. The early work of Beck and Strohmaier (1987) demonstrated that viruses from European FMDV type A outbreaks in the 1980s (Iberian Peninsula in 1983 and Italy and East Germany in 1984) were closely related to the European A5 vaccine viruses (which were isolated during the early 1960s); however, one virus from Aachen, Germany in 1976 appeared to be exotic to Europe. Outbreaks of type A in Europe which have occurred since the cessation of routine vaccination in 1991 (Albania and the former Yugoslav Republic of Macedonia in 1996) were shown to have been introduced from Asia (Knowles and Samuel, 1998). In later studies,

contemporaneous type A virus isolates from Saudi Arabia and Iran were shown to be genetically distinct from each other and to be unrelated to the West Germany outbreak virus of 1984 (Samuel and Knowles, 1988; Marquardt and Adam, 1989).

Further Asian type A viruses were examined by nucleotide sequencing in a limited study by Armstrong *et al*. (1994) who demonstrated differences between Indian field viruses isolated in Gujarat in 1987 and their relationship to the A22/Iraq/24/64 reference virus. It was not until recently that more extensive studies were undertaken on viruses isolated in India; into 21 genetic groups using partial VP1 sequences. They failed to find any correlation between genetic group and geographical location, which probably reflects the extensive movement of animals within India.

A study (Tosh *et al*., 2002a) compared 83 Indian type-A viruses, isolated between 1977 and 2000 with 37 other available type A sequences. They described 10 major genotypes (designated I-X) with the Indian isolates being distributed in four of the genotypes (I, IV, VI and VII). At least two of these genotypes (VI and VII) were co-circulating in different Indian States. The study also revealed differential geographic distribution of genotypes; some (genotypes I and VII) were recovered from large geographical areas, sometimes even across continents, while others were more restricted. Three Indian isolates from 1986 were closely related to European A10 viruses; a derivative of A10/Holland/42 had been used as a vaccine strain in India. Phylogenetic analysis of 51 type A viruses from Africa showed that they were extremely diverse, but could be grouped into six genotypes, whose members were geographically linked (Knowles *et al*., 1998).

In Africa, serotype A predominantly occurs in countries of North Africa where the disease is endemic (Ferris and Donaldson, 1992; Knowles *et al*., 1998). Sporadic reports of this serotype in North Africa (Morocco, Algeria, Libya, Tunisia and Egypt) and Southern sub-continental regions

(Namibia, Angola, Malawi and Zambia) appear to be due to introductions from other continents (Knowles *et al*., 1998). Six distinct topotypes have been identified, five of which are unique to Africa (Knowles *et al*., 1998). These endemic lineages are represented by topotype I, which is restricted to West Africa, whilst topotypes III-VI are distributed throughout the East African region. Topotype II represents the European/South American virus lineage that was introduced on four separate occasions to the continent (Knowles *et al.,* 1998). The presence of two independent evolutionary lineages (topotypes) within single countries is of particular interest within serotype A. Kenya and Ethiopia have topotypes II and IV, respectively within their borders, whilst in Malawi both exotic (topotype II) and endemic (topotype III) lineages have been recorded (Knowles *et al*., 1998). In countries of North Africa (Algeria, Morocco, Tunisia and Libya) and Southern and Central Africa (Angola and Malawi), the exotic virus serotype occurring are related to historical European and South American reference strain (Knowles *et al*., 1998). Ko'nig *et al*. (2001) compared the VP1 sequences of 16 type A viruses isolated in Argentina (between 1961 and 1992) with the South American vaccine strain A24/Cruzeiro/Brazil/55. They found these viruses to be genetically diverse; however, they were not compared with contemporaneous type A viruses from other South American countries nor to viruses from other parts of the world.

Similarly, Araujo *et al.* (2002) described considerable diversity between nine type A viruses isolated during a 2-year period (1994-1995) in the State of Sao Paulo, Brazil; they belonged to three distinct genetic lineages which also differed from A24/Cruzeiro/Brazil/55. In a collaborative study between laboratories in Argentina, Brazil and the United Kingdom (the National Institute of Agricultural Technology (INTA), Argentina; the Servicio National de Sanidad y Calidad Agroalimentoria (SENASA), Argentina; the Pan-American FMD Centre

(PANAFTOSA), Brazil; and the WRLFMD at the Institute for Animal Health, Pirbright, UK) the complete or partial VP1 sequences of over 40 South American FMD type A viruses were examined. The extensive diversity of this serotype over a 44-year period (1955-1998) and in six South American countries was, thus, confirmed (Piccone *et al*., 2000). Foot and mouth disease type A viruses which caused recent extensive outbreaks in Argentina, Uruguay and Southern Brazil in 2001 have been shown to be very closely related to each other and to viruses isolated previously from that area. It has been demonstrated that numerous genetic groups of FMDV serotype A exist by partial or complete sequencing of the VP1 gene of over 500 viruses (Knowles and Davies, 2000; Marquardt and Freiberg, 2000; Piccone *et al*., 2000; Ko'nig *et al*., 2001; Nayak *et al*., 2001; Tosh *et al*., 2001a). However, comparison of almost 300 complete, or nearly complete, VP1 sequences has shown that type A viruses can be grouped into three major geographically restricted genotypes, (i) Euro-SA; (ii) Asia and (iii) Africa, although occasional spread between these continents may take place.

2.7.3 Foot and mouth disease virus serotype C

Foot and mouth disease virus type C has a very limited distribution compared with most of the other serotypes. Historically, it has been recorded in Europe, South America, North Africa, Angola and Southern Asia. However, it now appears to be limited to the Indian sub-continent and only appears intermittently. Serotype C appears to have disappeared from the world as a whole, with the exception of Kenya (Kitching, 2002a). It is not clear why type C is apparently disappearing, although vaccination and control campaigns have been responsible for its eradication in Europe and South America, where the last outbreaks occurred in 1989 (Italy) and 1994 (Argentina), respectively. However, this is not the case in Asia. Foot and mouth disease type C viruses isolated in Europe and South America were originally classified into five antigenic subtypes, C1-C5 (Davie, 1962) based on relationships obtained using the complement

fixation test (Davie, 1964). The C1 subtype consisted of an early German isolate from approximately 1926 (CGC) and later European isolates from 1953 to the 1980s. The C2 subtype was represented by an early Uruguayan vaccine strain isolated in 1944 (C/Pando) and a field strain from the UK isolated in 1953 (C997). The C3 subtype comprised exclusively South American isolates represented by the vaccine strains C3/Resende/Brazil/55 and C3/Indian/Brazil/71. The C4 subtype contained a single isolate, C4/Tierra del Fuego/Argentine/66 and the C5 subtype contained only South American isolates represented by C5/Argentine/69. All these viruses were later compared phylogenetically using VP1 sequences (Marti'nez *et al*., 1991). Historically, serotype C is the rarest of the FMDV types to have occurred in Africa, having been recorded only in three countries, namely: Ethiopia, Kenya and Angola. Published sequence data is presently limited to a 1996 strain from Kenya, which when compared phylogenetically to type C viruses occurring world-wide, appears to belong to an African/Middle East-specific topotype (Reid *et al.*, 2001a). There is need for further genetic characterization to determine whether this serotype was introduced to East Africa from the Middle East, or whether the reverse scenario holds true.

2.7.4 Foot and mouth disease virus serotype Asia 1

Antigenically, FMD Asia 1 viruses have been considered less diverse than those belonging to types O, A or C. only three antigenic subtypes were defined in the 1960's (Davie, 1964). A study of 44 FMD Asia 1 viruses isolated throughout Asia between 1954 and 1990 showed that their 3 terminal VP1 sequences were less variable than the same region in other FMDV serotypes (Ansell *et al*., 1994). Although two major genotypes could be distinguished, relationships were generally above 85% and it was concluded that all the isolates could be included in a single topotype (Ansell *et al.*, 1994). In a small study conducted by Tulasiram *et al*. (1997) analysis of partial VP1 sequence data showed that FMD type Asia 1 viruses from two field outbreaks in

Southern India were closely related to each other when compared with an isolate from the North and all the three isolates were distantly related to the vaccine virus (Asia 1/IND/63/72).

The epizootic of FMDV Asia 1 which spread across Iran and Turkey in 1999, and which eventually reached the eastern part of Greece in 2000, was shown to have its origins in the mid-1990s in Pakistan, India and Bangladesh (Knowles and Davies, 2000; Marquardt *et al*., 2000). It was also demonstrated that this strain had presumably not spread. Periodically epizootics of FMD appear to spread from the Indian sub-continent across Iran and Turkey to threaten European borders (e.g Asia 1 in 1972/1973), but it is not clear what circumstances are necessary for these events to occur.

Muthuchelvan *et al.* (2001) showed that Asia 1 viruses clustered in two groups within a single genotype. The prototype strain from Pakistan (Asia 1/PAK/1/54) formed one group and the other was formed by isolates from Bangladesh, Bhutan, India, Israel and Nepal. In a more comprehensive study of 61 Indian type Asia 1 viruses (isolated between 1985 and 1999), Gurumurthy *et al.* (2002)showed that the viruses could be grouped into four genotypes (genotypes I-IV). All the 61 isolates from India belong to a single genotype (genotype-II) which they further subdivided into three lineages (B1, B2 and B3). The viruses of the lineage B1 and B3 were found to be more prevalent before 1996 while the viruses of lineage B2 appeared to be new variants responsible for most of the recent outbreaks.

2.7.5 Southern African Territories serotypes

The natural host for the Southern African Territories (SAT) viruses is the African buffalo (*Syncerus caffer*) in which overt disease is rarely observed (Hedger, 1976; Thomson, 1995). These animals are usually persistently infected in the oro-pharynx, sometimes with multiple serotypes and often for long periods (5 years has been recorded) (Condy *et al*., 1985). The SAT serotypes are normally confined to sub-Saharan Africa. However, a number of outbreaks of SAT

1 have been recorded in the Middle East (1962-1965 and 1969-1970) and, on one occasion (1962), even Greece. Recently type SAT 2 spread into Saudi Arabia and Kuwait, but has since been eradicated. A number of studies have been published concerning the molecular epidemiology of the SAT serotypes (Thomson *et al*., 1992; Vosloo *et al*., 1992; Vosloo *et al*., 1995; Vosloo *et al*., 1996; Thevasagayam, 1996; Keet *et al*., 1996; Bastos, 1998; Bastos *et al*., 2000, 2001; Reid *et al.*, 2001a; Vosloo *et al*., 2001), however, most of these have been confined to the situation in southern Africa.

2.7.5.1 Southern African Territories serotype 1

Serotype SAT 1 is widespread throughout sub-Saharan Africa and has also been introduced into the Middle East periodically, but has not become established in that region (Ferris and Donaldson, 1992). Genetic characterization of viruses from African buffalo in Southern and East Africa has shown that distinct evolutionary viral lineages occur (Bastos *et al*., 2001; Vosloo *et al*., 1995). Buffalo in Southern Africa are the source of infection for other cloven-hoofed species (Bastos *et al*., 2001; Dawe *et al*., 1994). Three distinct southern Africa lineages have been defined (Bastos *et al.*, 2001; Reid *et al*., 2001a). The identification of three unrelated topotypes occurring within a single country (Zimbabwe) was worthy of note (Bastos *et al*., 2001). A retrospective analysis of viruses from West Africa revealed that a further two SAT 1 topotypes occur in the continent, bringing the total number of SAT1 topotypes identified in Africa to six (Sangare, *et al*., 2004). This number may increase when further virus representative of Central and North-East Africa are characterized.

2.7.5.2 Southern African Territories (SAT) serotype 2

Serotype SAT2 occurs throughout sub-saharan Africa and has made recent incursions into the Middle East from North-East Africa (Bastos and Sangare, 2001). This serotype has been studied

more intensively than the other two SAT serotypes, given the frequent association of the serotype with outbreaks of the disease in Southern and West Africa (Sangare *et al*., 2004; Vosloo *et a*l., 1995), but have also conclusively shown that buffalo in defined areas of southern Africa transmitted SAT2 virus to cattle and impala within their immediate vicinity (Bastos *et al*., 2000; Hargreaves *et al.,* 2003). The conclusion of a comprehensive survey of SAT2 type viruses from all major regions on the continent has revealed the presence of eleven distinct topotypes (Bastos *et al*., 2003). Four topotype occur in Southern Africa, two in East Africa, two in Central Africa, one in the Horn of Africa and two in West Africa. These results stress the diversity of the endemic African populations, the complexity of control through vaccination and the need for regional FMD management programmes. As with SAT1, the disease situation in some countries is complicated by the presence of numerous topotypes, e.g Zimbabwe and Kenya. Control of the disease in these countries is further exacerbated by the presence of other serotypes and large numbers of wildlife hosts (Vosloo *et al.,* 2002).

2.7.5.3 Southern African Territories (SAT) serotype 3

Serotypes SAT 3 has the most restricted distribution with few topotypes of the three SAT types. Nonetheless, five different topotypes have been identified to date, despite that the viruses studied are being derived from just seven countries, namely; South Africa, Zimbabwe, Zambia, Namibia, Botswana, Malawi and Uganda (Bastos, 2001). Three of the five topotypes occur within different regions of Zimbabwe, whilst the remaining countries have a single topotype within their borders (Bastos, 2001; Reid *et al.,* 2001a). In the Southern African Region, SAT3 is the serotype least frequently associated with outbreaks of FMD in domestic or wild cloven-hoofed animals (Thomson, 1994).

2.8 Pathogenesis

Susceptible livestock may be infected by FMDV as a result of direct or indirect contact with infected animals or with an infected environment. When infected and susceptible animals are in close proximity, the aerial transfer of droplets and droplet nuclei is probably the most common mode of transmission (Alexandersen *et al.,* 2003b). Long-range airborne transmission of virus is an uncommon but important route of transmission, requiring the combination of the following factors:

i. The animal species
ii. The number and location of the transmitting and recipient animals
iii. Favourable topographical and meteorological conditions

2.8.1 Minimal doses and route of infection

2.8.1.1 Direct and indirect contact

Direct contact is the most common mechanism of spread of FMD, and this may take different forms such as:

i. Mechanical transfer of virus (through cuts, abrasions or mucosae) from infected to susceptible animals
ii. Infection via the deposition of aerosols (droplets or droplet nuclei) in the respiratory tract of susceptible animals. In certain situation, especially in pigs which are mostly resistant to aerosol exposure (Alexandersen and Donaldson, 2002), contact transmission may be facilitated by physical contact with infected excretions or secretions, including vesicular fluid or vesicular epithelium containing relatively large amounts of FMDV (Alexandersen *et al.,* 2002a). Pre-existing damage to the integument make livestock highly susceptible to infection through the damaged skin, such is common in pigs kept on concrete floors, or under intensive conditions which may lead to fighting. Also, in cattle feeding on thorny materials (penetrating the tissues of the mouth); or diary cows with

teats damaged by milking machines; and in sheep suffering from foot-rot (Alexandersen *et al.,* 2003b).

Susceptible livestock can be infected through indirect contact via contaminated personnel, vehicles, and all classes of formites. Furthermore, activities such as shearing, de-worming and rounding up for lambing or for clinical examination and blood sampling (procedures common during FMD epidemics), may increase the risk of indirect spread of the virus and of its entry through damaged epidermis (Alexandersen *et al.,* 2003b).

2.8.1.2 Airborne transmission

Under certain climatic conditions, short-distant aerosols transmission may be extended to long-range airborne transmission. This is especially important when pigs are the source of infection, because of the major domesticated species, the pig liberates the largest quantities of airborne virus (Sellers and Parker, 1969; Donaldson and Ferris, 1980; Alexandersen and Donaldson, 2002; Alexandersen *et al.,* 2002a).

Ruminants excrete fewer viruses in their breath but, in contrast to pigs, are highly susceptible to infection to the respiratory route. Thus, ruminants may be infected experimentally by airborne exposure to as little as 10 tissue culture 50% infective doses (TCID50) whereas pigs require more than 10^3 TCID50. Furthermore, to infect pigs consistently the dose has to be delivered at a high concentration (Donaldson *et al.,* 1970; Donaldson and Ferris, 1980; Gibson and Donaldson, 1986; Donaldson and Alexandersen, 2001; Alexandersen and Donaldson, 2002). Consequently, airborne FMD spread from pigs to cattle and sheep downwind is the most likely pattern. Cattle are more readily infected than sheep by the airborne route because they inhale more air in a given time (Alexandersen *et al.,* 2003b). Reports show that FMDV isolates vary greatly in the distance over which airborne spread can occur (Donaldson *et al.,* 2001; Alexandersen and Donaldson, 2002; Donaldson and Alexandersen, 2002). These authors reported that some

isolates, e.g the type O UK strain, are likely to spread more than 20km by the wind, even when many infected pigs provide the source. However, other isolates, in particular C Noville, have the potential to spread up to about 300km by wind (Gloster *et al.,* 1981; Gloster *et al.,* 1982; Donaldson *et al.*, 1982; Sorensen *et al.,* 2000; Sorensen *et al.,* 2001). Such long-distance airborne spread is likely to occur only in circumstances where the biological and physical loss of infectivity in the plume is minimal due to the specific climatic and topographical conditions. Favourable conditions include a relative humidity of 55% or more and minimal mixing of the air by turbulence and convection. Such conditions are typically seen when there is a continuous steady or slight wind, cloud cover and level topography (Alexandersen *et al.,* 2003b). It was reported that natural aerosols from infected animals contain large, medium and small particles excreted as droplets and droplet nuclei in the breath. The respiratory tract is the source of the infectious particles, but the mechanism of virus release is not understood (Alexandersen *et al.,* 2003b). This report described that from pigs, 35-70% of the infectivity is associated with large particles (>6μm diameter), 20-40% with medium sized particles (3-6μm diameter) and 10-25% with small particles (<3μm diameter). The diameter and mass of the particles determines the sites of deposition in the respiratory system. Larger droplets will be affected by gravity and will tend to sediment rapidly; however, particles of less than 6μm diameter will not be greatly affected by gravity and therefore can be transported over long distances (Gloster *et al.,* 1981). These are particles which contain high amounts of FMDV and are most likely to be deposited in the upper (nares) and middle to upper regions (pharynx, trachea, bronchi) of the respiratory tract. Similarly, smaller particles descending in the trachea and bronchi will be taken upwards towards the pharynx. Donaldson *et al.* (1987) concluded that the particle size therefore determines the deposition and concentration of virus in the pharynx (a predilection site for FMDV replication).

After initial replication in the pharynx, the virus spreads through the lymphoid system into the general circulation. However, animal exposed to artificially generated aerosols of small (<3μm) particle size have a different pathogenesis of infection since the initial route of infection will be mainly through the walls of the lower respiratory system and thence to the pulmonary circulation and general circulation, leading to infection and replication at distant predilection sites (Donaldson *et al.,* 1987).

In addition to excretion of virus in the breath of infected animals, aerosols may be created by the splashing of infected milk and urine, by the use of high pressure hoses to clean areas contaminated with infectious secretions, excretions and lesion materials, and by the process of spraying infected slurry on pastures (Alexandersen *et al.,* 2003b), however, the infectivity of, and therefore risk of spread by such aerosols is likely to be much less than that of aerosols exhaled by infected animals.

2.8.1.3 Transmission by oral route

Several recent past epidemics of FMD particularly in countries normally free from the disease have been linked to the entry of virus in contaminated materials which has subsequently been fed to animals (Knowles *et al.,* 2001b), such as the South Africa 2000 and UK 2001 outbreaks that was attributed to feeding unheated waste food to pigs, and the Japan 2000 outbreak to the feeding of contaminated fodder. It is worthy of note that animals are relatively insensitive to experimental infection by the oral route, the dose for pigs being about 10^4-10^5 and for ruminants about $10^{5-}10^6$TCID50 (Sellers, 1971). These doses are much higher than those required to infect by the airborne route (Donaldson, 1987). However, it should also be noted that animals with abrasions of the epithelium in and around the mouth may be infected by smaller doses (Donaldson, 1987). Infection by contaminated waste food may therefore be facilitated by sharp objects, such as piece of bones.

2.8.1.4 Nasal instillation and pulmonary inoculation

Infections have been initiated by these methods in experimental studies. Generally, the dose that will result into infection by nasal instillation is much larger than that given as an aerosol, around 10^4-10^5TCID50 for cattle and sheep (McVicar and Sutmoller, 1976), and the portal of entry is probably the pharynx. However, after nasal instillation only a small proportion of the dose is likely to reach the pharynx, since most of it will be swallowed or will exit through the nares (Alexandersen *et al.,* 2003b). The susceptibility of pigs to nasal instillation is known, but the high resistance of pigs to airborne infection may suggest that it is relatively low. Conversely, however, as pigs are relatively susceptible to oral infection, it is possible that intranasally instilled virus could infect through the mouth, especially when there are pre-existing oral abrasions (Alexandersen *et al.,* 2003b). The hypothesis that the lung is a specific site for entry and replication of the virus has been postulated by various authors (Eskildsen, 1969, Sutmoller and McVicar, 1976; Brown *et al.,* 1996). There is no doubt that infection can be initiated by an artificial aerosol deposited in the lung or following the introduction of virus directly into a bronchus or bronchiole.

However, in the opinion of Alexandersen *et al.*(2003b), it is possible that virus administered by these methods can reach the circulation directly without local replication, and thereafter initiate infection at the usual target sites. In their experiments, (Alexandersen *et al.,* 2001) have consistently failed to find any indication of significant replication of FMDV in the lungs. Pigs infected by the airborne route (by a natural aerosol from infected pigs or by contact with infected pigs) showed no signs of pulmonary replication. Similarly, in a study in cattle infected by needle inoculation, no indication of viral replication in the lung was fund. Other workers have found no evidence to support the proposal that the lung is a predilection site for initial infection and replication (Burrows *et al.,* 1981). During the acute phase of the disease the virus can be found in

virtually all organs including the lungs as a consequence of the high level of viraemia, and consequently, organs should be regarded as sites of active viral replication only when they show a higher concentration of virus or viral RNA than that found in blood sampled at the same time (Alexandersen *et al.,* 2003b).

2.8.1.5 Parenteral inoculation (intradermal, intravenous and intramuscular)

These routes have been used in experimental studies to initiate infection and have occasionally been incriminated as causing iatrogenic disease due to the use of contaminated instruments (e.g needles) or medicinal products (Beck and Strohmaier, 1987), such as the injection of an FMDV-contaminated pituitary extract and administration of FMD vaccines containing live virus as a consequence of the use of inappropriate inactivants.The intradermal injection of virus is dependable for establishing infection in experimental studies on FMD, for example, when donors are required for pathogenesis studies or when vaccine protency is to be measured. This route of infection targets the highly susceptible epithelial regions of the tongue in cattle or of the coronary bands or heel bulbs of the feet in sheep and pigs respectively (Henderson, 1952, Burrows, 1966; Burrows. 1968b). These are sensitive and reliable routes, usually with a very short incubation period. Although artificial, these methods simulate infection through damaged skin, by-passing the protective effect of the intact integument. The dose to establish infection may be as low as 100 TCID50, although dose of around 10^4-10^5 TCID50 may be required to give consistent results under experimental conditions (Alexandersen *et al.*, 2003b).

The intravenous inoculation appears to be less efficient and more variable than the intradermal/subdermal methods described, but it also results in a relatively short incubation period (Henderson, 1952). Intramuscular inoculation is a relatively inefficient method and may require a dose of 10^4 TCID50 or more (Burrows *et al.,* 1981; Donaldson *et al*., 1984).

2.8.1.6 Scarification

Although now rarely used experimentally, it is carried out by applying a suspension of virus to scarified skin (Alexandersen *et al.,* 2003b). The protective effect of intact integument was shown in an experiment in which exposure of the bovine tongue to $10^{7.8}$ infectious units for 10 minute did not lead to FMD unless the tongue was first scratched with a needle (Cottral *et al.,* 1965).

2.8.2 Incubation period of foot and mouth disease

The incubation period for FMD is highly variable, and depends on the strain and dose of virus, the route of transmission, the animal species and the husbandry conditions. The incubation period for farm-to-farm airborne spread and for farm-to-farm spread by indirect contact ranges from 4 to 14 days (Sellers and Forman, 1973). The incubation period for farm-to-farm spread resulting from direct contact may range from 2-14 days (Garland and Donaldson, 1990). For within farm spread the incubation period is generally 2-14 days but may be as short as 24h, especially in pigs and under very high challenge conditions (Kitching, 2002b; Kitching and Hughes, 2002). When spread is occurring within a herd or flock, the typical incubation period is 2-6 days, although this may be as short as 1day or as long as 14 days under certain conditions (Alexandersen *et al.,* 2003b).

Under experimental conditions, the mean incubation period was 3.5 days for continuous, direct cattle-to-cattle contact and 2 days for intensive sheep-to-sheep contact (Alexandersen *et al.,* 2002a; Alexandersen *et al.,* 2003a). Pigs were readily infected by direct pig-to-pig contact exposure, with a mean incubation period of 1-3 days depending on the intensity of contact (Alexandersen *et al.,* 2003a). These differences confirm the strong relation between dose and length of incubation period. Under field conditions the dose of FMDV will be influenced by many factors such as stocking density (intensive or extensive management), whether the animals are housed and if so, whether in well or poorly ventilated surrounding, the extent to which animals are handled and other common farming practices (Alexandersen *et al.,* 2003b).

2.8.3 Sites of primary infection

Several evidence points to the pharyngeal area as the usual primary site of infection except for those occasions when the virus gains entry into the skin or mucosa through cuts or abrasions (Alexandersen *et al.,* 2003b). The importance of the pharynx has been demonstrated for cattle by means of both direct and indirect contact infection, intranasal instillation and airborne exposure (Garland, 1974; McVicar and Sutmoller, 1976; Burrows *et al.,* 1981). The dorsal soft palate and the roof of the pharynx, just above the soft palate, are sites of particular significance. Some workers have demonstrated these same primary sites of infection for pigs infected by contact or airborne virus, and also for cattle infected by contact or by needle-inoculation using the rRT-PCR and in-situ hybridization (Zhang and Kitching, 2000; Zhang and Kitching, 2001; Alexandersen *et al.,* 2001; Oleksiewicz *et al.,* 2001). In sheep, Burrows (1968b) reported that the tonsils played a role of particular importance in the initial infection.

Virus may be demonstrated in the pharynx for 1-3 days before a vireamia can be detected (McVicar and Sutmoller, 1976; Burrows *et al.,* 1981; Alexandersen *et al.,* 2002b; Alexandersen *et al.,* 2003a) and is the means by which virus is distributed to secondary sites. Under certain circumstance, for example after injection of virus into the skin or tongue or when infection takes place through the damaged skin, replication will take place at the site of entry. After initial replication and spread through regional lymph nodes (Henderson, 1948) and into the circulation, a number of secondary sites will be infected especially the skin (both with and without hair) and the epithelia of the tongue and mouth, i.e cornified epithelia, where the main viral amplification occurs. There is no evidence to suggest that the lung is either a primary or secondary site of viral amplification (Burrows *et al.,* 1981; Alexandersen *et al.,* 2001). Nasal mucosa has also been suggested as a site of initial replication (Korn, 1957) but no evidence exists to support this hypothesis (Alexandersen *et al.,* 2003b).

2.8.4 Secondary sites of replication

The earliest sites of FMDV infection and replication in contact-exposed animals appear to be in the pharynx (Garland, 1974; McVicar and Sutmoller, 1976; Burrows *et al.,* 1981; Alexandersen *et al.,* 2003b). Viral replication may reach a peak as early as 2-3 days after exposure (Alexandersen *et al.,* 2001; Oleksiewicz *et al.,* 2001). Data indicate that after initial replication the virus enters through regional lymph nodes and into the bloodstream.

The greater part of the viral amplification occurs subsequently with the cornified stratified squamous epithelia of the skin (including the feet and mammary gland) and mouth (including the tongue), or in the myocardium of young animals (Alexandersen *et al.,* 2003b). Although some viral replication occurs in the epithelia of the pharynx, it is much less than in the skin and mouth during the acute phase of the disease. In comparison with the skin and mouth, other organs (e.g salivary glands, kidneys, liver, spleen, lymph nodes, lung and nasal mucosa) produce no more than negligible amounts of virus (Burrows *et al.,* 1981; Alexandersen *et al.,* 2001; Oleksiewicz, 2001). It has been suggested that a secondary phase of replication occurs in lymph nodes (Alexandersen *et al.,* 2003b). An *in vitro* study has indicated that FMDV infectivity may persist in macrophages for 10-24h (Rigden *et al.,* 2002). However, experimental investigation *in vivo* (Burrows *et al.,* 1981) suggest that lymph nodes as well as lymphocytes and macrophages (including alveolar macrophages) play little or no part in FMDV replication, and that any virus present in lymphoid organs is produced elsewhere, i.e. in the pharynx and the epithelia of the mouth and skin (Alexandersen, *et al.,* 2003b).

2.8.5 Excretion of virus by infected animals

All secretions and excretions become infectious during the course of the disease and some contain significant titres of virus before the development of clinical signs (Hyslop, 1965; Scott *et al.,* 1966; Sellers *et al.,* 1969; Sutmoller and McVicar, 1973; Garland, 1974). Thus, saliva, nasal

and lachrymal fluid, milk and expired breath may contain virus during the prodromal period. Urine, feaces also contain virus but to a lesser extent. It appears that faeces contain only small amount of virus (Parker, 1971), but once voided they are likely to be contaminated further by admixture with desquamated lesion material, vesicular lymph, saliva, milk and urine.

In an experimental study of steers indirectly exposed to infected pigs and sampled daily for virus isolation, it was found that oral saliva and probang samples from the pharynx were first to contain new virus isolation, it was found that oral saliva and probang samples from the pharynx were first to contain new virus (Garland, 1974). Studies in sheep demonstrated that virus was detectable in the breath 1-2 days before the appearance of clinical signs (Sellers and Parker, 1969) and subsequently in the blood, followed by the nasal fluid. By contrast, the peak of airborne viral excretion in cattle and pigs occurred during the viraemic phase, after generalized lesions had developed (Donaldon *et al.,* 2001; Alexandersen and Donaldson, 2002).

Virus is also excreted in milk and semen (Burrows, 1968a) from shortly before clinical signs appear and through the clinical phase, in a pattern that largely mirrors the viraemia profile. Large amounts of virus are excreted in vesicular fluid, in desquamated vesicular epithelium and, in cattle, also in saliva (Hyslop, 1965; Scott *et al.,* 1966). There is also excretion, but to a much lesser extent, in faeces (Burrows, 1968a; Parker, 1971; Garland, 1974), in a pattern that reflects the peak of viraemia, lesion and clinical disease. A sharp decline in viral excretion and load occurs around day 4-5 of clinical disease, when a significant circulating antibody response is detectable. However, it is important to note that although all secretions and excretions (other than oesophageal-pharyngeal fluid in ruminants) are free of detectable infectivity at 10-14 days post-infection, virus already excreted during the pre-clinical and acute clinical phase can survive in the environment for weeks or even months (Alexandersen *et al.,* 2003b), depending on the

nature of material contaminated (manure, fodder, bedding, foot wear, clothing, equipment, vehicles other formites) and the ambient conditions (weather, moisture, relative humidity, temperature, pH). There have been isolated reports of survival of virus for extended periods (e.g on hay for at least 200 days, and in faecal slurry for 6 months (Hyslop, 1970), few quantitative data are available on the persistence of FMDV in the environment (Cottral, 1969; Sanson, 1994; Donaldson, 1997). In general terms, the environmental factors that favour survival of virus are moist conditions, a neutral pH and low temperature (Alexandersen *et al.*, 2003b).

2.8.6 The antibody response

The FMDV elicits a rapid response in either infected or vaccinated animals. Virus-specific antibodies protect animals in a serotype-specific manner against reinfection, or against infection in the case of vaccination, and protection is generally correlated with high levels of neutralizing antibodies (McCullough *et al.*, 1992). The strong association between the capacity of a vaccine to induce FMDV-specific antibodies and the capacity to protect against challenge has been documented (Hamblin *et al.*, 1986; Van Maanen, 1990). Antibody response is directed to epitopes on the three external structural proteins, and good protective immunity is apparent between 7 and 14 days after either infection or vaccination.In cattle, the immunoglobulin G1 (IgG1) response predominates over IgG2 (Mulcahy *et al.*, 1990; Salt *et al.*, 1996a; Capozzo *et al.*, 1997), and antibody, including IgA, can be detected in upper respiratory secretions early in infection (Salt, 1993; Salt *et al.*, 1996a). The neutralization of virus within the host may occur by mechanisms similar to those occurring in *in vitro* neutralization; however, there is a suggestion that macrophages may play a role in clearing the virus from the infected animal by phagocytosis of opsonized virus (McCullough *et al.*, 1988; McCullough *et al.*, 1992; Ridgen *et al.*, 2003). The efficiency with which macrophages can phagocytose FMDV is enhanced when the virus is complexed with antibody of the appropriate specificity and affinity (McCullough *et al.*, 1992).

Specific T-cell antiviral responses, involving CD^{4+} and CD^{8+} cells, have been observed in cattle and swine following either infection or vaccination (Garcia-Valcarcel *et al.,* 1996; Childerstone *et al.,* 1999; Bautista *et al.,* 2003), and it has been suggested that cell-mediated immunity is involved in clearance of virus from persistently infected animals (Hott *et al.,* 1997; Childerstone *et al.,* 1999).

The induction of anti-FMDV antibody correlates with a lymphoproliferative response in cattle and swine (Collen and Doel, 1990; Saiz *et al.,* 1992), and is T-cell dependent in mice (Collen *et al.,* 1989). A study of the early acute phase of FMDV infection of swine, prior to the detection of antibody, demonstrated a transient lymphopenia by 2 days after infection involving CD^{4+}, CD^{8+}, CD^{4+}/CD^{8+} T cells or apoptosis and thus may be caused by alteration of lymphocyte trafficking (Bautista *et al.,* 2003). In addition, T-cell function, as measured by response to mitogens, is either reduced or eliminated (Bautista *et al.,* 2003). Both the number of lymphocytes and the altered T-cell function return to normal levels by 4 days after infection. These results suggest that T-cells play a role in virus protection and that reduction of both T-cell numbers and function enhances viral pathogenesis by allowing the virus to spread within the host, leading to increased viral shedding into the environment. A number of studies have shown that interferon (IFN)-α,-β, and -γ may be involved in the host defence against FMDV infection (Brown *et al.,* 2000; Alexandersen *et al.,* 2002c; Zhang *et al.,* 2002). Other cytokines, in addition to the IFNS may also play a role in the host response. Studies of swine immunized with a conventional FMD vaccine shows that vaccinated pigs did not appear to exhibit a systemic inflammatory response, but chemotactic activity of plasma on peripheral blood leukocytes increased within the first week after immunization (Rigden *et al.,* 2003). Furthermore, in pigs that either were only vaccinated or were vaccinated and challenged, levels of interleukin-6 (IL-6), IL-8, and IL-12 in plasma

increased after vaccination and/or challenge, suggesting monocyte/macrophage activation (Barnett *et al.*,2002a). Although the levels of IL-6 and IL-8 did not appear to be related to protection of pigs upon challenge, IL-12 levels were higher in vaccinated pigs, which were protected from contact challenge, suggesting a role for cytokine-induced monocyte cell activity in protection from acute-phase disease (Barnett *et al.*, 2002a).

2.8.7 Survival of food mouth disease virus

Foot and mouth disease virus is moderately stable but can readily be inactivated by appropriate disinfectants and heat (Alexandersen *et al.*, 2003b). In general, most strains are stable within the pH range 7.0-8.5, especially at lower temperature, but increasingly labile at pH values outside this range (Bachrach *et al.*, 1957; Bachrach, 1968). Foot and mouth disease virus is resistant to detergents and organic solvents such as ether and chloroform. The acidity produced in carcass meat during rigor mortis in cattle will inactivate the virus. Such acidity is variable in pig meat (pork) and has not been examined in detail for sheep meat (Alexandersen *et al.*, 2003b), The pH in bone marrow, lymph nodes and certain organs and offal does not decline during rigor mortis; virus can therefore be found in such material (especially if refrigerated or frozen) for an extended period of time, and may cause new outbreaks if fed to livestock as unheated waste food (Donaldson, 1987).

Airborne virus is stable at humidity above 55-60% and drying will inactivate most of the virus. The drying of fluids or organic materials containing virus will also inactivate a relatively large proportion, but surviving virus may be more stable after drying, thereby creating a residual infectivity. Sunlight has little or no direct effect on infectivity; any loss is indirect and occurs mainly through the effects of drying and temperature (Donaldson, 1987). Survival of the virus in the environment will depend on the nature of the material (e.g. desquamated epithelium, secretions, excretions),the initial concentration of virus in the material, the strain of the virus, the

humidity, the pH and the temperature, and will therefore be highly variable under field conditions (Alexandersen *et al.,* 2003b). Several survival times of FMDV have been reported; up to 20 weeks on hay or straw; up to 4 weeks on cow's hair at 18-20°C; up to 14 days in dry feaces; up to 39 days in urine; up to 6 months in slurring in winter; 3days on soil in summer and up to 28 days in autumn (Alexandersen *et al.*, 2003b).

2.9 Clinical Variation in Foot and Mouth Disease

All species of cloven-hoofed animals are susceptible to FMD, including domestic livestock and wild ungulates such as buffalo, antelope and warthogs. Clinical signs are essentially similar in all species although the severity may vary considerably (MAFF, 1986). The disease is characterized by fever and vesicles (blisters), which progress to erosions in the mouth, nares, muzzle, feet or teats. Typical clinical signs include depression, anorexia, and excessive salivation, serous to mucopurulent nasal discharge, lameness and reluctance to move. Abortion may occur in pregnant animals due to high fever (FMDV does not cross the placenta). Death in young animals is due to severe myocardial necrosis (CFSPH, 2007).

Foot and mouth disease virus strains may differ in their virulence for different species e.g, the O Taiwan 1997 strain caused severe lesions in pigs, but no clinical disease in ruminants (Dunn and Donaldson, 1997). The marker for the severe virulence of the O Taiwan 1997 strain for pigs and the absence of virulence for cattle is associated with changes in the 3A gene of the virus (Knowles *et al.*, 2001a). Virulence of FMDV strains may also vary between breeds of animal and sometimes within a breed, probably because of genetic or physiological factors (Alexandersen *et al.*, 2003b).

In cattle, clinical signs are generally obvious, an initial pyrexia of up to 40°C lasting one or two days is followed by the development of vesicles on the tongue, hard palate, dental pad, gums, lips, muzzles, coronary band, interdigital space and teats. Profuse salivation, mucoid to

mucopurulent nasal discharge, loss of condition and drop in milk yield may also occur in infected cattle (Kitching, 2002b). The vesicles in the mouth rupture rapidly, usually within 24 hours, leaving shallow erosions surrounded by shreds of epithelium. On the tongue, the vesicles coalesce and may displace a large proportion of the dorsal epithelium. The vesicles may remain intact on the feet for two or three days before rupturing, depending on the terrain. The rupture of vesicles, especially on the feet or teats, may predispose the affected areas to secondary infections, which may complicate and prolong the healing processes (Alexandersen *et al.*, 2003b).

Although animals will generally recover from the acute clinical phase of the disease there may be permanent loss of performance e.g. chronic lameness, permanent drop in milk yield, poor weight gain and poor quality 'staring' coat. A large proportion of cattle will go on to become persistently infected carriers of FMD (AVIS, 2002).

Clinical disease is usually severe in pigs, and the early signs include acute lameness, blanching of the skin around the coronary band, reluctance to stand, adoption of a dog-sitting posture, depression, loss of appetite and fever (Kitching and Alexandersen, 2002). The lesions of the feet may include the shedding of claws ("thimbling"), and the accessory digits may be affected, as well as pressure points on the knees and hocks (Alexandersen *et al.*, 2003b). The severe lesions of the coronary bands may often lead to a separation of the horn during the acute inflammatory state. This results in the formation of a ring in the horn that becomes visible below the coronary band approximately 1week after the first appearance of clinical disease (Alexandersen *et al.*, 2003b). In pigs, mouth lesions are not as common as in cattle and when present are most often located on the tongue, either far back on the dorsum or as tiny lesion at the tip (Alexandersen *et al.*, 2003b).

In sheep and goats the signs may be severe but are generally much more subtle than in pigs and cattle. In mild cases the lesions are superficial and transient, and heal rapidly, with lameness being the first indication of the disease in these species (Kitching and Hughes, 2002; Alexandersen *et al.,* 2003b). An affected animal develops fever and may separate itself from the rest of the flock. However, under field situation, lameness due to other causes may conceal the presence of FMD (Kitching and Hughes, 2002). Serological field surveys and experimental investigations have shown that FMD in small ruminants may be clinically inapparent in a significant proportion of animals (Donaldson and Sellers, 2000). Vesicles may develop in the interdigital cleft, on the heel bulbs and on the coronary band, but they rupture rapidly and their appearance may be hidden by the coexisting presence of foot rot (Kitching and Hughes, 2002). Fluid filled vesicles, which are readily seen in cattle, are rarely observed in the mouth of small ruminants, probably because the thinness of the lingual epithelium causes superficial lesions to rupture early, leaving shallow erosions, most commonly on the dental pad, adjacent to the incisors, but also on the tongue, hard palate, lips and gums, which usually heal within a few days (Hughes *et al.,* 2002; Alexandersen *et al.,* 2003b). Vesicles may also be seen on the teats of milking sheep and goats and rarely on the vulva and prepuce (Kitching and Hughes, 2002). Vesicular disease may not develop in some infected sheep (Gibson *et al.,* 1984; Hughes *et al.,* 2002).

The age of lesions can be assessed be examining the stage of their development according to the following established criteria (Alexandersen *et al.,* 2003b): development of vesicles from days O to 2; rupture of vesicles on days 1-3 (initially having fragments of epithelia attached); followed by sharply marginated erosion (day 2-3); with the sharpness lost from day 3; serofibrinous

exudation on days 4-6; and the beginning of repair with a marked fibrous tissue margin at 7 or more days.

2.10 Pathology

The characteristic lesions of FMD are single or multiple fluid-filled vesicles/bullae from 2mm to 10cm in diameter. Early lesions range from a small pale area to a fluid filled vesicle, sometimes coalescing with adjacent lesions to form bullae (Kitching, 1992). The vesicles rupture leaving red eroded areas, which may then be covered with a gray fibrinous coating. This coating becomes yellow, brown or green and is replaced by new epithelium with a line of demarcation that gradually fades. Occasionally, there may be lesions which appear necrotic instead of vesicular. “Dry” lesions are more common in pig oral cavity (CFSPH, 2007).

The location and prominence of FMD lesions varies with the species. In cattle, numerous erosions, ulcers or vesicles may be found in the oral cavity (Kitching, 2002b). In pigs, sheep and goats, these lesions may be more common on the heel, coronary band and interdigital cleft of the feet (Kitching and Alexandersen, 2002; Kitching and Hughes, 2002). Some lesions may extend to the feet (Kitching, 1992). Coronitis may be seen on the hooves, and animals with severe disease may slough their hooves or claws. In addition, vesicles may be found in other locations including the teats or udder; pressure points of the legs, ruminal pillars, prepuce or vulva (CFSPH, 2007). In young animals, cardiac degeneration and necrosis can cause gray or yellow streaking in the myocardium; these lesions are sometimes called “tiger heart” lesions (Donaldson and Seller, 2000; Kitching, 2002b; Kitching and Alexandersen, 2002; Kitching and Hughes, 2002).

2.11 Epidemiology

Foot-and mouth disease was found worldwide after World War II. The last U.S. outbreak was in 1929. Endemic areas are Asia, Africa, the Middle East, and parts of South America. Epidemics

have occurred in recent years in Taiwan, 2000; South Korea, 2002; Japan, 2000; Mongolia, 2000; Uruguay, 2000; Russia, 1995; China, 2000; Britain, 2001, 2003 and 2007; France, 2001 and the Netherland, 2001 (Leforban and Gerbier, 2002; Correa Melo *et al.*, 2002; Sakamoto and Yoshida, 2002). North and Central America, Australia, and New Zealand have been free for many years (CFSPH, 2007).

Foot and mouth disease endemic areas of the world are high-risk zones for introducing FMD to countries free of the disease and for the origin of new FMDV lineages. Therefore, a continuing surveillance of FMDV is needed for the early recognition and understanding of emerging risks or changes in the global FMD situation (Klein, 2009). Foot and mouth disease is endemic in nearly all countries of Africa, but majority of outbreaks are unreported (Ferris and Donaldson, 1992) because of inadequate/non-existent diagnostic laboratories (Vosloo *et al.*, 2002). Due to the extensive livestock-raising systems practiced in most parts of Africa, FMD is viewed differently compared to the more developed parts of the world in which intensive livestock production system is practiced and highly vulnerable to the effects of FMD (Vosloo *et al.*, 2002). Factors such as lack of infrastructure and financial resources, ineffective animal health authorities and political instability in most African countries contribute to their inability to face trans-boundary diseases. Also, most government in these countries give low priority to animal diseases and control compared to other sectors of the economy. Due to the recent global food/economic crisis, there is the realization that efficient livestock production is necessary to ensure food safety and economic development in the longer term.

In Africa FMDV is maintained by cattle and African buffalo. Available evidence indicates that although other domestic and wild species become infected, they are unable to maintain the infection for more than a few months in the absence of cattle and African buffalo (OIE 2012). In

Southern Africa, with the exception of Angola, the SAT1, SAT2, and SAT3 serotypes are almost exclusively prevalent, however, serotypes O and A have been reported (Knowles and Samuel, 1998; Sangare *et al.,* 2001). In Central Africa, Serotypes O, A, SAT1 and SAT2 have been responsible for most outbreaks of FMD. Serotypes SAT3 and C has been reported in this region. Outbreaks in cattle due to five serotypes prevalent in Africa (A, O, C, SAT1 and SAT2) have been recorded in East Africa, although SAT3 was once reported in a carrier buffalo in Uganda (Hedger, *et al.,* 1973, Kitching, 2002a). In West Africa, only serotypes O, A, SAT1 and SAT2 were recorded between 1958 and 2008 (Ferris and Donaldson, 1992). A lack of the knowledge of the epidemiology of FMD viruses negatively affects the ability to control the disease in this sub region (Bizimana, 1994). Also, in this region, the disease is endemic in livestock and outbreaks occur regularly in the absence of any known wildlife maintenance hosts. It is therefore possible that the disease is maintained in small ruminants and even cattle during inter-epidemic periods (Sangare *et al.,* 2003).

The presences of large numbers of nomads who migrate with their livestock probably also play a major role in spreading infection across country borders. This is supported by genetic studies of types A, O and SAT2 that have shown that a single epizootic often affects two or more neighboring countries in West Africa (Knowles and Samuel, 1998; Bastos and Sangere 2001; Sangere, *et al.,* 2001).

In Nigeria, FMD was first reported in 1924 as sporadic outbreaks attributed to type O virus (Libeau, 1960). Other outbreaks have been reported by Nawathe and Goni, (1976); Owolodun, (1971), and Durojaiye, (1981). These outbreaks resulted in the identification of other serotypes (A, SAT1 and SAT2) but surprisingly no new report of serotype O has been made ever since the first until recently when serotypes O and SAT2 were recorded (Knowles *et al.,* 2008).

Interestingly, the type O virus reported was found to be related to type O viruses from Sudan whilst the SAT2 viruses were related to a SAT2 type in Sudan and Niger republic (Knowles *et al.,* 2008), suggesting that each new serotype was associated with trade cattle entering Nigeria from neighboring countries. Serotypes A and SAT1 was last reported in Nigeria in 1979 and 1981 respectively (Ferris and Donaldson, 1992), however, report of nucleotide sequence of serotype A have been documented recently (Knowles*et al.,* 2008).

In the transhumance husbandry system in West Africa (Nigeria in particular), where livestock move freely over a long distance within and across international borders, and frequently intermingle with other domestic livestock and possibly wildlife on route, due to the concentration of stock at rivers, watering points and lake shores, where grazing is possible during the driest part of the year, the risk of exposure and virus transmission due to contacts increases. Worldwide, cross-border cattle movements have been shown to be one of the main reasons for the spread and persistence of FMD (Rweyemamu *et al.,* 2008; Sumption *et al.,* 2008; Khounsy *et al.,* 2009). Several studies that demonstrate the link between livestock trade and the geographical spread of FMDV have been documented (Gibbens and Wilesmith, 2002; Perry *et al.,* 2002; Ortiz-Pelaez *et al.,* 2006). In Nigeria, there is extensive unregulated cross-border livestock (cattle, sheep and goats) movements most especially during religious festivals , where thousands of these animals are imported from neighboring countries of the West (Niger) and Central (Chad, Cameroon) Africa. Religious practices, insufficient livestock production to meet local demand, and price differences encourage livestock trade into Nigeria

2.11.1 Role of small ruminants in the epidemiology of foot and mouth disease

Although some areas of the world with large sheep and goat populations, such as Australia, New Zealand, North America and parts of South America, continue to remain free from FMD, the disease is endemic or sporadic in many of the countries of Asia, Africa and Eastern Europe

(Kitching, 1998). During the late 1980s and early 1990s an epidemic spread across North Africa from Libya and Tunisia to Morocco. The virus, type O, is believed to have originated from the Middle East and to have had predilection for sheep (Taylor and Tufan, 1996). Outbreaks also occurred in Turkey during 1995 and 1996 (Kitching, 1998), in which 20% of the total cases were associated with sheep and goats (Taylor and Tufan, 1996). In recent years, Great Britain and most other EU members have been free from FMD, the exceptions being Italy, which reported 57 outbreaks in 1993, and Greece, which had epidemics in 1994 and 1996 (Kitching, 1998). The 1994 Greek epidemic originated from the movement of infected sheep from the island of Lesvos. The causal agent was a type O, similar to endemic strains in the Middle East. A total of 95 outbreaks were finally recorded.

The epidemic was controlled by 'stamping out' and movement restrictions. No evidence of a predilection of the strain for sheep and goats (as seen in the Middle East) was recognized, as cattle present on affected premises showed high morbidity. During the course of the outbreaks, 12450 sheep, 4738 goats, 1241 cattle and 139 pigs were killed, but no vaccination was implemented. Thirty-nine outbreaks of FMD type O also occurred in Greece during 1996, probably as a consequence of the illegal importation of live sheep from turkey. Approximately 5000 sheep and goats, 1800 cattle and 30 pigs were destroyed from clinically affected flocks/herds or following contact with infected animals. These outbreaks serve to demonstrate several important points: firstly, the role of the Middle East as a reservoir of FMD, and the potential for spread from the region (via Eastern Europe and countries that border the Mediterranean to the rest of Europe); and secondly, the probable role of sheep and goats in disease transmission (88.4% of the region's livestock are small ruminants) (Anon., 1993). The large number of live animal imports into the Middle East from FMD endemic areas results in the

frequent introduction of new strains of virus (Yadin and Cloudia, 1995). Strains that circulated during the 1990s seemed to have a predilection for sheep and goats although they spread into the dairy herds, in spite of regular vaccinations (Kitching, 1994). Studies in Nigeria (Obi &Newman, 1988; Ehizibolo, 2010) and South America (Fernandez *et al.*, 1975a; Fermandez *et al.*, 1975b), where communal farming of cattle, sheep and goats also occurs, identified a high prevalence of antibodies against the virus-infection-associated (VIA) antigen (indicative of active infection with FMDV rather than vaccination) in sheep and goats in the absence of clinical signs. This suggests that small ruminants have an important role in the epidemiology of FMD elsewhere in the world.

2.11.2 Role of wildlife in the epidemiology of foot and mouth disease

The presence of large numbers of wildlife, particularly in Southern and East Africa complicates the situation of FMD. Some of these wildlife species act as intermidiaries in disease transmission whilst buffalo have been shown to be able to maintain and transmit SAT type viruses to other cloven-hoofed animals in close proximity (Bastos *et al.*, 2000; Condy *et al.*, 1985; Hargreaves *et al.*, 2003). African buffalo (*Syncerus caffer*) act as maintenance hosts of the SAT type viruses, with individual animals maintaining the virus for up to 5years, and isolated herd for up to 24years (Condy *et al.*, 1985) although persistence in individual buffaloes is probably not lifelong (Hedger, 1976).

With buffalo populations, SAT viruses are efficiently maintained, but the disease typical of FMD is rare (Thomso*n et al.*, 2001). Also, carriers ensure persistence of the viruses, thus transmission of the virus from acutely infected buffalo as well as carriers is well established (Vosloo *et al.*, 1996). Sexual transmission between carrier animal is also possible (Bastos *et al.*, 1999). Wherever African buffalo populations are present, SAT type viruses are likely to occur.However, wildlife is scarce in the arid regions of North-East Africa and West Africa, but it

has not been proved how SAT1 and SAT2 viruses are maintained in inter-epidemic periods. Domestic animals probably maintain the infections independently of the wildlife agent, just as the case for serotypes O and A (Vosloo *et al.*, 2002).

Although impala (*Aepyceros melampus*) and other antelopes are susceptible to FMDV, they are not capable of maintaining SAT viruses for long period of time, and there is no evidence that they become carriers (Thomson, 1997). Therefore, species of wildlife other than buffalo are probably incidental hosts and their importance in the epidemiology of FMD in regions where they are found lies in their ability to serve as intermediaries in the transmission of SAT viruses from buffalo to domestic livestock (Bastos *et al.*, 2000).

2.11.3 Role of carrier cattle in the epidemiology of foot and mouth disease

The carrier is defined as an animal from which livevirus can be recovered after 28days following infection (Kitching, 2002b). Over 50% of ruminants exposed to live FMDV become carriers (Donaldson, 1987). The establishment and duration of carrier state depends on the host species, strain and serotype of FMDV and even the breed of host species (Kitching, 2002b). The African buffalo may carry virus for over 5years, cattle for over 3years, sheep for up to 9months (Salt *et al.*, 1996b), goats and wild ruminants for shorter period and for South American camelids, no carrier state exists (David *et al.*, 1993). The virus persists in the basal layer cells of the pharyngeal epithelium, particularly of the dorsal soft palate (Zhang and Kitching, 2001).

The mechanism by which the virus changes from a lytic agent, which destroys the host cells, into one that can establish a persistent infection is not known. A mutation possibly reduces the ability of the persistent virus to shut down host cell metabolism. A further back-mutation may then restore the lytic action of the virus, resulting in elimination. However, this is not yet proven.

The SAT viruses occasionally spread out of Africa into the Middle East and most recently into Saudi Arabia (Kitching, 2002b), however, although the serotypes A, O and Asia 1 persist in this

regions, in spite of limited attempts at control, the SAT viruses die out. This implies that the cattle, sheep and goats are unable to maintain the SAT serotypes, or conversely, these serotypes require a particular host species (African buffalo) to be maintained. Similarly, the distribution of Asia 1 serotype suggests that the virus has been constrained from establishing itself outside Asia (Kitching, 2002b).Whether the geographical restriction of serotypes and even strains of FMDV is related to the ability of the virus to establish a persistent state of infection (Carrier state) in particular susceptible species or breed is not known, but if that is the case, it presents a powerful tool for considering the importance of the carrier in the epidemiology of FMD.

Field observations have indicated that carrier cattle can transmit FMDV and also precipitate new outbreaks of the disease (Vosloo *et al.,* 1996; Thomson, 1997). This underscores the need for caution in the movement of cattle that have been previously infected with FMD. The definitive identification of carrier animals requires the recovery of FMDV from live animals. In these animals, the epithelium of the pharynx is the predilection for the virus and a probing cup can be used for sampling for virus isolation (Kitching, 2002b).

The test for antibodies to non-structural proteins (NSPs) is a significant advance in the detection of carrier animals. Carrier animals also have antibodies to FMDV which can be detected in the serum (OIE, 2012) and in the saliva (Kitching, 2002b).

2.11.4 Molecular epidemiology

Foot and mouth disease viruses display high levels of genetic and antigenic variation (Sobrino *et al.,* 2001). The molecular epidemiology of FMD has been studied by the phylogenetic analysis of partial nucleotide sequences of the viral genome (Knowles and Samuel, 1995). Generally, phylogenetic analysis is a statistical inference of relationship, using a probability model of nucleotide and amino-acid substitution (Evolutionary model) (Klein, 2009). The most studied genome region is the VP1-the most variable of the capsid polypeptides. RNA sequence

differences between the VP1 genes of the 7 serotypes range from 30-559 and within a serotype, different sub-groups (topotypes) are defined by difference of up to 15 to 20% (Knowles and Samuel, 1998; Samuel and Knowles, 2001a). Phylogenetic analysis was used to define the genetic relationship between Nigerian FMD viruses and those collected from neighboring countries. Sequence coding of the FMDV VP1 protein has been used to investigate the relationship between different isolates of the virus (Beck and Strohmaier, 1987). As a rule of thumb, viruses with <5% of nucleotide differences in the VP1 sequence are considered as the same strain (Samuel and Knowles, 2001) and those with >15% as unrelated (Samuel *et al.*, 1988), although because of the greater variation in the SATs, a higher threshold of 20% has been suggested (Knowles and Samuel, 2003). From the phylogenetic neighbor-joining trees constructed, it was possible to infer the genetic relationships of isolates, and how FMD viruses could be dispersed between countries in sub-Saharan Africa at different times and places.

The serotypes of FMDV have an average of 86% nucleotide sequence identity to each other across the whole genome, (Fry *et al.,* 2005) but the VP1 coding region is substantially more variable and shows only about 50-70% identity (Knowles and Samuel, 2003). Serotypes O, A, C and Asia-1 have been further classified into genotypes based on up to 15% difference in VP1 coding sequences (Knowles and Samuel, 2003; Jamal *et al.,* 2011). Phylogenetic analysis of genome region that lie outside the outer capsid coding genes do not correlate with serotypes and are therefore less useful for epidemiological studies. Pirconavirus genome can undergo intra and inter-typic homologous recombination (King *et al.*, 1985; McCahon *et al.*, 1985; King, 1988) and it had been suggested that cross over sites might be located in areas of high RNA secondary structure (Wilson *et al.*, 1988).

A sequence data-base of FMDV is held at the World Reference Laboratory for FMD (WRLFMD) located at Institute of Animal Health Laboratory Pirbright, UK, which currently has more than 2000 partial or complete VP1 sequences representing all 7 serotypes. Sequences of new isolate are compared with those stored at the database and with which phylogenetic trees are constructed. This enables the laboratory to monitor movement and trace the origin of different virus strain. For example it is documented that the causative strain of the 2001 outbreak of FMD in UK is of Asian origin which was noticed in 1994 in Saudi Arabia. It was a genetic lineage that had not been previously identified (Samuel and Knowles, 2001a).

2.12 Diagnosis of Food and Mouth Disease

2.12.1 Field diagnosis

Foot and mouth disease is diagnosed based on clinical signs (high temperature, excessive salivation, formation of vesicles on mouth, nose, interdigital spaces and coronary band on the feet).However, clinical signs cannot be distinguished from vesicular lesions caused by vesicular stomatitis and swine vesicular disease (Bachrach, 1968; Kitching, 2002a; Kitching and Alexandersen, 2002; Jamal and Belsham, 2013), and thus laboratory diagnosis is necessary. In addition, FMDV infection of sheep and goats can be difficult to detect clinically (Geering, 1967; Donaldson and Sellers, 2000). Furthermore, the determination of the serotype involved in field outbreaks cannot be ascertained on the basis of clinical signs, but has to be established within laboratories to permit proper control/vaccination programs to be followed. Also, sensitive diagnostic assays are necessary to distinguish vaccinated from infected or convalescent animals, for trade purposes for countries that may have used vaccination as part of their control program and to identify carrier animals (Grubman and Baxt, 2004).

2.12.2 Differential diagnosis

Foot and mouth disease cannot be distinguished clinically from other vesicular diseases including vesicular stomatitis, swine vesicular disease and vesicular exanthema (Kitching, 1992).

In domesticated animals, the symptom may also resemble foot rot, traumatic stomatitis, and chemical and thermal burns. In cattle, oral lesions can resemble rinderpest, infectious bovine rhinotracheitis, bovine viral diarrhea, malignant catarrhal fever and epizootic hemorrhagic disease. In sheep, the lesions can be confused with blue tongue, contagious ecthyma, and lip and leg ulceration(Kitching, 1992).

2.12.3 Laboratory diagnosis

Diagnosis of FMD is by the demonstration of FMD viral antigen or nucleic acid in samples of tissue or fluid and the detection of specific humoral antibody response in the absence of any history of vaccination (OIE, 2012).Prompt sensitive and specific laboratory diagnosis and identification of the serotype of the viruses involved in disease outbreak is essential due to rapidity of spread of FMD and the serious economic consequences that can arise (Jamal and Balsham, 2013).

2.12.4Methods of laboratory diagnosis

Various techniques have been used to diagnose FMD and to ascertain the serotype of the virus. Some of the diagnostic methods are described below:

2.12.4.1 Identification of agent

This involves tests for antigen, the virus and genome.

2.12.4.2 Antigen enzyme-linked immunosorbent assay (AgELISA)

The complement fixation test (CFT) was the test of choice for diagnosis and typing of FMDV until 1970s. However, in order to overcome the problems of its low sensitivity and difficulty in interpretation of its results due to pro-and anti-complement activities, it has largely been replaced by the enzyme-linked immunosorbent assays (ELISAs) for antigen detection and virus typing (Roeder and Le Blank Smith, 1987). Given a satisfactory sample, a positive result for FMD can be obtained in 3-4 hours by an antigen ELISA (Ag ELISA), which also identifies the virus

serotype present (Hamblin *et al.*, 1984; Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988; Ferris *et al.*, 1990). However, less satisfactory samples may yield weak, inconclusive or negative results, and small amounts of virus must be amplified in cell cultures.

Monoclonal antibody (Mab)-based ELISAs have also been developed for diagnosis of FMD and virus typing in epithelial tissue suspensions (Morioka *et al.*, 2009; Chen *et al.*, 2012). Recently, a sandwich ELISA using recombinant integrin αvβ6 (a receptor for FMDV) for virus capture and serotype–specific monoclonal antibodies as detecting reagents was compared with the conventional polyclonal antibody-based sandwich ELISAs for the identification and serotyping of all the seven types of FMDV. The integrin/Mab ELISA recognized FMDVs of wide antigenic and molecular diversity from all seven serotypes. Although some FMDVs could not be detected, the assay showed greater specificity than the conventional polyclonal ELISA while retaining test sensitivity (Ferris *et al.*, 2011).

2.12.4.2 Virus isolation

The concentration of the virus in the sample may be too low to be detected by ELISA; it has to be amplified in susceptible cell cultures. Primary cultures (such as bovine thyroid cells and porcine or ovine kidney cells) or cell line (such as BHK or IBRS-2) are considered to be generally suitable for isolation (De Castro, 1964; De Castro and Pisani, 1964; Snowdon, 1966; OIE, 2012). Cultures showing a cytopathogenic effect are confirmed by Ag ELISA of the cell culture supernatants (Hamblin *et al.*, 1984; Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988; Ferris *et al.*, 1990). For most FMDV strains the bovine thyroid (BTY) cell system is about 10times more sensitive than other cultures (Snowdon, 1966; Burrows *et al.*, 1981), however, certain pig-adapted strains e.g. the O Taiwan 1997 strain, grow more easily in IBRS-2 cells (Dunn and Donaldson, 1997). Virus isolation in BTY and IBRS-2 cell cultures

essentially detects all positive samples with more than 1-5 infectious units per ml or per 0.1g, however, if poor specimens are submitted, or if the transport conditions are less than optimal, a small proportion of samples may give negative results for infectivity but positive results by ELISA or RT-PCR, as seen in UK samples in 2001 (Alexandersen *et al.*, 2003b). The production of consistent quality, ready-for-use primary cells is laborious, time-consuming and expensive. Furthermore, virus isolation requires the presence of infectious virus, which depends on sample quality. Two 48 hours passage (i.e 4 days) of each test inoculums may be required before a final result can be declared. Moreover, some FMDVs fail to grow in a specific cell type. Thus absence of apparent growth does not mean absence of the virus and therefore suspected samples have to be further investigated using another testing system (Jamal and Belsham, 2013). Additional disadvantages include the problems associated with obtaining and maintaining a regular supply of cells; possible contamination of cell cultures and the necessity to confirm any apparent virus growth by ELISA. All these may delay the initiation of control measures to contain outbreaks (Jamal and Belsham, 2013).

2.12.4.3 Reverse transcription-polymerase chain reaction

The reverse transcription-polymerase chain reaction (RT-PCR) offers the advantage of fast, sensitive and reliable diagnostic tool for FMD. A variety of RT-PCR methods have been reported in recent years for the early detection of FMDV RNA in epithelium, cell culture isolates and other tissues using universal primers for all seven serotypes (Meyer *et al.*, 1991). Typing of FMDV by RT-PCR was first demonstrated by Rodriguez *et al.* (1992) for the differentiation of the serotypes O, A and C. Serotype specific primers have since been designed for the detection of all seven serotypes by RT-PCR (Vangrysperre and De Clercq, 1997; Callens and De Clercq, 1997). Primers designed for these assays target various regions of the virus genome, including the 5´ UTR, the open reading frame and the 3´ UTR. However, evaluation of available sets of

primers, designed for universal and serotype-specific diagnosis of FMDV, on a variety of field samples, representing all the seven serotypes of FMDV, has shown that no single primer set is capable of diagnosing the disease or typing of the virus. In order to improve the diagnostic sensitivity of RT-PCR, multiplex assays, incorporating more than one set of primers have been developed (Giridharan *et al.,* 2005; Bao *et al.,* 2008).

However, differentiation/serotyping could only be made for certain groups of serotype or individual isolates. Thus the conventional RT-PCR is not sufficiently sensitive and specific to replace methods using virus propagation in cell culture and ELISA. A real time/quantitative RT-PCR (rRT-PCR) methods have recently been developed which do not require post-PCR processing (e.g gel analysis) and the signals can be monitored directly as the target cDNA is being amplified. Other advantages of the rRT-PCR include high thoroughput capability and the ability to quantify the genetic material in the starting sample. A TaqMan assay has been shown to be very robust and as effective for primary detection of FMDV as virus isolation in conjunction with antigen ELISA (Reid *et al.,* 2003). Currently, two different rRT-PCR TaqMan assays are in common use, one targeting the internal ribosomal entry site (IRES) within the 5´UTR (Reid *et al.,* 2002) and the second targeting the 3D (RNA polymerase) coding sequence (Callahan *et al.,* 2002).

The speed and accuracy of detection of the rRT-PCR assay was further improved by coupling the assays with robotic methods for extraction of nucleic acid from the samples and for set up of the assays (Jamal and Belsham, 2013). For this reason, the assay has become highly suitable for the diagnosis of the primary index case and for use in an ongoing outbreak. The rRT-PCR assays are currently used as a routine test for FMD diagnosis and quantification of the virus in many developed countries. However, these assays are not designed to discriminate between serotypes

of FMDV. Although these assays exploit highly conserved regions across all the seven serotypes of FMDV, serotype biases still exist within both of these assays. The 5´UTR assay has been shown to be more sensitive in detecting serotype A viruses, whereas, the 3D assay has greater sensitivity for detecting the SAT viruses (King *et al.,* 2006). Recently, Tam *et al.* (2009) reported fluorescence-based multiplex rRT-PCR assays for the detection of FMDV and virus typing. The assay was found to have greater sensitivity for detection but some cross-reactivity between some serotypes was also noted. Further work is in progress to develop rRT-PCR assays for serotyping of the virus.

2.12.4.4 Reverse transcription loop-mediated isothermal amplification

The loop-mediated amplification (LAMP) was developed, and enables tests to be conducted in the field using inexpensive tools. LAMP amplifies specific nucleotide sequences at a constant temperature and thus does not require a thermocycler. The assay is based on the principle of DNA amplification by an autocycling strand displacement reaction. The assay is performed using a set of two specially designed inner primers and two outer primers and DNA polymerase with high strand displacement activity (Notomi *et al.,* 2000). The primers recognize 6 independent target sequences in the initial stage and 4 independent sequences during the later stages of the LAMP reaction. The reaction is carried out in less than an hour using a standard water bath or heating block and the results can be visualized with the naked eye. The advantage of its simple operation, rapid reaction and potential for visual interpretation without instrumentation make the technique attractive for field use in endemic countries. The RT-LAMP assay that has been developed for FMDV detection can be used in a high thoroughput system (Duke *et al.,* 2006). This assay has, however, not yet been extensively evaluated for its ability to replace or supplement the techniques currently in use.

2.12.4.5 Chromatographic strip test

Virus isolation combined with ELISA and RT-PCR assays are reliable and accurate for the diagnosis of FMD but the shipment of samples from the field to the laboratory and the poor quality/amount of submitted samples can result in hindrance of early diagnosis of the disease. A rapid and specific test for disease diagnosis at the site of a suspected outbreak may allow timely implementation of control measures. A MAb-based chromatographic strip test for FMD diagnosis was developed and was found to be at least as sensitive as the conventional antigen ELISA for the detection of FMDV in epithelial suspensions tested and had an equivalent 100% sensitivity on the cell culture supernatants of FMDV serotypes O, A, C and Asia-1 (Reid *et al.*, 2001b). Further research is underway to develop chromatographic strip tests capable of ascertaining the serotype of the virus.

2.12.4.6 Serological tests

The demonstration of specific antibody in non-vaccinated animals where vesicular condition is present is sufficient for a positive diagnosis. This is useful in mild cases or where epithelial tissue cannot be collected (OIE, 2012).

Serological test for FMD are performed in support of four main purposes (OIE, 2012).

Namely:

i. To certify individual animals prior to import or export (i.e for trade)
ii. To confirm suspected cases of FMD
iii. To substantiate absence of infection
iv. To demonstrate the efficacy of vaccination

Serological tests for FMD are of two types; those that detect antibodies to viral structural proteins (SP) and those that detect antibodies to viral nonstructural proteins (NSPs). The viral structural proteins tests are serotype-specific and detect antibodies elicited by vaccination and infection.They include virus neutralization (VN) test (Golding *et al.*, 1976), the solid-phase

competitive ELISA (SPCE) test (Mackay *et al.,* 2001; Piaba *et al.,* 2004) and the liquid-phase blocking ELISA (LPBE) test (Hamblin *et al.,* 1986; Hamblin*et al.,* 1987). In the World Reference Laboratory (WRL) in Pirbright, UK, the LPBE was routinely used for FMD antibody detection (Hamblin *et al.,* 1986; Hamblin *et al.,* 1987), the sensitivity of this test is close to 100% and the specificity is 95%. The relatively low specificity of the LPBE makes it less optimal for large scale screening purposes; a SPCE was developed with a high sensitivity and specificity (99.8%) (Mackay *et al.,* 2001; Piaba *et al.,* 2004). The VN test is used for testing inconclusive antibody results obtained by ELISA (Golding *et al.,* 1976), and it is currently considered as the definitive "gold standard" for the final assessment of such results (OIE, 2012).

These tests are serotype-specific and are highly sensitive, providing that the virus or antigen used in the test is closely matched to the strain circulating in the field. They are the prescribed tests for international trade and are appropriate for confirming previous or ongoing infection in non-vaccinated animals and for the monitoring of immunity conferred by vaccination in the field (OIE, 2012). As various primary cells and cell lines with variable degrees of sensitivities are used in VNTs, they are more prone to variability than other serological tests. Furthermore, VNT is slower (takes 2-3 days to provide results), subject to contamination and requires restrictive biocontainment facilities in contrast to other serological tests that uses serotype-specific polyclonal or monoclonal antibodies, quicker to perform and are not dependent on tissue culture system and the use of live viruses (OIE, 2012; Jamal and Belsham, 2013).

The detection of antibody to the nonstructural proteins (NSPs) of FMDV has been used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated (Berger *et al.,* 1990; De Diego *et al.,* 1997; Sorensen *et al*., 1998a). The NSPs are expressed only by replicating viruses. Inactivated vaccines are purified to

remove cellular proteins and NSPs, and therefore only animals that have been infected with live virus should develop antibodies to these proteins (Berger *et al.,* 1990; Bergmann *et al.,* 1993). Conventionally, the detection of antibody to NSPs has been carried out by measuring antibody to the virus infection-associated antigen (VIAA; the viral RNA polymerase protein 3D) using agar gel immunodiffusion (AGID) (McVicar and Sutmoller, 1970; Dawe and Pinto, 1978). The VIAA-AGID test has now largely been superseded by assays that measure antibody to FMDV NSPs produced by recombinant techniques in a variety of in-vitro expression systems (Mackay *et al.,* 1997; Meyer *et al.,* 1997).

Currently, the polyproteins 3ABC and 3AB appear to be most promising as diagnostic antigens (Bergmann *et al.,* 1993; Mackay *et al.,* 1997). Antibody to these polyproteins is the single most reliable indicator of infection (De Diego *et al.,* 1997; Sorensen *et al.,* 1998b). The simultaneous detection of antibody to several NSPs (L, 2C, 3A or 3D protein) in a single test by ELISA (Mackay *et al.,* 1997) or by enzyme-linked immune-electrotransfer blot (ELTB), a type of Western blot (Bergmann, *et al.,* 1993) is useful for confirmation of animals positive to 3AB or 3ABC.

2.13 Collection of Samples

The type of sample recommended for testing is based on knowledge of the amount of virus present in various tissues, excretions and secretions (Alexandersen *et al.,* 2003b). The material of choice for laboratory diagnosis of FMD, especially the ELISA methods is epithelium from an unruptured or freshly ruptured vesicle (OIE, 2012), as these materials contain high titres of FMDV. The area should be washed with water or phosphate buffered saline (PBS) if heavily contaminated with food (in the mouth) or feaces (on the feet) but disinfectants must not be used (AVIS, 2002). If unruptured vesicles are found, the straw-coloured vesicular fluid can be withdrawn with a syringe. Pieces of epithelium and/or vesicular fluid should be suspended in

transport medium, which is a mixture of equal amounts of glycerol and 0.04M phosphate buffer pH 7.2-7.6, preferably with antibiotics added. Tissue culture medium or phosphate buffered saline can be used if 0.04M phosphate buffer is not available, but it is important for the pH of the glycerol/buffer mixture be in the range of 7.2-7.6 (OIE, 2012).

The more sensitive methods (cell culture and RT-PCR) are appropriate for samples likely to contain smaller amounts of infectivity of viral RNA, e.g blood, swabs, milk, probang samples, tissues and feaces (Alexandersen *et al.,* 2003b). Samples should be labeled and kept refrigerated or in ice (Kitching and Donaldson, 1987). If vesicles are not available, blood may be collected for virus isolation in tissue culture or antibody detection. For virus isolation, whole unclotted blood is required while serum is required for antibody detection (AVIS, 2002). Virus isolation from blood may not be successful if ageing of lesions suggests that the disease has been present for more than 3 to 4days (AVIS, 2002).Esophageal-pharyngeal fluid samples can also be collected by probing cup from ruminants, or as throat swabs from pigs in transport medium for virus isolation or RT-PCR. These samples should be refrigerated or frozen immediately after collection (Kitching and Donaldson, 1987).

Foot and mouth disease virus excretion in milk is often at high titre, can take place for up to 4days before the onset of clinical signs and continues for several days after typical lesions appear. Therefore, milk samples from lactating animals suspected of FMD is useful. Milk should be collected into plain glass tube and kept at 4°C until submitted to the laboratory or frozen in the possibility of delay to prevent a fall in pH which may result in inactivation of the virus (AVIS, 2002).

2.14 Control of Foot and Mouth Disease

In the late 19th and early 20th centuries, FMD outbreaks occurred sporadically in Europe with devastating consequences (Barteling and Vreeswijk, 1991). By the early 1950s however, some

countries in Western Europe were experiencing frequent FMD outbreaks per year (Brown, 1992). At this time, disease control was by animal movement inhibition, slaughter of infected animals, and disinfection. Following a concerted effort in the 1930s in Germany, especially by Waldmann *et al.* (1937), an inactivated FMD vaccine was developed (Brooksby, 1982). Vaccine was produced by inactivation of live virus with formalin in the presence of aluminium hydroxide gel. Virus for the Waldmann vaccine was obtained by infecting at slaughter house and collecting epithelium and vesicular fluid (Brooksby, 1982). From this method, only limited numbers of animals could be infected as a result, could not meet the vaccine need in Europe to control the disease. Frenkel developed a method to produce virus by infecting bovine tongue epithelium obtained at the time of slaughter of healthy animals (Frenkel, 1947). This method paved the way for FMD vaccine commercialization. The observation that FMDV replicates in BHK-21 cells led to more commercially viable large scale production of virus in suspension cultures (Grubman and Baxt, 2004).

Current vaccines are whole-virus produced in cell culture, inactivated by treatment with aziridines such as binary ethylenemine, and mixed with adjuvant (Bahnemann, 1975). Most countries have established vaccine banks which contain concentrated antigen against virus serotypes, stored in gaseous phase of liquid nitrogen (Doel, 2003), and provide member countries with immediate source of vaccine. However, there are a issues and limitations with the use of the vaccine in emergency control programs, including the following (Grubman and Baxt, 2004):

i. High containment facilities are required for the production of vaccine
ii. Most virus preparation used for vaccines are concentrated cell culture supernatants from FMDV-infected cells and depend on the manufacturer, contain various amounts of contaminating viral NSPs. Vaccinated animals develop antibody responses against

contaminating proteins, in addition to viral structural proteins, making it difficult to reliably distinguish vaccinated from infected or convalescent animals with currently approved diagnostic tests.

iii. The vaccine does not induce rapid protection against challenge by direct inoculation or direct contact. Thus, there is adaptive immune response

iv. Vaccinated animals can become long-term carriers following contact with FMDV,

2.15 Alternative Vaccine Strategies

Because of the concerns and limitations of the killed vaccine, researchers over the years have attempted to develop alternative FMD vaccines (new marker vaccines) that do not require infectious virus.

2.15.1 Proteins and peptides

A number of strategies have been employed based on information concerning the FMDV capsid structure, including the prominent surface exposure of VP1 and the immunologically important VP1 G-H loop. Some of these strategies included the use of VP1, either isolated from purified virus or produced by recombinant DNA techniques (Bachrach *et al.*, 1975; Kleid *et al.*, 1981); the use of VP1- derived peptides (Strohmaier *et al.*, 1982) or chemically synthesized VP1 fusion proteins (Kit *et al.*, 1991); inoculation with DNA expressing VP1 epitopes alone (Wong *et al.*, 2000) or co-administered with DNA encoding IL-2 (Wong *et al.*, 2002); and use of transgenic plants infected with a recombinant tobacco mosaic virus expressing VP1 (Wigdorovitz *et al.*,1999). These strategies present a limited subset of viral immunogens to the vaccinated animal, and although they often induce high titers of neutralizing antibodies, they do not always achieve protection against virus challenge in livestock (Mulcahy *et al.*, 1990). The immunogenicity of these subunit vaccines appears to be due to their ability to present sequential epitopes from the immunologically important VP1 G-H surface loop (Grubman and Baxt, 2004).

Although immunodorminant in many assay, these epitopes are not the only neutralizing epitopes on the virion (Crowther *et al.,* 1993), nor are they uniformly recognized in all host species (Mateu *et al.,* 1995).

2.15.2 Live attenuated vaccines

Attenuated strains of FMDV are produced by passage in non-susceptible species, such as mice, rabbits and embryonated eggs, until their virulence for cattle is reduced (Bachrach, 1968; Brooksby, 1982). Grubman and Baxt (2004) noted that field studies with these viruses had been performed in Africa, the Middle-East, and South America. While in some cases the attenuated vaccines included a degree of protection, it was found that viral strains attenuated for a given host were often virulent in other susceptible species. Furthermore, it has been difficult to obtain viruses that are both attenuated and immunogenic. In addition, a major concern with live attenuated vaccines is the possibility of reversion to virulence. More recently, attempts to develop attenuated vaccines have utilized genetic engineering to mutate regions of the genome or delete a protein-coding region (Mckenna *et al.,* 1995).

2.15.3 Empty viral capsids

Other experimental FMD vaccines have targeted immunogens that contain the entire repertoire of immunogenic sites present on intact virus but lack infectious nucleic acid (Belsham *et al.,* 1991; Grubman *et al.,* 1993). This strategy involves the molecular cloning of the regions of the viral genome necessary for the synthesis, processing, and assembly of the viral structural proteins into empty viral capsids (P1-2A-and $3C^{pro}$- coding regions). These structures are naturally produced in FMDV-infected cell culture systems, are antigenically similar to virus particles, and are as immunogenic as virions in animals (Grubman *et al.,* 1985). Animals

inoculated with this type of vaccine could be easily distinguished from infected or convalescent animals by using currently approved technology, since the regions of the genome coding for the NS proteins used in the diagnostic assays to detect infection are not present in the empty capsid cDNA construct. In preliminary studies, FMDV capsid structures were expressed in *Escherichia coli* or in recombinant baculovirus-infected cells and inoculated into animals. Even though the products offered some protection, they did not reach the efficacy of the current inactivated whole-virus vaccine because only small amounts of antigen were obtained (Belsham *et al.,* 1991; Grubman *et al.,* 1993). To enhance the expression and delivery of empty capsid constructs, alternative vector systems that allow expression of FMDV capsid structures in infected cells and potentially induce both humoral and cell-mediated immune responses have been examined (Grubman and Baxt, 2004). DNA inoculation based strategy designed to produce empty capsids in inoculated animals (Beard *et al.,* 1999; Benvenisti *et al*., 2001) is one approach utilized. Similar studies using cDNA that encodes the entire viral genome but contains a mutation at the cell binding sites were also performed (Ward *et al.,* 1997; Beard *et al.,* 1999). Another very efficient, antigen delivery system utilizes a live vector for foreign gene expression (Imler, 1995; Russell, 2000) and have been used to deliver FMDV capsid proteins (Mayr *et al.* 2001; Moraes *et al*., 2002; Wu *et al.,* 2003).

2.15.4 Antiviral approach

While both the current vaccine and the human adenovirus-vectored (Ad5) vaccine can induce complete protection by 7 days, it is imperative to block or reduce virus shedding as rapidly as possible in emergency outbreak situations to contain the outbreak (Grubman and Baxt, 2004). Several researchers have demonstrated that in cells pre-treated with IFN-α/β, productive replication of FMDV is inhibited by the IFN-induced gene products, double-stranded RNA-dependent protein kinase and 2-5 A synthetase/RNase L (Ahl *et al.,* 1976; Chinsangaram, *et al.,*

1999; Chinsangaram, *et al.,* 2001). IFN is one of the first line of host cell defence against viral infection (Vilcek and Sen, 1996) and can rapidly induce a non-specific protective response against all FMDV serotypes thus far tested (Ahl *et al.,* 1976; Chinsangaram, *et al.,* 1999; Chinsangaram, *et al.,* 2001). Since effective protection requires the vaccine to be matched to the outbreak strain and induce a rapid immune response, antiviral therapy could circumvent these inherent problems of FMD vaccination. Thus, IFN-α/β may be useful as an anti-FMD agent for the prophylactic treatment in susceptible animals (Grubman and Baxt, 2004). Treatment with IFN-α/β requires multiple high-dose inoculations because clinical studies in humans have demonstrated that the protein (IFN) is rapidly cleared (Lukaszewski and Brooks, 2000; Santodonato *et al.,* 2001). For IFN therapy to be effective in an FMD outbreak, it must be delivered in one inoculation, in combination with vaccination, so as to induce rapid protection prior to the onset of the vaccine-induced adaptive immune response (Grubman and Baxt, 2004). To meet these goals, Chinsangaram, *et al.* (2003) constructed a replicative-defective Ad5 containing porcine IFN-α (Ad5-pIFN-α). Delivery via a viral vector would result in endogenous expression of IFN over a period of time, and the amount delivered could be controlled by the dosage of the recombinant virus. Swine inoculated with one dose of this recombinant virus were completely protected when challenged 1 day later by direct inoculation of FMDV (Chinsangaram, *et al.,* 2003).

Also, additional studies demonstrate that, treatment with Ad5-pIFN-α alone can protect swine from challenge for 3 to 5 days and can reduce viraemia, virus shedding, and disease severity when administered 1 day post challenge. Furthermore, a combination of pIFN-α and vaccination can provide both immediate and long-term protection (Moraes *et al.,* 2003). In a more recent study with cattle, Ad5-IFN-α treatment failed to completely protect the animals from FMDV

infection; however, disease was delayed and less severe compared to that in control animals (Wu *et al.,* 2003). This lower level of protection of cattle compared to swine appears to be correlated with reduced levels of IFN detected in the plasma (Wu *et al.,* 2003). There have also been some limited attempts to develop antiviral drug therapy that affects specific viral protein targets (Kleina and Grubman, 1992).

2.16 Types of Foot and Mouth DiseaseVaccines

Both conventional vaccines and vaccines formulated for emergency use are based on cell culture derived preparations of whole virus, which are chemically inactivated by the use of inactivants of the first order such as aziridines like binary ethylenimine (Bahnemann, 1990; Barteling and Vreeswijk, 1991) and blended with a suitable adjuvant (Doel, 1999; Smitsaart *et al.,* 2004). Vaccines formulated with the adjuvants aluminium hydroxide and/or saponin provide protective immunity in ruminants (cattle, sheep and goats) but are poor in conferring similar response in pigs. Mineral oil adjuvanted vaccines, in various emulsification forms, were successfully developed for use, and afforded protection in Swine (Doel *et al.,* 2003).

Foot and mouth disease vaccines can be monovalent or polyvalent (multivalent) in relation to the serotype of antigen (De Clercq *et al.,* 2008). There is a constant requirement to monitor contemporary outbreak strains of FMDV, to check the suitability of the available vaccines and identify the appearance of antigenically novel strains (Barnett *et al.,* 2001; Paton *et al.,* 2005). The degree of protection afforded by a vaccine depends on both the potency of the vaccine and the antigenic relationship between the vaccine strain and the field strains (De Clercq *et al.,* 2008). The vaccines needed in each country depend on the virus pool responsible for infections in that country (FAO, 2009). The types of vaccines available from commercial sources have remained virtually unchanged for several decades and there has been less investment in research

and development by manufacturers than was formerly the case (Barnett *et al.,* 2002; Barnett and Carrabin, 2002).

Current developments in the area of improved vaccine types are based on: understanding the innate and cellular immune parameters (Barnard *et al.,* 2005); induction of mucosal immunity (Summerfield *et al.,* 2004); improving adjuvancy (Smitsaart *et al.,* 2004); FMDV recombinant proteins (Wang *et al.,* 2003); fusion proteins (Sun *et al.,* 2003) or proteins expressed in plants (Dus Santos *et al.,* 2005); chimeric marker vaccines (Baranowski *et al.,* 2001); consensus sequence peptides (Wang *et al.,* 2002; Rodriguez *et al.,* 2003; Beignon *et al.,* 2005); vector-based approaches that express part or all of the FMD empty capsid and/or cytokines, such as interferon-alpha (Berinstein *et al.,* 2000, Moraes *et al.,* 2007); DNA vaccination strategies including protein antigen boost (Shao *et al.,* 2005; Li *et al.,* 2006) or the use of FMDV minigenes (Borrego *et al.,* 2006); cytokine and Toll-like receptor mRNAs in the nasal-associated lymphoid tissues (Zhang *et al.,* 2006). It often takes 5-10 years before a research idea makes its way to a commercial product.

The ultimate objective of improved vaccine investigations is the development of vaccines that induce a life-long immunity very shortly after administration (Doel, 2005) and that are capable not only of preventing clinical disease but also infection and the possibly arising carrier state, thus blocking the further transmission of infection. Other points of high importance are long-term vaccine stability, less dependency on the cold chain and induction of broad spectrum immunity (serotype independent pan-vaccines) (De Clercq *et al.,* 2008).

The FMD vaccines may also be classified as either “standard” or “higher” potency vaccines (Barnett *et al.,* 2002; Barnett and Carrabin, 2002). Standard potency vaccines are formulated to contain sufficient antigen to ensure that they meet the minimum potency level required. High

potency vaccines are formulated with an increased amount of antigen such that the potency is in excess of the minimum requirements to provide particular features such as more rapid onset of immunity and a wider spectrum of immunity against relevant field viruses (Barnett *et al.,* 2002; Barnett and Carrabin, 2002). There is evidence that highly potent vaccines can induce cross-protection against heterotypic strains (Brehm *et al.,* 2008).

2.17 Emergency Vaccine Bank

Foot and mouth disease virus is an antigenically variable virus consisting of serotypes and dozens of subtypes. Immunity to one subtype will not protect against the others and in some cases, immunity to one subtype will not protect against other members of the same serotype. A number of countries have established vaccine banks containing concentrated antigens that can be mobilized for use during an outbreak. These banks contain several subtypes and serotypes of virus to help ensure that they will be effective against the likely introduced strains of virus (Doel, 1999). The finding that viral antigen could be inactivated, concentrated and stored at a low temperature (typically in the vapour phase over liquid nitrogen) for extended period with little or no loss of immunogenicity provided the technical basis for the establishment of vaccine banks (Adamowicz *et al.,* 1974).

Since the first FMD vaccine bank was established in Denmark in 1976, many more banks have been created worldwide to provide vaccines for emergency application in cases of disease outbreaks (Forman and Garland, 2002). The North American Vaccine Bank (NAVB) located at the Plum Islam Animal Disease Centre, USA was inaugurated in 1982, for the United States of America (USA), Canada and Mexico. In 1985 an International Vaccine Bank (IVB) was established in the United Kingdom and located at the Institute for Animal Health, Pirbright, UK, to serve as a contingency supply for the UK, Australia, New Zealand, Findland, Ireland, Norway and Sweden. The European Union Vaccine Bank (EUVB) was established in 1991 to service the

member States with the antigens stored in two national institutes in France and Italy (Garland, 1997; Leforban, 1995; Ryan, 2001). In 2001, Denmark discontinued the national maintenance of the first established bank and to rely upon EUVB in future (Forman and Garland, 2002).

The establishments of vaccine banks have a number of advantages over conventional, pre-formulated vaccines including flexibility in the formulation of mono-or polyvalent vaccines, the level of incorporation of antigen and the type of adjuvant used, all of which can be tailored to best meet the epidemiological characteristics of an emergency. In addition, banked antigen can be formulated and used directly in times of need. This contrasts with pre-formulated vaccine which has advantages of immediate availability, but disadvantages of limited shelf-life if unused before the expiry date which is within 12 to 18 months of testing and release (Forman and Garland, 2002).

2.18 Foot and Mouth DiseaseVaccination Strategies

Vaccination strategies will vary considerably depending on local situations and objectives, e.g. mass vaccination or vaccination targeting specific animal sub-populations or zones, highrisk situations, ring vaccination around outbreaks and buffer or protection zones around free zones (FAO/OIE, 2012).Strategies for the control of FMD implemented by various countries tend to reflect the patterns of disease incidence. Thus, countries free from the disease place reliance on a policy of preventing entry of virus through strict control of importation of livestock and animal products and in the event of an outbreak, the imposition of movement controls, slaughter of infected and in-contact animals, carcasses disposal and disinfection. This is the so-called ‘stamping out’ policy and does not involve vaccination (FAO/OIE, 2012).

However, some countries such as South America continue to vaccinate after being officially declared free: their World Organization for Animal Health (OIE) status in that case is ‘free’ with vaccination. Other countries free without vaccination include in their national contingency plans

the holding of or access to vaccine banks or reserves that could be used in an emergency, should stamping out fail to limit the extent of spread (FAO/OIE, 2012).

The devastating consequences of some recent outbreaks of FMD, in particular the United Kingdom and Dutch epidemics of 2001, have shifted political opinion in favour of the implementation of vaccination in future epidemics and accordingly many veterinary services have modified their national contingency plans to accommodate this possibility (FAO/OIE, 2012).

In countries where the disease is sporadic, the usual practice in the event of outbreaks is to carry out emergency vaccination, stringent zoo-sanitary measures and the slaughter of infected stock. In countries where FMD is endemic, but at a low level of incidence, control programmes based on routine vaccination of cattle have had considerable success (Correa Melo, *et al.*, 2002; Dekker, 2008). On the other hand, in endemic regions with a high incidence of FMD, the strategic vaccination of valuable animals e.g. high producing dairy animals may be the only affordable control measures. Globally, however, the highly endemic regions are those inhabited by the world's poorest livestock owners (Correa Melo, *et al.*, 2002; Dekker, 2008).

Several critical factors need to be considered in planning and design of an FMD vaccination programme if it is to achieve optimal results (Garland and De Clercq, 2011), which include: at least 80% vaccination coverage; vaccination campaigns should be completed in the shortest possible time; vaccination should be scheduled to allow for interference from maternal immunity; vaccines should be administered in the correct dose and by the correct route; monitoring the efficacy of vaccination; and complying with manufacturer's instruction. A more recent report has recommended 100% vaccination coverage (FAO, 2011).

Some national FMD vaccination programmes vaccinate all bovines three times a year and all sheep and goats once a year (Hamond, 2011). In South America, cattle under two years of age are vaccinated twice a year while those above two years are vaccinated once a year. Vaccination of cattle is twice a year in India. Also, vaccination of cattle, sheep and gaots is done twice, with pigs vaccinated once a year in China. Most other regions have no official policy on vaccination against FMD (Hamond, 2011).For FMD vaccine to be effective, the strain or strains in the vaccine must antigenically match those circulating in the field. Although there is evidence that highly potent vaccines can induce cross-protection against heterotypic strains (Brehm *et al.*, 2008), however, because of their prohibitive cost, they are not used in endemic regions.

2.19 The Global Foot and MouthDiseaseControl Strategy

The Progressive Control Pathway for Foot and Mouth Disease (PCP-FMD) is essentially a development tool intended for countries where the disease is endemic and where a clearer knowledge of the local situation (epidemiological factors, circulating virus strains etc.) and the preparation and implementation of control programmes to reduce the impact of the disease are needed (FAO, 2011). The PCP-FMD approach was developed by Food and Agriculture Organization (FAO) and the European Commission for the control of FMD (EuFMD) and finalized together with the World Organization for Animal Health (OIE) in 2010 (Sumption *et al.*, 2012).

This pathway includes a set of criteria to assist FMD-endemic countries to progressively reduce the level of FMDV circulation and to mitigate the impact of FMD (FAO, 2011). The PCP-FMD consists of a set of FMD control activity stages, that if implemented, should enable countries to progressively increase the level of FMD control to the point where an application for OIE endorsement of a national control programme (in an advanced phase of stage 3) or official freedom from FMD with or without vaccination (end of stages 4 and 5, respectively) may be

successful and the status sustainable (FAO, 2011). Each stage has well-defined outcomes which can be achieved through a variety of activities. The specific activities required to achieve the outcomes have, however, not been prescribed. This non-prescriptive approach has the advantage that each country can adopt an approach according to its national/regional requirements and capabilities to achieve the outcomes. Understanding the "local" epidemiology of FMD and the active monitoring of the virus circulation are the foundations of the PCP-FMD and activities to meet these requirements are required in all stages (FAO, 2011). Determination of the factors responsible for maintenance and spread of the disease and knowledge about circulating subtypes of FMDV are essential for effective control of the disease.

Inadequate laboratory diagnostic capacity and its implications for the PCP-FMD in Easter Africa have been described recently by Namatovu *et al.* (2013). In order to monitor FMDV circulation effectively, each participating country should, therefore, have sufficient FMD diagnostic and surveillance capacity. Progression along the PCP is based on an annual assessment of evidence-based activities related to FMD epidemiology and control measures undertaken by the country. The organizational infrastructure of each country is required to be progressively strengthened to ensure that the activities required to monitor and control FMD are implemented (Bartels *et al.*, 2012).

Countries where the disease is endemic with no reliable information on the disease status are classified as in stage 0. In order to move from stage 0 to 1, a comprehensive study on the epidemiology of FMD is required to be planned (FAO, 2011).

Stage 1 assists in identifying appropriate control options. Countries in stage 1 are in the process of developing their control strategies in at least one animal production section based on a comprehensive assessment of the epidemiology and control options. Progression from stage 1 to

2 requires a risk based FMD control plan. Thus stage 2 involves the implementation of the chosen control policy. Countries in stage 2 have implemented a risk-based FMD control strategy that aims to reduce disease in at least one animal production sector. In order to move from stage 2 to 3, an aggressive strategy to eliminate FMD needs to be developed. Countries in stage 3 have adopted a control plan to progressively reduce/eliminate virus circulation in at least one region/production system (FAO, 2011). This requires very significant national capacity and ongoing investment including the ability to ensure maintenance of sufficient herd immunity in critical populations to prevent FMD virus circulation. Moving to stage 4 requires that FMD is controlled to an extent that it is not endemic in domestic livestock. If a country decides to continue along the FMD-PCP to stage 4 and beyond, it may ask the OIE for endorsement of its national FMD eradication program. Progression to stage 4 would thus indicate attaining officially recognized FMD free status with vaccination by the OIE for the whole or part of the country. Countries in stage 4 have maintained zero circulation with no incursions of FMD (FAO, 2011). Vaccination plays a vital role in controlling FMD for countries in stages 2-4.

2.20 Economic Impact of Foot and Mouth Disease

The presence of FMD creates problems for all livestock owners who are connected to a population infected either geographically or through input or output market chains (the livestock value chain). Therefore, FMD creates a negative externalities of all those connected to the problem (Rushton, 2009). FMD is widely prevalent, with the disease circulating in an estimated 77% of the global livestock population. In this population, it affects a large proportion of animals during an outbreak and affects many species.

2.20.1 Direct impacts

The direct impacts of FMD may be classified as invisible losses. The visible losses are the production losses due directly to FMD and these include:-

i. Reduced milk production, affecting both the humans and calves that depend on it. This can account for 33% of losses in endemic settings. Although FMD typically has a short-term effect on animal's health, chronic FMD typically reduces milk yields by 80% (Balman and Terrazas, 1976; Barasa *et al.,* 2008; Bayissa *et al.,* 2011).

ii. Reduce livestock growth

iii. Mortality in young stock, typically to between 2-5%

iv. Loss of traction power where draught animals are used. If this occurs during harvest the effects can be particularly severe (James and Ellis, 1978; Perry *et al.,* 1999).

v. Abortion, the cost of a single abortion is high as the farmer will have to pay to keep the cow without it producing anything for another year or more, or cull the animal.

Visible production losses are most prominent in pigs in intensive production systems followed by dairy cattle. These two systems are important sources of animal protein in poor countries (Delgado, *et al.,* 1991). Extensive systems of production do not have such pronounced losses, and some species such as sheep and goats show limited clinical symptoms and minor economic losses. In the invisible losses, fertility is affected leading to the need to have a herd structure that contains extra breeding animals (Rushton, 2009).

2.20.2 Indirect impacts

This can be categorized as additional costs (control costs) and revenues foregone; where the presence of the disease limits market opportunities, both at local, national and international levels, and the use of technologies, particularly improved breeds and more intensive production systems. The cost of control measures carried out by the veterinary services, such as vaccination, outbreak control and sometimes culling and compensation are borne by the tax payer. Some national FMD vaccination programmes vaccinate all bovines three times a year and all sheep and

goats once a year, this limits resources available to combat other diseases. In endemic settings significant amounts are spent on privately funded vaccination and control (Sutmoller, *et al.*, 2003; Barasa *et al.*, 2008; Forman *et al.*, 2009). In Africa, it has been estimated that more is spent controlling FMD than any other veterinary disease (Gall and Laboucq, 2004). In addition to the costs of vaccination and culling there are also costs incurred with the need for controlling movement and performing diagnostics for the confirmation of disease presence or absence (Rushton, 2009).Livestock trade is limited as farmers affected by FMD receives lower prices for their stock, those wishing to purchase animals from FMD free herds face a restricted supply.

Countries infected with FMD cannot trade live animals with FMD free countries. The countries with the best prices for meat are FMD free (i.e. EU, USA, Japan) (James and Rushton, 2002). Trade in livestock products, fruits and vegetable can also be affected by FMD status (James and Rushton, 2002). The FMD status of nations that a country trades with also affects a country's ability to trade with FMD free countries irrespective of its own status (James and Rushton, 2002). Lack of access to lucrative markets restricts the development of commercial farming, consequently employment and tax revenue from the area is limited by FMD status.

Impacts at the national level ultimately impact on the individual farmer and vice-versa. Similarly, impacts on the livestock producer have ripple markets effects along the entire market chain, impacting on other players such as, abattoirs, dairies etc. (Gall and Leboucq, 2004). High productivity breeds are typically more susceptible to FMD. The risk of FMD therefore restricts the use of these breeds and prevents the development of more intensive production system based on these breeds (Rushton, 2009). Pastoralist and agro-pastoralist communities are highly dependent upon livestock for milk, meat and as assets of economic and social value. In a

questionnaire survey study, Gall and Leboucq (2004) found FMD to have the greatest impact on poverty of all the ruminant bacterial and viral diseases.

2.21 Public Health Significance of Foot and Mouth Disease

Foot and mouth disease is a zoonosis, a disease transmissible to humans, but it crosses the species barrier with difficulty and with little effect (Prempeh, *et al.*, 2001). Considering the high incidence of the disease in animals, both in the past and in recent outbreaks worldwide, it occurrence in man is observed to be rare (Bauer, 1997). The circumstances in which the disease occurs in humans are not well defined, though all reported cases have had close contact with infected animals (Armstrong *et al.*, 1967). Meat and Milk of infected animals are safe to consumers (PUCES, 2008); they may be dangerous as sources of infection to susceptible animals. Person to person spread has not been reported (Prempeh, *et al.*, 2001; Mitchell, 2001). However, humans can unknowingly transmit the virus to susceptible animals if they come in contact with infected livestock, soil or objects that have been contaminated with FMDV (PUCES, 2001; USDA, 2008). The virus can easily be carried on clothing, shoes and personal items.

Humans exposed to FMDV can also harbor the virus in their throat or nasal passages for several days (USDA, 2008). Despite considerable possible exposure among those handling affected animals, the risk of human exposure is very low (Prempeh, *et al.*, 2001; Mitchell, 2001). The incubation period in humans is 2-6 days, and the symptoms have mostly been mild, short-lived and self-limiting, mainly uncomfortable tingling blisters on the hands, but also fever, sore throat and blisters on the feet and in the mouth including the tongue (Bauer, 1997; CFSPH, 2007; USDA, 2008). Patients have usually recovered from the disease a week after the last blister formation (Prempeh, *et al.*, 2001).

Foot and mouth disease should not be confused with the human disease called hand, foot and mouth disease. This is an unrelated and usually mild viral infection, principally of children, caused by Coxsackie A virus **(**CFSPH, 2007**).**

CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 Study Areas

The study was conducted in Kaduna and Plateau States, located in the North Western and North Central geopolitical regions of Nigeria respectively (Figure 3.1). Animal husbandry is common to the inhabitants of Kaduna and Plateau States and these two States are considered to be major cattle routes linking north to the western and eastern parts of Nigeria.Furthermore, acessibility and livestock population were also factors for choosing these two States.

Kaduna state lies between latitude $9^{o}30'0''N$ and $11^{o}0'0''N$ and longitude $6^{o}0'00''E$ and $9^{o}\ 0'0''E$ and shares boundaries with Katsina, Kano and Zamfara States to the North, Plateau and Bauchi States to the East, Nasarawa State and Federal Capital Territory to the South and Niger State to the West (Figure 3.2). Administratively, Kaduna State is divided into three Federal Senatorial Zones (Central, Northern and Southern) and 23 Local Government Areas (LGAs), with at least seven LGAs in each senatorial zone(KDSG, 2008). Kaduna is nestled within the gently undulating terrain of the central plains of Northern Nigeria. Climatic type is characterized by a

rainy season (about 6 months), followed by a dry and dusty harmattan season lasting from November to February. The rainy season is preceded by a short hot dry spell with mean maximum daily temperature of between 35^{o}C and 40^{o}C. Annual total rainfall is about 1,272mm and rain falls mostly from May to October. Kaduna state has a human population of over 6,066,562 people according to the 2006 census figures while agriculture is the main stay of the economy of Kaduna state with about 80% of the people actively engaged in animal and crop farming (KDSG, 2008).

Plateau State of Nigeria lies between longitude 8^{o} 22N, 8^{o} 35E and Latitude 10^{o} 26N, 10^{o} 40E with a total land area of 26,899 square kilometers. The state is bordered in the North West by Kaduna State, in the North East by Bauchi State, in the South West by Nassarawa State and in the South East by Taraba State (Figure 3.3). Though situated in the tropical zone, Plateau State has a near temperate climate with an average temperature of between 18 and 22°C, while the annual rainfall varies from 131.75 cm in the Southern part to 146 cm on the Plateau (NBS, 2009).Harmattan winds cause the coldest weather between December and February.The warmest temperatures usually occur in the dry season months of March and April. Plateau State has a favourable climate for both livestock and crops and therefore has a very high population of livestock. Administratively, Plateau State is divided into three Federal Senatorial Zones (Central, Northern and Southern) and seventeen Local Government Areas (LGAs), with at least five LGAs in each senatorial zone. It has an estimated population of aboutthree million people (2006 National Population Census) with over 30 ethnic groups of which a large percentage are mostly engaged in farming (NBS, 2009).

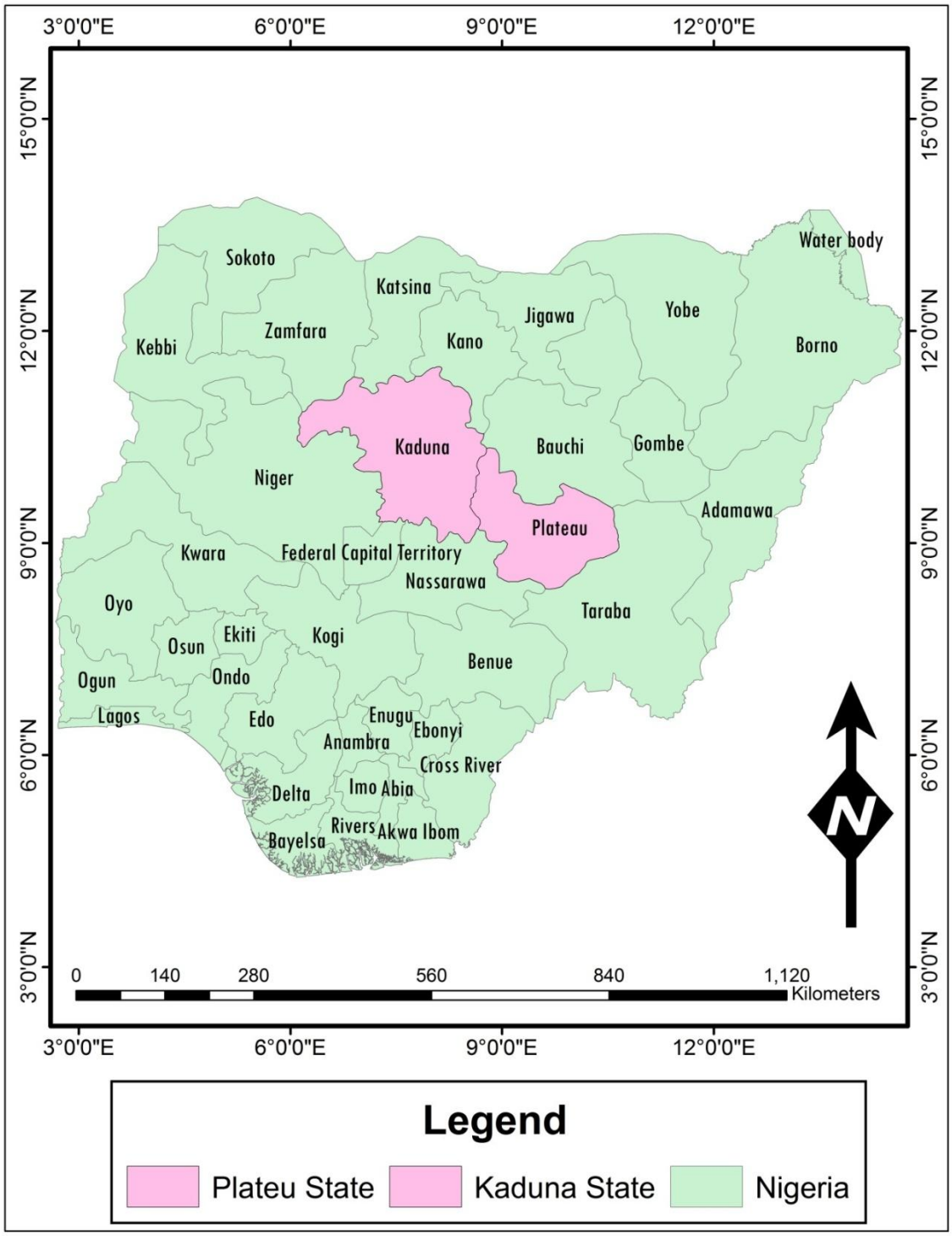


Figure 3.1: Map of Nigeria Showing the Kaduna and Plateau States (Source: Modified From Administrative Map of Nigeria).

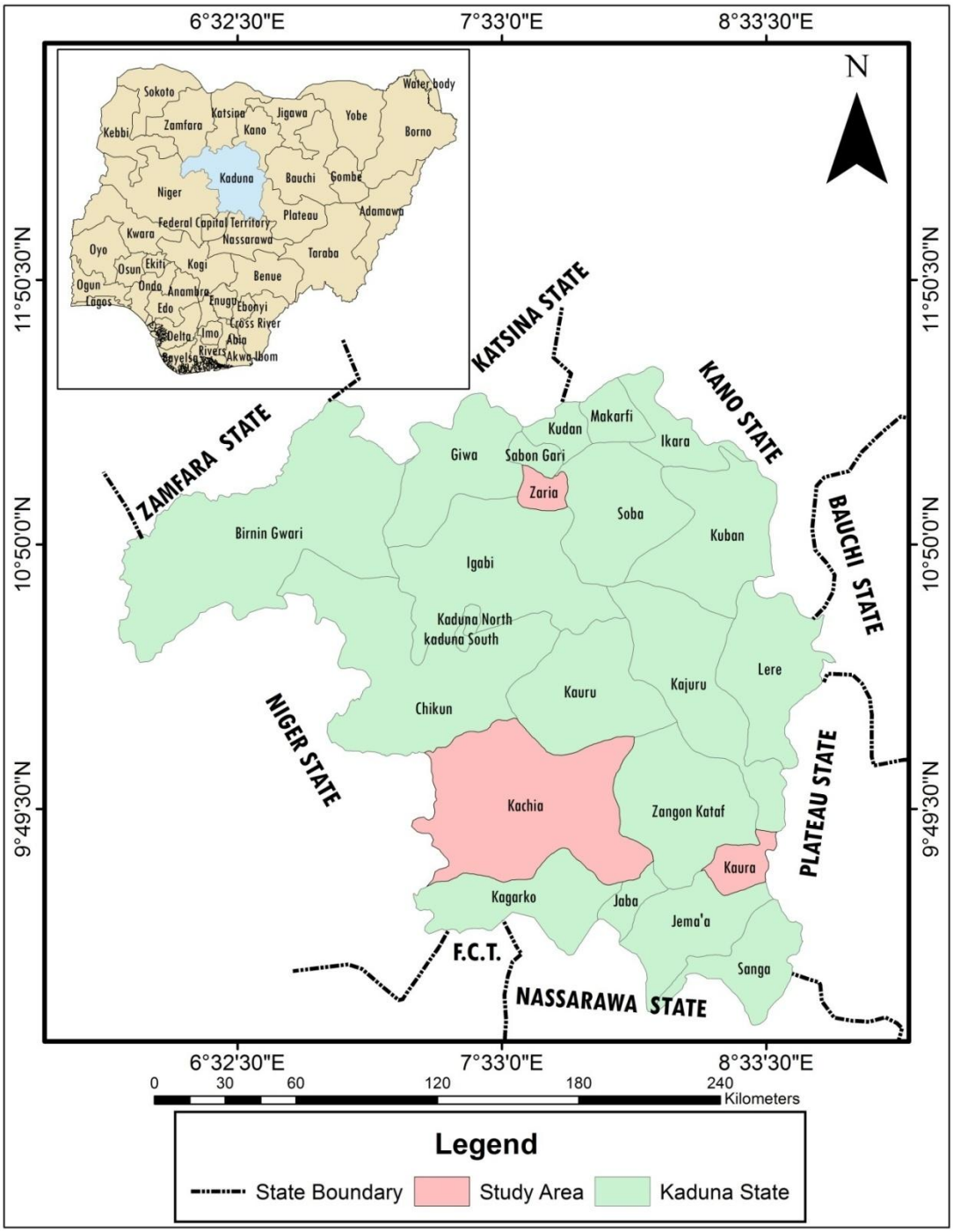


Figure 3.2: A geographical map of Kaduna State showing the study areas. (Source: Modified from the administrative map of Kaduna State).

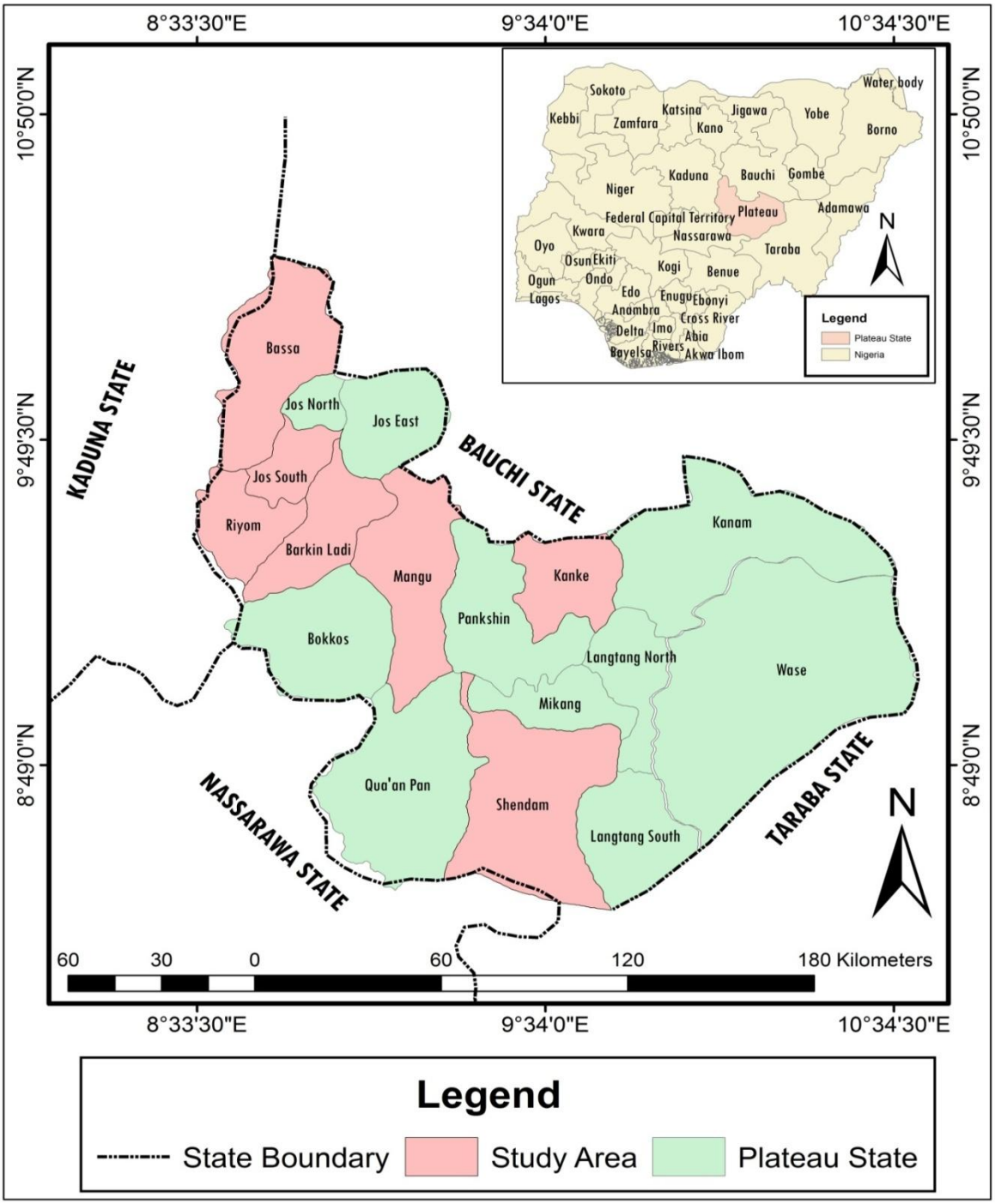


Figure 3.3: A geographical map of Plateau Stateshowing the study areas. (Source: Modified from the administrative map of Plateau State).

3.2Sampling Techniques, Sample Size and Sample Collection

3.2.1 Sampling techniques

This is a cross-sectional descriptive and analytical study. The multi-stage sampling technique for the survey study and a targeted sampling technique forsuspected FMD outbreak investigation (Toma *et al.*, 1999) was employed.

In Kaduna State, the survey for blood sample collection was carried out in Kachia grazing reserve (KGR), which comprises of six contiguous administrative blocks containing 774 households (cattle owners), with about 80-188 household per block.The survey tool box program was used to select 40 households/herds for blood sample collection. The ultimate sampling unit was the individual animals in selected herds. All ruminant animals in a selected herd were sampled. In Plateau State, cattle herds, within five LGAs (Barkin-ladi, Jos-South, Bassa, Mangu and Shendam) across the three senatorial zones of the State were studied.In the first stage, a random selection of at least one Local Government Area (LGA) from each of the three senatorial zones of the state was carried out. From each selected LGA, a random selection of at least five cattle herd was done, and within each selected herd, a proportion of animals were randomly selected depending on the herd population.

All herds were eligible irrespective of herd size. However, herds whose owners were uncooperative were excluded. Sampled animals were properly restrained. Five milliliters of blood was collected via the jugular vein in a syringe and allowed to clot. Samples were kept in a Coleman box and transported to the laboratory in National Veterinary Research Institute Vom, and centrifuged at 1100g for 5 minutes. Sera were aliquoted into 1.8mls cryovials (Nunc), frozen and stored at -20^{o}C.

3.2.2 Sample size

The sample size was calculated through conventional statistical methodsbased on an estimated 36.5% sero-prevalence rate for Plateau State (Ehizibolo, 2010).

A total of 2007 blood samples were collected from ruminant animals in Kaduna and Plateau State, Nigeria, between 2009 and 2015. Of these, 1926 were sera collected from a survey study in KGR and Plateau, whilst 81 sera were collected from outbreak investigations in Kaduna State. From the 1926 sera, 1321 (1155 cattle and 166 sheep) were from KGR, Kaduna State whilst 605 (456 cattle, 109 sheep and 40 goats) were from Plateau State (Bassa, Barkin-ladi, Jos South, Mangu and Shendam).

3.2.3 Sample collection

Clinical materials were collected from outbreaks following notification by the Fulani herdsmen or veterinary/para-veterinary field officers in the studied areas. Epithelial tissue samples (n=103) from erupted vesicles and oro-pharyngeal (probang) samples (n=10) were collected from suspected FMD clinical cases in Kaduna and Plateau State into virus transport medium (50% glycerol and 50% PBS; pH 7.2). Of these, 50 samples (40 epithelium and 10 probang) were from FMD-suspected cases in 3 LGAs (namely Kachia, Kaura and Zaria) in Kaduna State; and 63 epithelium samples were from suspected cases in 5 LGAs (Barkin-ladi, Jos-South, Bassa, Riyom and Kanke) in Plateau State. These were stored at -70^{o}C until required. Import permits were obtained from the Federal Agency for the Safety of Food Chain, Brussels, Belgium and the United States Department of Agriculture (USDA) – Veterinary Services, Maryland, USA and export permit was obtained from Federal Livestock Department, Federal Ministry of Agriculture and Rural Development, Abuja, Nigeria, and the samples were shipped in dry-ice to the Vesicular Diseases Unit, Veterinary and Agrochemical Research Centre (CODA-CERVA), Belgium and the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island Animal Disease Center (PIADC), USA, for laboratory analyses.

3.3 Analysis of Serum Samples

3.3.1 Foot and mouth disease virus 3ABC ELISA

The PrioCHECK® FMDV NS antibody test enzyme-linked immunosorbent assay (ELISA) kit (Prionics Lelystad B.V., The Netherlands, product code 7610450), was used according to the manufacturer's instructions for detection of antibodies to the highly conserved non-structural 3ABC protein of FMDV (Sørensen *et al*, 1998). Briefly, the ELISA kit contains plates pre-coated with the FMDV 3ABC nonstructural antigen captured by anti-3ABC monoclonal antibody (mAb). Test and control sera were added to plates as directed, followed by the addition of a secondary monoclonal antibody-horseradish peroxidase (mAb-HRP) detector and substrate chromogen (O-phenylene-diamine dihydrochloride (OPD)). Anti-3ABC antibodies, if present in sample sera, block the binding of the secondary mAb-HRP detector thereby decreasing color development. Results are determined based on percent inhibition (PI). 3ABC ELISA positives were tested on solid phase competitive ELISA (SPCE) and the virus neutralization test (VNT) for serotype determination.

3.3.2 Solid phase competitive ELISA

Sera positive for the 3ABC NSP ELISA were subsequently screened using an in-house antibody ELISA, the serotype-specific solid phase competitive ELISA (SPCE), based on the methodology and reagents developed at the World Reference Laboratory for FMD (WRLFMD), Pirbright laboratory with some modifications. The FMD virus O_1 Manisa, A22 Iraq 24/64, A Iran 96, SAT 1 ZIM 25/89, SAT 2 ZIM 3/97, SAT 3 ZIM 4/99 provided by the WRLFMD, Pirbright, UK were used as antigens. Guinea pig and rabbit immune sera towards the above viruses were also produced at WRLFMD, Pirbright, UK. All incubations were at 37^{o} C, and plates were washed three times between steps using wash buffer (0.015M Na_2 $HP0_4$, 0.0025M KH_2, 0.5M NaCl, 0.05% Tween-20). Serotypes were run on separate plates, and plates were coated with serotype-specific guinea pig sera diluted in carbonate bicarbonate (0.035M $NaHCO_3$, 0.015M

$Na_2CO_3.10H_2O$) coating buffer for 1 h. 30 min and subsequently reacted with the corresponding antigen diluted in ELISA buffer (PBST phenol red) for 1 h.

Each plate included in duplicate, two wells with a cut off sera, one well with a weak positive control sera, two wells with a negative control sera, one well with a positive control sera and two wells of virus control (buffer). For serotype screening, test, control and guinea pig sera were added to the plates in 1/10 dilution in ELISA blocking buffer (10% negative bovine serum, negative porcine serum and negative rabbit serum). Following 1hour incubation, horseradish-peroxidase conjugated to polyclonal rabbit anti-guinea pig immunoglobulin (IgG) diluted 1/1000 in ELISA blocking buffer was added for 1 h. The substrate chromogen (10mg O-phenylene-diamine dihydrochloride (OPD) suspended into 25ml of citrate phosphate buffer, activated with 30% hydrogen peroxide) was added and incubated in the dark for 15min. the ELISAs were stopped using sulphuric acid (H_2SO_4) and plates were read on a microplate reader at 490nM. PI ≥40% was considered positive.

3.3.3 Virus neutralization test

The virus neutralization test (VNT) was performed on serum samples according to OIE (2009), using the following FMDV serotypes: A Iraq 4/90, O1 Manisa, C1 Noville, SAT-1 Rho 5/66, SAT-2 Zim 5/81, and SAT-3/Rho 7/34. Briefly, an equal volume of sera diluted 2-fold was incubated at 37°C (5% CO_2) for 60 min with a standardized quantity of each virus, between 32 and 100 $TCID_{50}$. Following the addition of swine kidney (SK-6) cells, the test was incubated at 37°C (5% CO_2) for 3 days. Serum samples were considered positive to the virus strain that produce the highest neutralizing titres. The Spearman-Karber method was used to determine the 50% endpoint antibody titer.

3.4 Analysis of Tissues

3.4.1 Virus isolation

Tissues were prepared as 10% w/v homogenates in PBS (1.05mM KH_2PO_4, 155.5mM Nacl, 2.96mM $Na_2HPO_4.7H_20$, pH 7.4) in 2ml micro centrifuge tubes, using tissue lyser (Type 85220, Retsch Gmbh, Rheinische, Germany). The homogenized tissue was centrifuged at 10, 000 x g for 10minutes and filtered through 0.2µm filter. Virus isolation (VI) was performed using swine kidney (SK-6),Instituto Biologico Rim Suino-2 swine kidney (IBRS-2), baby hamster kidney (BHK-21) and fetal lamb kidney (FLK) cell-lines. Confluent monolayers of cell culture in 24-well plates (Thermoscientific, Waltham, MA, USA) containing 1ml of reserved media (1x minimum essential medium (Gibco, Life Technologies) with antibiotics (gentamycin) and 2% fetal calf serum) were used. Media were removed from appropriate wells, and 0.3ml of sample filtrate was added, and incubated for 30minutes at 37^oC for adsorption. Inoculum was aspirated from the wells then 1ml/well of media (minimum essential medium + antibiotic without fetal calf serum) was added and incubated at 37^oC for 72hours. The monolayers were monitored daily for the presence of cytopathic effect (CPE). Wells with CPE were harvested into labeled cryovials and stored at -80^oC until tested by the real-time reverse transcription polymerase chain reaction (rRT-PCR) for FMDV identification and by antigen-capture ELISA (AgELISA) for virus serotyping. Wells without CPE were frozen at -80^oC and thawed, and the lysate (1ml) collected into 2ml micro centrifuge tubes and centrifugd at 5000 x g for 20minutes; 0.3ml of this lysate was inoculated into confluent monolayer in 24-well plates containing reserved media for second passage. A sample was considered negative for wells without CPE after three passages.

3.4.2 Foot and mouth disease virus AgELISA

The cell culture isolates were serotyped using AgELISA test as described by Dulac *et al.,* 1993. Rabbit polyclonal antisera to FMDV types O1 BFS 1860 and 01 Manisa, A5/22/24 (Combination) and A Col 85, C3 Resende, SAT 1 BOT 1/68, SAT 2 ZIM 5/81, SAT 3 ZIM 4/81 and Asia 1 CAM 9/80 were used to coat the wells and guinea pig polyclonal antisera to the same

FMDV serotypes were used as detector antibodies in the assays. The conjugate, MP (ICN Kappel) was a commercial Rabbit anti-guinea pig conjugate with horseradish peroxidase.The normal bovine serum was from Hyclone Cosmic CalfTM. The normal rabbitserum was obtained from Sigma. After coating microplates (96 wells U-bottom), 50µl of samples and controls were dispensed into appropriate wells and incubated for 1hour at 37^{o}C. Plates were washed and 50µl/well of reconstituted guinea pig serum was added, and incubated at 37^{o}C for 1 hour. This was followed by the addition of 50µl/well of anti-guinea pig conjugate and incubating at 37^{o}C for 1hour. The substrate chromogen (10mg O-phenylene-diamine dihydrochloride (OPD) suspended into 25ml of citrate phosphate buffer, activated with 30% hydrogen peroxide) was added (50µl/well) and incubated for 15min. the reaction was stopped with the addition of Sulphuric acid (H_2SO_4) and plates were read on a micro-plate reader at 490nM. Positive test was determined by percentage inhibition.

3.4.3 RNA extraction

The RNA from cell culture isolates was extracted using the Nucleospin Viral RNA extraction kit (Macherey-Nagel, Duren, Germany) according to manufacturer's instructions. Viral lysis was carried out by the addition of 150 µl of each respective sample to 600 µl 30-60% guanidine thiocyanate (RAV1) dissolved in 1mg carrier RNA, and incubated at 70^{o}C for 5minutes. Binding condition was adjusted by adding 600µl of absolute ethanol (96-100%) to the lysis solution and mixed by vortexing for 10-15s. Viral RNA was bounded by placing the spin column in 2ml collecting tube with the loading of lysed sample step-wise and centrifuging at 8000 x g for 1mins. Three washing steps followed thereafter; first was the addition of 500 µl of wash buffer, guanidine hydrochloride (RAW) to the spin columnand centrifuged at 8000 x g for 1min; followed by the addition of 600µl buffer RAV 3 (100ml ethanol added into RAV 3), which was also centrifuged at 8000 x g for 1min; then the addition of 200µl of buffer RAV 3 to the column

and centrifuged at 11000 x g for 5mins to remove ethanolic buffer completely. The column was placed in a well labeled 1.5ml micro centrifuge tube and viral RNA was eluted by the addition of 50µl Rnase free water (pre-heated at 70^{o}C) and centrifuged at 11000 x g for 1min. The extracted RNA was stored at -80^{o}C until analysed.

3.4.4 Real-time polymerase chain reaction assay

The extracted RNA were tested for the presence of nucleic acid of FMDV by rRT-PCR, using oligonucleotide primers and probe targeting a conserved region within the FMDV RNA polymerase (3D) gene (Callahan *et al.*, 2002). The location and sequence of the primers and probes are shown in Table 3.1. The probe was labeled with a 5'-reporter dye, 6-carboxyfluorescein, and a 3'-quencher, tetramethylrhodamine. The rRT-PCR was performed on a Light Cycler® 480 (Roche, Diagnostic Ltd, Rot Kreuz, Switzerland) in 2 steps; the denaturation step, with a mixture 11.48µl total volume containing primer mix of 3.48µl and 8µl RNA sample with a denaturation condition of 95^{o}C for 3mins and 4^{o}C for 1min. the second step is the PCR step with a mixture of 20µl total volume containing probe mix of 10µl and 10µl sample (denaturation product), with 3-stages of PCR condition; reverse-transcription at 50^{o}C for 30mins/1cycle, inactivation at 95^{o}C for 2mins/1cycle amplification at 95^{o}C for 10s/50cycles and cooling at 4^{o}C for 2mins. Positive result was determined by the threshold value (C_t). A $C_t$value ranging from 12.0-20.0 was considered positive for FMDV 3D gene from cell culture isolate.

Table 3.1: Sequences of oligonucleotide primers and probes used for reverse transcription-PCR, real-time-PCR and sequencing

0ligonucleotide	Sequence (5'→3')	Sense	Location	Serotype	Method used
EUR-2B52R	GACATGTCCTCCTGCATCTGGTTGAT	–	2B	O, A, C	RT-PCR
O-1C244F	GCAGCAAAACACATGTCAAACACCTT	+	VP3	O	RT-PCR
O-1C272F	TBGCRGGNCTYGCCCAGTACTAC	+	VP3	O	RT-PCR
O-1C283F	GCCCAGTACTACACACAGTACAG	+	VP3	O	RT-PCR
A-1C562F	TACCAAATTACACACGGGAA	+	VP3	A	RT-PCR
A-1C612F	TAGCGCCGGCAAAGACTTTGA	+	VP3	A	RT-PCR
SAT 2B208R	ACAGCGGCCATGCACGACAG	-	2B	SAT 1, SAT 2	RT-PCR
SAT1-1C559F	GTGTATCAGATCACAGACACACA	+	VP3	SAT 1	RT-PCR
SAT1U-OS	GTGTACCAGATCACTGACAC	+	VP3	SAT 1	RT-PCR
SAT2-1C445F	TGGGACACMGGIYTGAACTC	+	VP3	SAT 2	RT-PCR
SAT2-P1-1223F	TGAACTACCACTTCATGTACACAG	+	VP3	SAT 2	RT-PCR
M13F	GTAAAACGACGGCCAGT	+			sequencing
M13R	AACAGCTATGACCATG	-			sequencing
3D-Pf	ACTGGGTTTTACAAACCTGTGA	+	3D	Universal-p	rRT-PCR
3D-Pr	GCGAGTCCTGCCACGGA	-	3D	Universal-p	rRT-PCR
3D-tp	FAM-TCCTTTGCACGCCGTGGGAC-BHQ1		3D	Universal -p	rRT-PCR

(Ayelet *et al.*, 2009; Callahan *et al.*, 2002)

3.4.5 Reverse transcription polymerase chain reaction

The Reverse transcription polymerase chain reaction (RT-PCR) was performed according to the protocol described by Knowles *et al.* (2005). It is a 2-step PCR comprising of a reverse transcription (RT) step which involves the synthesis of a first-strand complementary DNA (cDNA), followed by the PCR amplification of the synthesized cDNA. The RT reaction mixture of 16µl containing the first-strand synthesis buffer (50mM Tris-HCl PH 8.3, 40mM KCl, 6mM $Mgcl_2$, 0.5mM TTP); dNTPs ½ (0.3mM); DDT (100mM); N6; Rnase inhibitor; Rnase free water and multiscribe reverse transcriptase were added to 4µl extracted RNA and incubated in a thermocycler at 37^{o}C for 1hour. Following the cDNA synthesis, the PCR amplification was carried out by adding 2µl RT-product to a 48µl PCR mix comprising of 5µl 10x ready mix buffer (20mM Tris HCl, PH8.3, 100mM KCl, 3mM $MgCl_2$, 0.002% gelatin); dNTP (0.3mM) ½ dilution; $MgCl_2$ (50mM); forward and reverse primers (15pmol/µl) 0.06 unit/µl TaqDNA polymerase and nuclease free water to give a 50µl total reaction volume. Three alternative primer combinations were used for the RT-PCR of FMDV serotype O viruses: O-lC244F/EUR-2B52R, O-lC272F/EUR-2B52R, and O-lC283F/EUR-2B52R. Two primer sets were used for each of the other serotypes: Serotype A (A-lC562F/EUR-2B52R and A-lC612F/EUR-2B52R); SAT 1 (SAT1-1C559F/SAT-2B208R and SAT1U-OSF/SAT-2B208R) and SAT 2 (SAT 2-lC445F/SAT-2B208R and SAT 2-Pl-1223F/SAT-2B208R). The primer sequences are presented in table 3.1. The thermal profiles used for the PCR of various serotypes were as follows: FMDV O: 94^{o}C for 5mins, 35 cycles of 94^{o}C for 60s, 60^{o}C for 10s, 72^{o}C for 90s followed by a final extension of 70^{o}C for 5mins. Conditions were the same for the other serotypes except that extension temperatures were 55^{o}C for A and 50^{o}C for SAT 1 and SAT 2.

3.4.6 Agarose gel electrophoresis

The RT-PCR cDNA amplicons were analyzed in a prepared 1.5% agarose gel electrophoresis in Tris acetate-EDTA (TAE, 0.04M Tris acetate, 1mM EDTA), stained with gel red and visualized on UV trans-illuminator (MacroVue, UV20, Hoefer, VilberLourmat, France). A 1Kbp DNA ladder (Invitrogen, Germany) was used to comparatively determine the molecular size of the PCR amplicons. A 5µl loading dye was added to 30µl PCR product. The solution was mixed by pipetting up and down before loading the gel.

3.4.7 Gel extraction

The PCR cDNA amplicons were purified from agarose gel by using QIAquick gel extraction kit (Qiagen Ltd, Crawley, West Sussex, UK) according to the manufacturer's instructions. The DNA fragment from agarose gel was excised and placed into a transparent 2ml micro centrifuge tube and 300µl of a solubulization buffer (buffer QG). This was incubated at 50^{o}C for 10mins (or until gel slice has completely dissolved), 100µl of isopropanol was added to the solution and mixed. The solution was applied to a QIAquick spin column placed in a provided 2ml collection tube to bind DNA and centrifuged at 13000 x g for 1min. Another 500µl buffer QG was added to the column and centrifuged as above. The flow through was discarded and 750µl buffer PE (wash buffer) was added and allowed to stand in the column for 2-5mins before centrifuging as above. Residual wash buffer is removed by a second spinning at 13000 x g for 1min. DNA was eluted by the addition of 50µl buffer EB (10mM Tris-Cl, pH 8.5) into the column placed into a clean 1.5ml micro centrifuge tube. The purified DNA was stored at +4^{o}Cuntil used.

3.4.8 DNA ligation (cloning) and chemical transformation

The purified DNA was ligated into a PCR 2.1-Topo vector (Life Technologies, Carlsbed, CA, USA) according to manufacturer's instruction. The reaction mixture with a final volume of 6µl (4µl fresh PCR product, 1µl salt solution and 1µl PCR 2.1-Topo vector) was incubated overnight at 11^{o} C. Ligated product (DNA + plasmid vector) was transformed into chemically competent

DH5α cells (*Escherichia coli*) by heat shock method using the Topo TA cloning procedure with some modifications. For each transformation reaction, one vial of DH5α competent cells (50µl) was removed from -80^{o}C and thawed on ice, 2µl of plasmid DNA was added, then mixed gently by swirling tube, and incubated on ice for 30mins. Heat shock cells at 42^{o}C for 30secs without shaking then replaced immediately on ice for 2-5mins and followed by addition of 250µl of SOC medium (2% tryptone, 0.5% yeast extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, & MgSO4) warmed at room temperature and incubated at 37^{o}C for 1hour. The bacterial cultures (250µl) were spread evenly over a pre-warmed Luria-Berteni (LB) agar plate(s) containing 100µg/ml ampicillin using a "hockey puck" spreader, and incubated at 37^{o}C overnight. An efficient cloning reaction produces several colonies. Agar plates were stored a 4^{o}C until required for further analysis.

3.4.9 Amplification and purification of recombinant DNA clones

At least 6 recombinant clones were randomly selected from each agar plate onto 25µl/50µl falcon tubes containing 10ml LB medium and incubated at 37^{o}C overnight for clone amplification. Recombinant clones that showrd evidence of growth were purified using QIA spin mini prep kit (Qiagen Ltd, West Sussex, UK) according to manufacturer's instructions. The 10ml bacterial overnight cultures were pelleted by centrifugation at 6800 x g for 3mins. The pelleted cells were re-suspended in 250µl buffer P1 (re-suspension buffer) and transferred to a micro centrifuge tube. Then 250µl of a lysis buffer (buffer P2) was added and mixed thoroughly by inverting the tubes 4-6 times until the solution becomes clear, followed by the addition of 350µl neutralization buffer (buffer N3, guanidine hydrochloride). This was also mixed as above until the solution turned colorless, then centrifuged at 17,900 x g for 10minutes. The supernatant was applied to the QIAprep spin column and centrifuged at 17,900 x g for 1min. The binding buffer (buffer PB, 500µl) was added to the spin column and centrifuged as above, after which 750µl of

the wash buffer (buffer PE) was added and the tubes were centrifuged twice at 17,900 x g for 1min to remove residual wash buffer. The plasmid DNA was eluted by the addition of 50µl of buffer EB (10mM TrisCl, pH 8.5), and centrifuged at 17,900 x g for 1min. and stored at -20^{o}C until tested.

3.4.10 Restriction enzyme analysis of recombinant DNA clones

The restriction enzyme, EcoR1 was used for the screening of the plasmid DNA clones of FMDV. A total reaction volume of 20µl comprising of 2µl reaction buffer, 0.5µl EcoR1 enzyme, 12.5µl distilled water mixed with 5µl of eluted plasmid DNA was incubated at 37^{o}C for 1hour. After incubation, the reaction mixture was mixed with loading dye and loaded on 1% agarose gel electrophoresis to verify the correct orientation of the insert (FMDV) and the plasmid. A single clone from each sample with correct insert-plasmid orientation was selected for sequence reaction.

3.4.11 DNA sequencing

The amount of recombinantDNA product was quantified using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific Inc., Wilmimgton, De, USA) prior to sequencing. The recombinant DNA was sequenced using Bright Dye Terminator Cycle Sequencing kit using the M13F/M13R oligonucleotide primers (primer sequence shown in table 3.1). A total reaction mixture of 20µl (comprising 3µl 5x Bright Dye sequencing buffer, 2µl Bright Dye, 1µl primer 1µl template DNA and 13µl DEPC treated water) was placed unto a thermocycler with thermal profile of 96^{o}C for 1min, 30 cycles of 96^{o}C for 10s, 50^{o}C for 5s and 60^{o}C for 4min.

The sequenced DNA was purified using the Big Dye XTerminator purification kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions. For a 20µl reaction size, 90µl/well of SAM solution was added followed by the addition of 20µl/well of Bright Dye XTerminator solution. The plate was sealed and mixed thoroughly on a plate mixer

for 30min, then centrifuged at 1000 x g for 2mins. DNA sequencing was performed on an ABI 3130 genetic analyzer (Applied Biosystems, Foster city, CA, USA). Nucleotide sequences covering the complete VP1-coding region were assembled and edited using chromas software package.

3.4.12 Phylogenetic analysis

The complete VP1 sequence was used for phylogenetic analysis, which was performed using the maximum likelihood optimality criterion as implemented in PAUP* version β10 (Swofford, 1998). Additional previously determined complete VP1 sequences from GenBank were included in the analysis. Parameters of nucleotide substitution were estimated using Modeltest, version 3.7 (Posada and Crandall, 1998). The sequences of 60 isolates reported here were submitted to GenBank for accession numbers.

3.5Asseement of Factors Associated with Foot and Mouth Disease

A pre-tested structured questionnaire (Appendix 1) was developed through discussion with field extension workers, researchers and livestock owners, in order to capture information related to epidemiological factors potentially associated with FMD prevalance. The questions covered herd information, production system, previous/recent FMD outbreak, herd movement, grazing system, number of contacts between herds and wildlife,number of contacts at watering point, animal purchases from the markets, how herdsmen respond to outbreak situation and several relevant questions. A total of 198 questionnaires were distributed to livestock owners/herdsmen from which blood samples were obtained from their animals and others who were willing to participate across the States. The questionnaire was composed in English and translated to the herdsmen in Hausa language. Each questionnaire took about 25-40 minutes to be administered.

3.6 Data Analyses

Questionnaire responses were used to assess herd management system and to identify factors associated with FMD prevalence at a herd level (herd level model), whereas the results of the

serological tests were used to assess the association between exposure to FMDV and epidemiological factors at the individual level (individual level model). Records with incomplete information were not included in the statistical analysis and for that reason, the herd level model included the information from 194/198 questionnaires, whereas the individual level model included data and results of analysis from 1922/1926 serological tests.

The dependent variables were FMD status (present, absence), as reported in the questionnaires by cattle farmers, for the herd level model and antibody status for FMD (positive, negative) detected by 3ABC-ELISA for the individual level model. Epidemiological factors included in the questionnaires were considered independent variables for the analysis. A threshold value of $P < 0.05$ in a two-by-two chi-square (χ^2) test and$P < 0.01$ was used in a two-by-two (bivariate) χ^2 test to screen out independent variables unlikely associated with the dependent variables. Variables that passed this initial screening were included in multivariate model using a backward-elimination procedure starting with the least statistically significant from the bivariate analysis. As each variable was removed from the saturated model, which includes all variables that passed the bivariate screening, the model was reassessed using a likelihood-ratio test (LLR). The LLR test compares the saturated model to the model with terms removed and assumes that the assessed model is nested within the saturated model. Variables that were no-longer significant were dropped and the deviance and its effect on other variables were reassessed. If the coefficient for a variable was altered by more than 10% (suggesting confounding), the variable was re-entered into the model (Hosmer and Lemeshow, 2000). Interaction between pairs of variables was assessed in the final models. The variables included in the final model were assessed for collinearity following the suggestion from Hosmer and Lemeshow (2000) that any variable with a correlation >0.9 should be further assessed. The Akaike's Information Criteria

(AIC) and the Bayesian Information Criteria (BIC) were used to compare between the non-nested competitive models as a final confirmation process and the final model was the one with the smallest AIC and BIC values.

The overall fit of the final models was assessed using Pearson's χ^2 statistic and the Hosmer-Lemeshow χ^2 statistic (Hosmer and Lemeshow, 2000). The regression diagnostics Delta D (ΔD) and influence ($\Delta\beta$) were calculated and plotted against the predicted probability of disease from the models. Statistical analyses were conducted using 'SPSS 17' software package (SPSS Corporation, Chicago, Illinois, USA). Missing value analysis was carried out to replace missing values that correspond to each variable.

CHAPTER FOUR
4.0 RESULTS
4.1 Risk Factors Analyses

In Table 4.1, a higher percentage, 99.5% of livestock farmers/herdsmen in Kaduna and Plateau State, essentially rear livestock for both meat and milk production. There was a statistical difference ($P<0.05$) in management system between nomadic fulanis (51.0%) who move southwards during dry season in search of pasturage and water sources, and settled fulanis (45.5%) as well as trade cattle rearers (3.5%). The percentage (98%) of herdsmen on common (free-range) grazing system was significantly ($P<0.05$) higher than those on enclosed (fenced) grazing (1.5%) and zero grazing (0.5%) systems. The finding also shows that a significant proportion ($P<0.05$) of herdsmen in the study areas (98.5%) do not practice any routine

vaccination against FMD, however, some few commercial farms (1.5%) embark on annual vaccination with imported vaccines from Botswana. The practice of keeping small ruminants together with cattle in the study area seems to be common. There was a significant difference ($P<0.05$) in the percentage of sheep (89.4%) reared with cattle at pasture compared to the keeping of goats (9.6%) with cattle by the herdsmen. Significant percentage (60.1%) of livestock farmers reported the prevalence of FMD in the administrated questionnaire. Also, 31% view FMD as an important disease in the past, 54.5% at present and another 13.4% views it as an important disease in the future. The differences between variables were statistically significant ($P<0.05$).

Table 4.1: Herdsmen's response to husbandry/management system and the status of FMD infection in Kaduna and Plateau States

Variable	Number of respondent	Percentage of total respondent	χ^2 p-value
Production System			
Dairy	1	0.5	< 0.001*
Meat	0	0	
Dual	197	99.5	
Herd Management			
Settled	90	45.5	< 0.001
Nomadic	101	51.0	
Trade	7	3.5	
Grazing System			
Common	194	98.0	< 0.001*
Enclosed	3	1.5	
Zero	1	0.5	
Other animal species kept with cattle			
Sheep	117	89.4	< 0.001

Goats	19	9.6	
Vaccination status of herds against FMD			
Vaccinated herds	3	1.5	< 0.001
Unvaccinated herd	195	98.5	
Previous outbreak/occurrences of FMD in locality			
Yes	119	60.1	< 0.001
No	76	39.9	
Do you consider FMD an important problem in your locality			
In the past	63	31.8	< 0.001
At present	108	54.5	
In future	27	13.6	

*Fisher's exact test

Table 4.2 shows the results of the analysis of livestock farmers/herdsmen's response to the likely exposure variables to FMDV infection. A significant percentage (77.8%, P<0.05) of herdsmen have contacts with other herds during grazing and (62.6%, P<0.05) do not have contacts with wildlife during transhumance. However, amongst the 36.9% of the herdsmen who encountered wildlife during transhumance, antelope (16.2%) and other wildlife types like Monkeys (n=24), Hares (n=30) and Hyena (n=11) which accounted for 20.7% were encountered. No respondent appears to encounter buffalo during transhumance. An important source of contact of the disease may be through the market. A significant proportion of herdsmen administered the questionnaire (71.7%, P<0.05) appear not to purchase livestock from the market, however, amongst the 25.5% who purchase livestock from the market, 6.6% had purchased livestock a month ago (as at time the questionnaires were applied), 10.6% purchased livestock a year ago and another 8.6% purchased beyond a year ago. The percentage of respondents that will not buy animals infected with FMD in the market were higher (94.5%, P<0.05) than those that bought infected animals from market. Also, it appears that a significant percentage of respondent in the study areas

(62.1%, P<0.05) do not sell sick animals infected with FMD to the market; however, 37.4% do sell animals infected with FMD to the market.

Thirty-five exposure variables were screened for association with the outcome variable, 'Prevalence of FMD reported by cattle farmers in Kaduna and Plateau States of Nigeria'. Ten variables passed the initial screening for use in the multivariable model (Table 4.3).

Table 4.2: Herdsmen's response to likely risk factor variables of FMD infection in Kaduna and Plateau State

Variable	Number of respondent	Percentage of total respondent	χ^2p-value
Contact with other herds during grazing			
Yes	154	77.2	< 0.001
No	44	22.8	
Contact with wildlife during transhumance			
Yes	73	36.9	< 0.001
No	124	62.6	
Types of wildlife encountered during transhumance			
Buffalo	0		< 0.001
Antelope	32	16.2	
Other†	41	20.7	
Any purchase made from the market			
Yes	51	25.8	< 0.001
No	142	71.7	
When was purchase made			
Last month	13	6.6	< 0.001
Last year	21	10.6	
Other	17	8.6	
Will you buy FMD infected animal from the market			
Yes	11	5.5	< 0.001

No	187	94.5	
Sell animals sick with FMD to the market			
Yes	74	37.4	< 0.001
No	123	62.1	

†Monkey (n = 24), Hyena (n = 11), Hare (n = 30)

Table 4.3: Association of some risk factors with prevalence of FMD as reported by cattle farmers in Kaduna and Plateau States

		95% C.I.for O.R.	
Variables	**O.R.**	**Lower**	**Upper**
Movement type			
Settled	0.511	0.274	0.952
*Nomadic	2.24	1.199	4.199
Trade	0.332	0.030	2.40
Herd Size			
≤50	0.473	0.243	0.92
≤100	0.823	0.445	1.52
*≥101	6.62	2.17	26.8
Grazing Type			
*Common	5.49	1.00	55.2
Enclosed	0.338	0.006	6.63
Zero	0.130	0.003	1.20
*Suspect FMD in Nearby	8.25	4.02	17.10
*Transhumance	2.09	1.12	3.91
*Wildlife Contact	2.53	1.33	4.82
Place of Animals at Night			
*Open field	3.80	1.57	9.63
Fenced Field	0.29	0.11	0.73
Cattle Market Pens	0.22	0.00	2.85
Number of Contacts within A herd			
1-2 contacts	0.541	0.291	1.01
3-4 contacts	1.15	0.616	2.15
*5-6 contacts	4.70	1.29	25.7
*Use of Drugs/Vaccines to Control FMD	2.10	1.04	4.33

*χ^2 2-sided P-value < 0.01

At the herd-level risk factors, ten variables passed the bivariate screening (P-value < 0.1) and were included as candidate variables in the multivariable model. Those ten variables were movement type (settled, nomadic and trade), herd size (≤50, 51–100 and >100), grazing type (enclosed, common pastures grazing and nomadic), suspected FMD nearby (yes and no), transhumance (yes and no), contact with wildlife (yes and no), overnight practice (open field, fenced field and cattle market pens), number of contacts within a herd (<3, 3–4 contacts and >4), use of drugs or FMD vaccines (yes and no) and presence of concomitant diseases (yes and no). The final model included five variables. Farms with suspected FMD nearby [odds ratio (OR): 9.5, 95% CI: 4.4–20.4, P < 0.01], with contact with wildlife (OR: 1.9, 95% CI: 0.9 –4.1, P < 0.01), that used drugs or FMD vaccines (OR: 3.2, 95% CI: 1.4–7.6, P < 0.01) and with >100 animals (OR: 5.8, 95% CI: 1.5–22.5, P < 0.01) were significantly associated with FMD (Table 4.4). Although not significantly associated with the outcome, grazing type was also retained in the final model because its removal caused a >10% change in the estimates of OR. No significant interactions were detected, and the final model fitted the data well (Hosmer–Lemeshow chi-squared P-value = 0.987).

At the individual-level risk factors, large ruminants (OR: 9.8, 95% CI: 5.2–18.4, P < 0.01), rather than small ruminants, and enclosed (OR: 3.4, 95% CI: 1.6–7.1, P < 0.01) or common pastures grazing (OR: 10.8, 95% CI: 5.1–22.3, P < 0.01), rather than nomadic grazing were associated with FMD-positive status to the 3ABC ELISA test (Table 4.5). The interaction between both variables was not significant, and the final model fitted the data well (Hosmer–Lemeshow chi-squared P-value = 0.11)

Table 4.4: Multivariate logistic regression model for risk factors associated with the prevalence of FMD reported by cattle farmers in Kaduna and Plateau state

Variables in the Equation	O.R.	95% C.I.for O.R. Lower	Upper
Suspected FMD Nearby	9.48	4.41	20.4
Wildlife Contact	1.94	0.932	4.06
Use of Drugs/Vaccines to Control FMD	3.21	1.36	7.58
Herd Size			
≤50	1.00	-	-
≤100	1.10	0.505	2.39
≥101	5.81	1.50	22.5
Grazing Type			
Zero	1.00	-	-
Common	7.76	0.7	80.4
Enclosed	0.358	0.007	17.1

Pearson's χ^2 P-value <0.01; Hosmer–Lemeshow χ^2 P-value = 0.987

Table 4.5: Multivariate logistic regression model for FMD categorical risk factors associated with the serological results of livestock herds in Kaduna and PlateauState

	O.R.	95% C.I.for O.R.

Variables in the Equation		Lower	Upper
Species (Large ruminants)	9.81	5.23	18.39
Grazing Type			
Zero	1.00	-	-
Enclosed	3.38	1.60	7.12
Common	10.78	5.07	22.9

Pearson's χ^2 P-value <0.01; Hosmer–Lemeshow χ^2 P-value = 0.11

4.2 Clinical Observation

Reported cases of suspected FMD defined in cattle herd as animals showing signs of lameness, with vesicular lesions on the feet and/or in the mouth also known as 'Boro' by the herdsmen were investigated in Kaduna and Plateau State between 2009 and 2015. FMD-like clinical signs were observed (Plate 4.1), as vesicular lesions in the mouth, muzzle; there was excessive salivation, lameness and nasal discharges or oral lesions such as erosion or ulceration of the oral mucosa and crusting of the muzzle.

4.3 Serological Analyses

Table 4.6 shows the summary of sera collected from ruminant animals in Kaduna and Plateau State, Nigeria between 2009 and 2014. Foot and mouth disease seroprevalence, as measured by the 3ABC ELISA, in Kaduna and Plateau States of Nigeria was estimated at 30.6% (589 of 1926) with a 95% CI = 26.6-34.6%. The proportion of positive cattle was 33.8% (545/1611) with a 95% CI = 29.8-37.8% and that of sheep was recorded as 16% (44/275), 95% CI = 12-20%.

Table 4.7 summarizes the result of 1321 sera collected in a survey study of FMD in apparently healthy agro-pastoralist livestock herd in Kachia grazing reserve with no FMD vaccination history,28.8% (380/1321) 95% CI = 24.8-32.8%were positive to the FMDV non-structural protein 3ABC. FMDV antibody was detected in cattle (30.6%, 95% CI= 26.6-34.6%) in all the contiguous blocks in KGR,and in sheep (15.7%, 95% CI= 11.7-19.7%) in blocks 1, 2 and 3 in KGR.

Plate 4.1. Clinical picture of FMD suspected cases in KGR. (A) Nasal discharge (black arrow) and mouth bleeding (white arrow); (B) severe erosive lesion (arrow) on the gum; (C) lesion (arrow) on the tongue; (D) erosion and ulceration (arrow) of the mouth; (E) lesion (arrow) on the muzzle (F) Un-ruptured vesicle (arrow) on the gum.

Table 4.6: Detection of antibodoies to FMDV from sera collected from ruminant animals in Kaduna and Plateau State from 2009-2014.

Location	No. of samples	FMDV 3-ABC ELISA positive (%)
Kaduna		
Cattle	1155	354 (30.6)
Sheep	166	26 (15.7)
Subtotal	**1,321**	**380 (28.8)**
Plateau		
Cattle	456	191 (41.5)
Sheep	109	18 (16.5)
Goat	40	0
Subtotal	**605**	**209 (34.5)**
Total	**1,926**	**589 (30.6)**

() percentage

Table 4.7: Detection of antibodies to FMDV from sera collected from Kachia Grazing Reserve (KGR), Kaduna state in 2012

Location (KGR)	No. of households sampled	No. of samples collected*	FMDV 3-ABC ELISA positive
Block 1	6	274 (270, 4)	51 (50, 1)
Block 2	14	462 (386, 76)	142 (130, 12)
Block 3	6	188 (150, 38)	75 (62, 13)
Block 4	9	274 (251, 23)	94
Block 5	4	89 (64, 25)	14
Block 6	1	34	4
Total	**40**	**1321 (1155, 166)**	**380 (354, 26)**

*: For locations in which samples were collected from species other than cattle, the species-specific number of samples and results are indicated (bovine, ovine).

Table 4.8 summarizes the detection of FMDV 3ABC non-structural proteins in 81 sera collected from suspected FMD outbreaks in KGR and Zaria, Kaduna State, affecting 13 cattle herds. The result shows that 53 (65.4%) of the tested animals were positive to FMDV 3ABC non-structural proteins by ELISA.

Table 4.9 shows the distribution of FMDV seropositivity in livestock herds across different locations of Plateau State. Out of a total of 605 serum samples tested, 209 (34.5%) were positive for antibodies to the FMDV non-structural proteins 3ABC. Of these, 41.9% (191/456) reactors were cattle and 16.5% (18/109) were sheep. FMDV non-structural proteins were not detected in the goat sera tested.

Table 4.10 shows the results of the serotype-specific test (SPCE) on 3ABC ELISA reactors in KGR. FMDV serotype O was the dominant serotype detected, 154/354 (43.5%) of the five serotypes assayed, followed by serotype A, 76/354 (21.5%). FMDV SAT 1 4/354 (1.1%), SAT 2 8/354 (2.3%) and SAT 3 2/354 (0.6%) were also detected in the cattle populations tested. The result also showed a multiple serotype combinations in some samples, with high percentage inhibition (PI) against a particular serotype with a lower PI against other serotypes. Multiple serotype combinations of O and A 57/354 (16.1%), O and SAT1 8/354 (2.3%), O and SAT2 5/354 (1.4%), O, A and SAT1 10/354 (2.8%) and O, A, SAT1 and SAT3 5/354 (1.4%) were detected. Serotypes O and A combination was the most dominant.

Table 4.8: Summary of serological investigation of FMD outbreaks in KGR and Zaria (Kaduna State) for FMDV 3ABC-ELISA from 2012-2014

Location	Date of sample collection	No. of herds affected	No. of samples collected	FMDV 3-ABC ELISA positive (%)
Kachia grazing reserve				
Block 1	22/6/12, 25/6/12	6	32	13 (40.6)
Block 2	25/6/12	2	8	6 (75)
Block 2	16/6/14	1	6	1 (16.6)
Block 4	9/7/12	2	6	6 (100)
Block 6	28/6/12	1	4	2 (50)
Zaria	4/3/13	1	25	25 (100)
Total		**13**	**81**	**53 (65.4)**

() percentage

Table 4.9: Summary of sera collected from Plateau State, Nigeria for FMDV 3ABC-ELISA assays in 2009 and 2014

Location	No. of herd sampled	No. of samples collected*	FMDV 3-ABC ELISA positive
Barkin-ladi	5	24	5
Bukuru	NR	6	5
Kwall	3	28	11
Kwata	5	51 (40, 11, 0)	12 (11, 1, 0)
Mangu	5	41	32
Miango	5	30 (17, 13, 0)	16 (9, 7, 0)
Shendam	5	16	15
Vom	2	336 (224, 72, 40)	59 (59, 0, 0)
Kadisi	5	56 (50, 6, 0)	45 (39, 6, 0)
Rukuba	2	17 (10, 7, 0)	9 (5, 4, 0)
Total		**605 (456, 109, 40)**	**209 (191, 18, 0)**

NR: Not recorded; *: For locations in which samples were collected from species other than cattle, the species-specific number of samples and results are indicated (bovine, ovine, caprine).

Table 4.10: Summary of sera collected from Kachia grazing reserve (KGR), Kaduna state for FMDV serotype-specific solid phase competitive ELISA (SPCE) in 2012.

Location (KGR)	3ABC ELISA positive	Single serotype SPCE positive [a]					Multiple serotype SPCE positive				
		O	A	SAT1	SAT2	SAT3	O & A	O & SAT1	O & SAT2	O,A & SAT1	O,A,SAT1 & SAT3
Block 1	50	16	11	1	1	0	9	0	0	1	0
Block 2	130	57	36	1	0	0	31	1	1	4	2
Block 3	62	32	12	2	0	2	12	5	1	4	1
Block 4	94	40	13	0	6	0	5	2	3	1	2
Block 5	14	6	4	0	0	0	0	0	0	0	0
Block 6	4	3	0	0	1	0	0	0	0	0	0
Total	**354**	**154**	**76**	**4**	**8**	**2**	**57**	**8**	**5**	**10**	**5**

[a] SPCE reactive sera (if serotype-specific percentage inhibition (PI) was ≥40 in absence of high PI against any other serotype).

In Table 4.11, the serotype-specific (SPCE) assay on the 3ABC reactors in sera collected from suspected FMD outbreaks in KGR and Zaria shows that FMDV serotype O was the most dominant serotype detected, 27/53 (50.9%), closely followed by serotype A,19/53 (35.8%). The frequency of occrrence of serotypes SAT1 and SAT2 detected were 1/53(1.9%) and 7/53 (13.2%) respectively. Samples with FMDV multiple serotype combinations of O and A, 3/53

(5.7%); O and SAT2, 5/53 (9.4%); O, A and SAT2,4/53 (7.5%); O, A, SAT2 and SAT3, 12/53 (22.6%), most dominant; and O, A, SAT1, SAT2 and SAT3, 6/53 (11.3%) were also recorded.

Table 4.12 shows the distribution of FMDV serotype neutralizing antibodies in cattle tested from KGR and Zaria by the virus neutralization test (VNT). The results indicated evidence of virus-neutralizing antibodies at various titres (range, <100->800) for more than one serotype of FMDV. Antibodies to serotype O were detected in Zaria and all the contiguous blocks in KGR except block 4. Lower tires to SAT1 were detected in KGR block 4 and in Zaria. Highest antibody titres were detected for serotypes O, A and SAT2 in KGR and for serotypes O and A in Zaria. Collectively, neutralizing antibodies against five FMDV serotypes O, A, SAT1, SAT2 and SAT3 were detected by VNT in Kaduna State, Nigeria.

Table 4.11: Summary of serological investigation of FMD outbreaks in KGR and Zaria (Kaduna State) for FMDV serotype-specific solid phase competitive ELISA (SPCE) in 2012 and 2014.

		Single serotype SPCE positive [a]					Multiple serotype SPCE positive				
Location	3ABC ELISA positive	O	A	SAT1	SAT2	SAT3	O & A	O & SAT2	O, A, & SAT2	O,A,SAT2 & SAT3	O,A,SAT1, SAT2 & SAT3
Kachia grazing reserve											
Block 1	13	4	5	0	3	0	0	1	2	5	2

Block 2	7	2	4	0	1	0	2	2	1	0	1
Block 4	6	3	1	1	1	0	0	0	0	1	1
Block 6	2	1	0	0	1	0	0	0	1	1	0
Zaria	25	15	9	0	1	0	1	2	0	5	2
Total	**53**	**27**	**19**	**1**	**7**	**0**	**3**	**5**	**4**	**12**	**6**

[a] SPCE reactive sera (if serotype-specific percentage inhibition (PI) was ≥40 in absence of high PI against any other serotype).

Table 4.12: Levels of FMDV serotype-neutralizing antibodies in cattle in KGR and Zaria, Kaduna State, Nigeria

	Kachia grazing reserve				
VNT Titre range	**Block 1**	**Block 2**	**Block 4**	**Block 6**	**Zaria**
<100			SAT1		SAT1
100-200		SAT1, SAT3			SAT2, SAT3
201-400	O, A	A		SAT3	O, SAT3
401-800	A, SAT2	O			A,O
>800	A	SAT2	SAT2	O	A, O

VNT, virus neutralization test

All sera tested from the various regions of Plateau State showed evidence of virus neutralizing antibodies at various titres (range, <100->800) to more than one serotype of FMDV, with the exception of sera from Vom region (Table 4.13). In sheep, antibody to serotype O was reported in Kwata, whilst antibody to SAT1, SAT2 and SAT3 was predominant in Miango. Also, lower titres to serotype O than those to SAT1, SAT2 and SAT3 were detected in sheep sera. In cattle, antibody to serotype O was more prevalent across the regions surveyed, whilst antibody to serotype A was detected in Bukuru, Mangu and Shendam. In contrast to sheep, highest antibody titres were detected against serotype O in cattle sera. Collectively, antibodies against five serotypes, A, O, SAT1, SAT2 and SAT3 were detected by VNT in Plateau State, Nigeria

4.4 Virological and Molecular Analysis

All the viruses isolated in KGR (N=21) in 2012 belonged to serotype SAT2 whilst the virus isolate from KGR in 2014 (n=1) belonged to serotype O. The virus isolates from Zaria and Kaura collected in 2013 belonged to serotypes O and A respectively (Table 4.14). No cytopathic effect (CPE) was observed after 48hours incubation on first and second passages for the probang samples on cell culture. Collectively, FMDV serotypes O, A and SAT2 were isolated from Kaduna State. All the isolates were positive for the FMDV 3D genes by the rRT-PCR with threshold values (C_t) ranging from 12.5-18.4 (Figure 4.1).

Table 4.13: Levels of FMDV serotype neutralizing antibodies in sera from cattle and sheep by location in Plateau State, Nigeria.

	Cattle sera								Sheep sera	
VNT Titre range	**Kwall**	**Kwata**	**Mangu**	**Shendam**	**Bukuru market**	**B/Ladi**	**Miango**	**Vom**	**Kwata**	**Miango**
< 100			A, SAT-2	SAT-2	A	O		O	O	
100-200	SAT-3	SAT-3	A		A	O, SAT-1				SAT-1, SAT-3
201-400	O			A, O			SAT-3			
401-800							O			SAT-2
>800		O	O	O	O					

Table 4.14: Results from tissue and probang samples from a vesicular disease outbreak in ruminants in Kaduna State in 2012-2014

Sampling	**Sample type**	**Number of herds affected**	**Date of sample collection**	**Number positive/tested**	**AgELISA**

location				VI	rRT-PCR	
Kachia grazing reserve						
Block 1	Gum	6	22/6/12	11/15	11/11	FMDV SAT2
	Probang*	3	22/7/12	0/10	0/0	NT
Block 2	Gum	2	25/6/12	3/4	3/3	FMDVSAT2
	Gum	1	16/6/14	1/4	1/1	FMDV O
Block 4	Tongue	2	9/7/12	6/7	6/6	FMDVSAT2
Block 6	Tongue	1	28/6/12	1/4	1/1	FMDVSAT2
Zaria	Gum	1	4/2/13	2/8	2/2	FMDV O
Kaura	Tongue	1	6/7/13	2/2	2/2	FMDV A

VI: virus isolation on swine kidney (SK-6) and fetal lamb kidney (FLK) epithelial cell lines; rRT-PCR: real-time reverse transcription-polymerase chain reaction; AgELISA: antigen capture enzyme-linked immunosorbent assay; NT: not tested; *included 2 samples from ovine.

The results of 63 tissue samples collected from suspected FMD outbreaks in cattle and sheep in Plateau State are presented in table 4.15. Out of 34 virus isolates, 21 were serotype A from Barkin-ladi (n=17) and Kwata (n=4); 8 belonged to serotype O (4 from Bukuru, 3 from Riyom and 1 from Rukuba); 2 belonged to SAT1 (from bukuru) whilst 3 isolates were SAT2 (from Dawarki). Serotype SAT1 was also detected by AgELISA in a mixed infection with serotype O in cultured supernatant from Rukuba, Riyom and Bukuru at a lower PI compared to serotype O. All isolates were positive to the FMDV 3D genes by rRT-PCR with C_t values ranging from 12.0-19.0 (Figure 4.2).

Figure 4.1 represents the results of rRT-PCR on cultured suspension of isolates from Kaduna State for the detection of FMDV 3D gene as shown by the threshold values generated by the Light Cycler® 480 software (Roche, Diagnostic Ltd, Rot Kreuz, Switzerland).

Figure 4.2 represents the results of rRT-PCR on cultured suspension of isolates from Plateau State for the detection of FMDV 3D gene as shown by the threshold values generated by the Light Cycler® 480 software (Roche, Diagnostic Ltd, Rot Kreuz, Switzerland).

Plate 4.2 shows the result of agarose gel electrophoresis of PCR amplicons of 4 FMDV isolates from Kaduna State. For each sample tested, specific cDNA amplicons equivalent to the expected sizes were amplified utilizing alternative serotype-specific primers for serotypes O, A and SAT2 at different annealing temperatures, manifested as discrete bands at the molecular sizes of about 1000bp. No amplicon was seen for the control lanes.

Table 4.15: Results of tissue and probang samples from a vesicular disease outbreak in ruminants in Plateau State from 2009-2015

Location	Species	Sample type	Number of herds affected	Date of sample collection	Number positive/tested		AgELISA
					VI	rRT-PCR	
Kwata	Bovine	Foot	5	15/11/09	2/3	2/2	FMDV A
	Bovine	Gum			2/2	2/2	FMDV A
Miango	Bovine	Foot	5	29/11/09	0/2	0/0	NT
	Ovine	Foot			0/1	0/0	NT
Bukuru	Bovine	Foot	NR*	19/11/09	0/5	0/0	NT
Barkin-Ladi	Bovine	Gum	5	20/11/09	3/3	3/3	FMDV A
	Bovine	Tongue			14/14	14/14	FMDV A

	Bovine	Gum	1	22/7/14	0/2	0/0	NT
Dawarki	Bovine	Tongue	1	18/8/13	3/4	3/3	FMDV SAT2
Rukuba	Bovine	Gum	2	2/9/14	1/10	1/1	FMDV O, SAT1
Riyom	Bovine	Gum	2	4/7/14	3/6	3/3	FMDV O, SAT1
Bukuru	Bovine	Gum	NR*	23/6/14	4/9	4/4	FMDV O, SAT1
Bukuru	Bovine	Gum	1	17/9/15	2/2	2/2	FMDV SAT1

VI: virus isolation on swine kidney (SK-6), IBRS-2 and fetal lamb kidney (FLK) epithelial cell lines; RT-PCR: real-time reverse transcription-polymerase chain reaction; AgELISA: antigen capture enzyme-linked immunosorbent assay; NR: not recorded; NT: not tested; *Cattle market

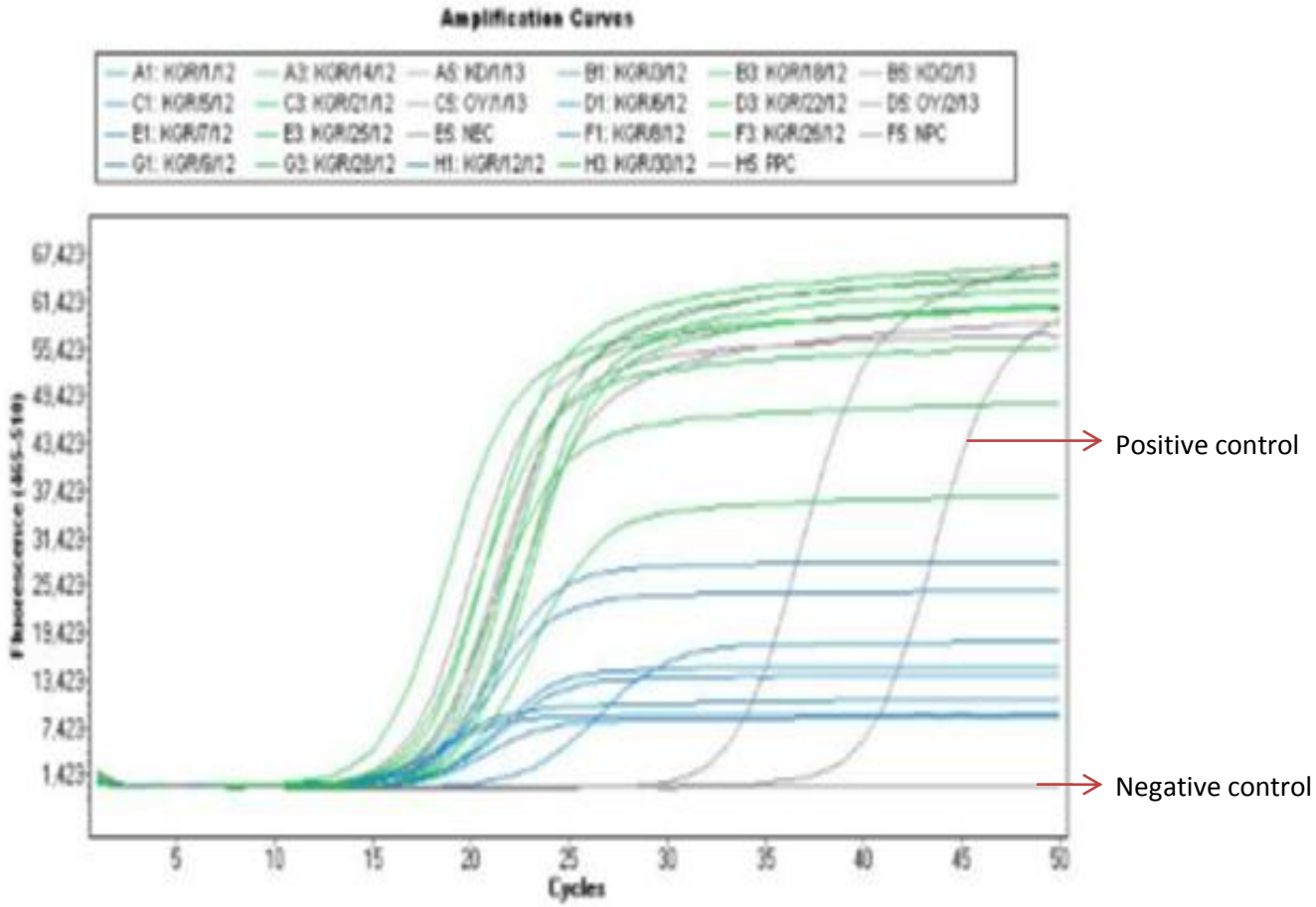


Figure 4.1: Real-time PCR amplification plot showing relative fluorescence against cycle number of samples from Kaduna State from 2012-2014.

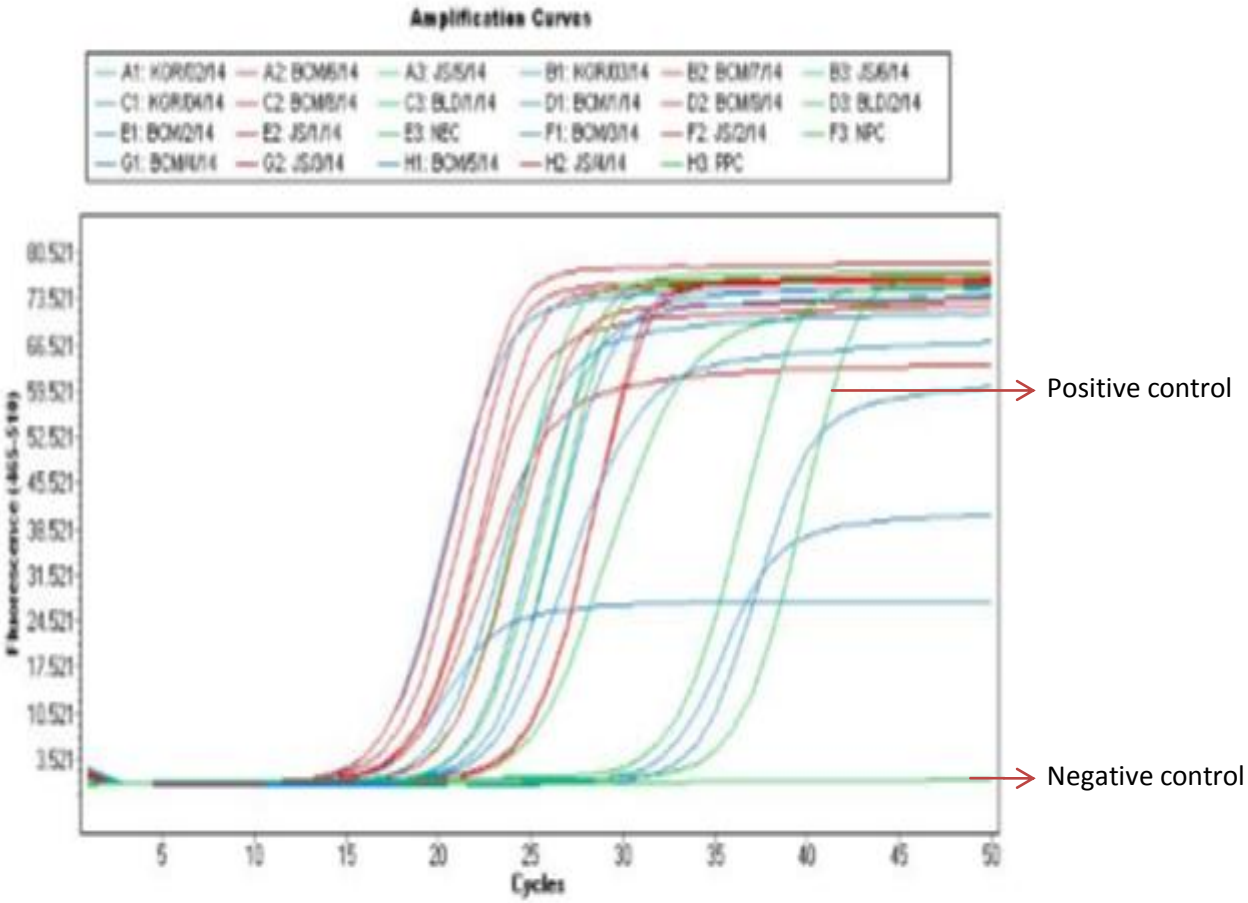


Figure 4.2: Real-time PCR amplification plot showing relative fluorescence against cycle number of samples from Plateau State from 2009-2014.

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

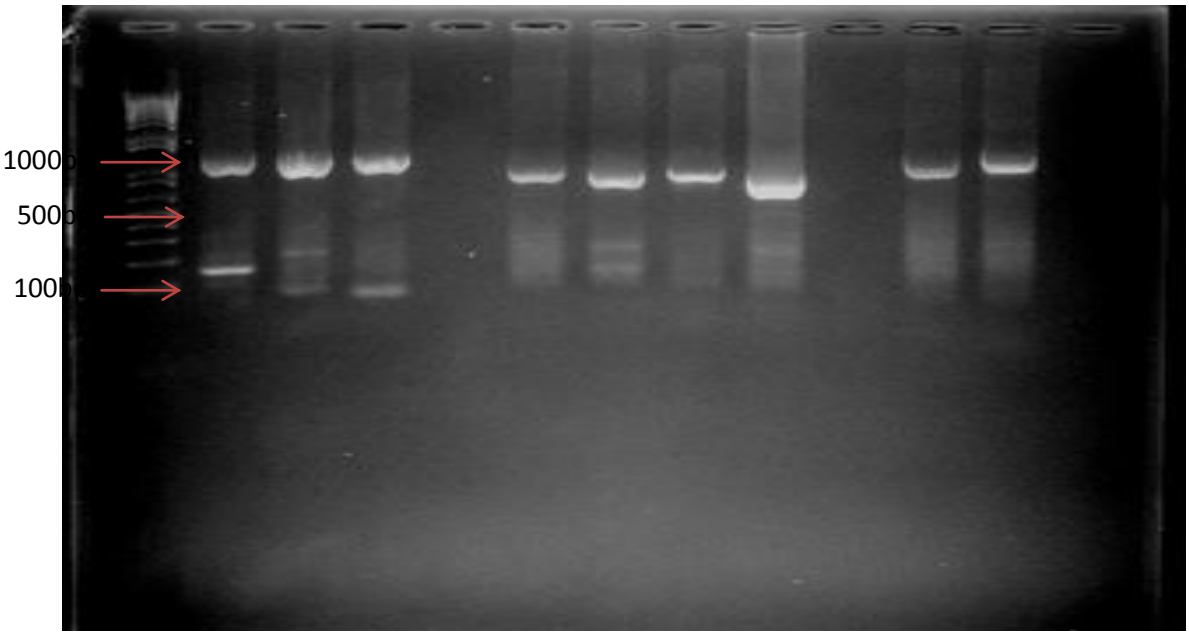


Plate 4.2: Agarose gel electrophoresis of amplified gene products of 4 isolates of FMDV outbreak in KGR using alternative serotype-specific primers for serotypes O, A and SAT2, separated on 1.5% agarose gel and stained with gel red. Lanes: (M) 1kbp DNA ladder; (1-3) sample 1(Z/1/13, FMD type O); (4, 9 & 12) negative controls (no amplicon); (5&6) sample 2(KD/1/13, type A); (7&8) sample 3(KD/2/13, type A); (10&11) sample 4(KGR/2/12, type SAT 2).

In Plate4.3, the results of agarose gel electrophoresis of PCR amplicons of 3 FMDV isolates from KGR, using2 alternative specific primers for FMDV serotype SAT2 was shown. For each sample tested, specific cDNA amplicons equivalent to the expected sizes were amplified utilizing alternative serotype-specific primers for serotypes SAT2, manifested as discrete bands at the molecular sizes of about 1000bp.

In Plate 4.4, the results of agarose gel electrophoresis of PCR amplicons of 6 FMDV isolates from KGR, using2 alternative specific primers for FMDV serotype SAT2 was shown. For each

sample tested, specific cDNA amplicons equivalent to the expected sizes were amplified utilizing alternative serotype-specific primers for serotypes SAT2, manifested as discrete bands at the molecular sizes of about 1000bp.

In Plate4.5, the results of agarose gel electrophoresis of PCR amplicons of 4 FMDV isolates from Plateau State, using 3 alternative specific primers for FMDV serotype Owas shown. For each sample tested, specific cDNA amplicons equivalent to the expected sizes were amplified utilizing alternative serotype-specific primers for serotypes O, manifested as discrete bands at the molecular sizes of about1000bp.

In Plate4.6, the result of agarose gel electrophoresis of PCR amplicons of 2 FMDV isolates from Plateau State is shown. For each sample tested, specific cDNA amplicons equivalent to the expected sizes were amplified utilizing alternative serotype-specific primers for serotypes O, observed as discrete bands at the molecular sizes of about 1000bp. However, no amplification was observed using specific primer sets for SAT1 on the same samples.

M 1 2 3 4 5 6 7

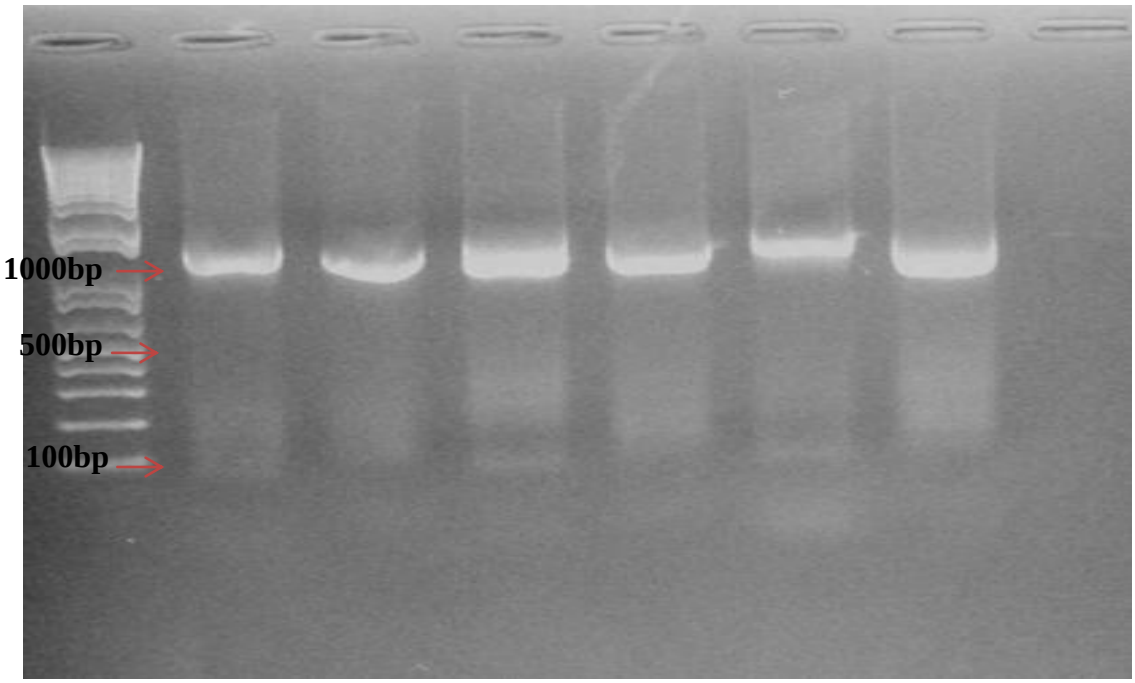


Plate 4.3: Agarose gel electrophoresis of amplified gene products of FMDV outbreak in KGR using FMDV serotype-specific primers for SAT2, separated on 1,5% agarose gel and stained with gel red. Lanes: M: 1kbp ladder; 1&2: sample 1(KGR/26/12); 3&4: sample 2 (KGR/28/12); 5&6: sample 3(KGR/30/12); 7: negative control

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

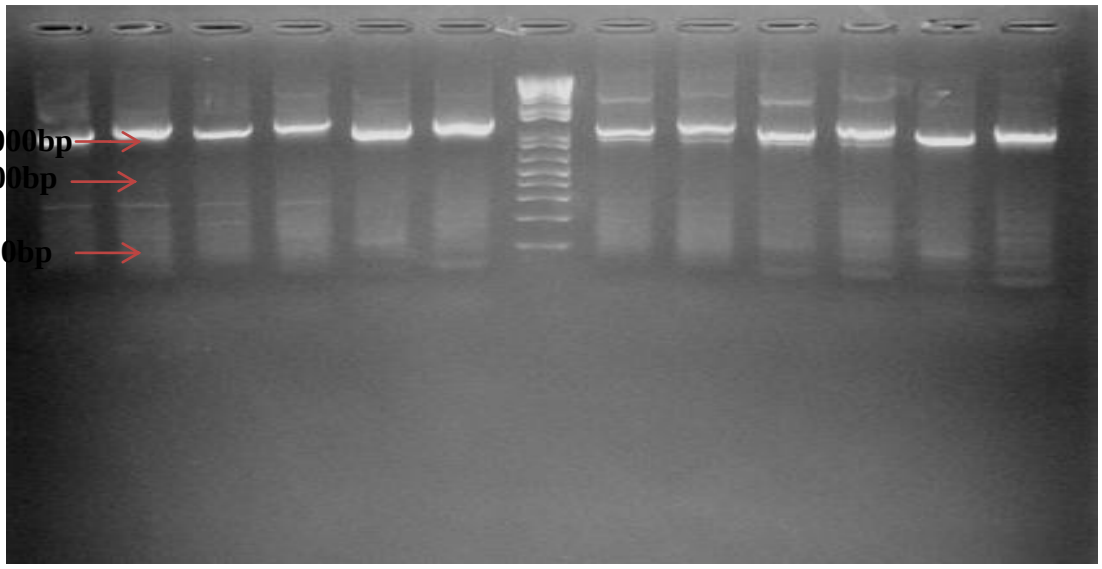


Plate 4.4: Agarose gel electrophoresis of amplified gene products of FMDV outbreak in KGR using FMDV serotype-specific primers for SAT2, separated on 1,5% agarose gel and stained with gel red. Lanes: 1&2, sample 1(KGR/3/12); 3&4, sample 2(KGR/5/12); 5&6,sample 3(KGR/6/12); M: 1kbp marker; 7&8,sample 4(KGR/7/12); 9&10, sample 5(KGR/8/12); 11&12, sample 6(KGR/12/12)

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

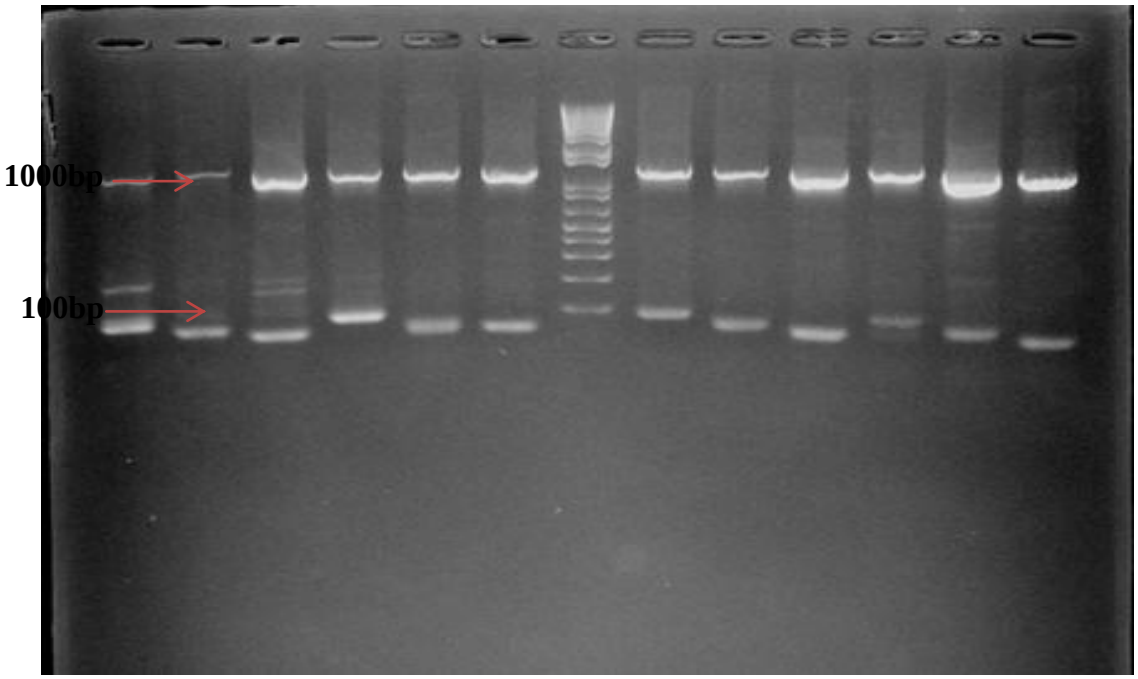


Plate 4.5: Agarose gel electrophoresis of amplified gene products of FMDV outbreak in KGR using FMDV serotype-specific primers for type O, separated on 1.5% agarose gel and stained with gel red. Lanes 1-3: sample 1(PL/BCM/1/14); 4-6: sample 2(PL/BCM/3/14); 7-9: sample 3(PL/BCM/7/14); 10-12: sample 4(PL/BCM/9/14);M: 1kbp marker.

1 2 3 4 5 6 M 7 8 9 10

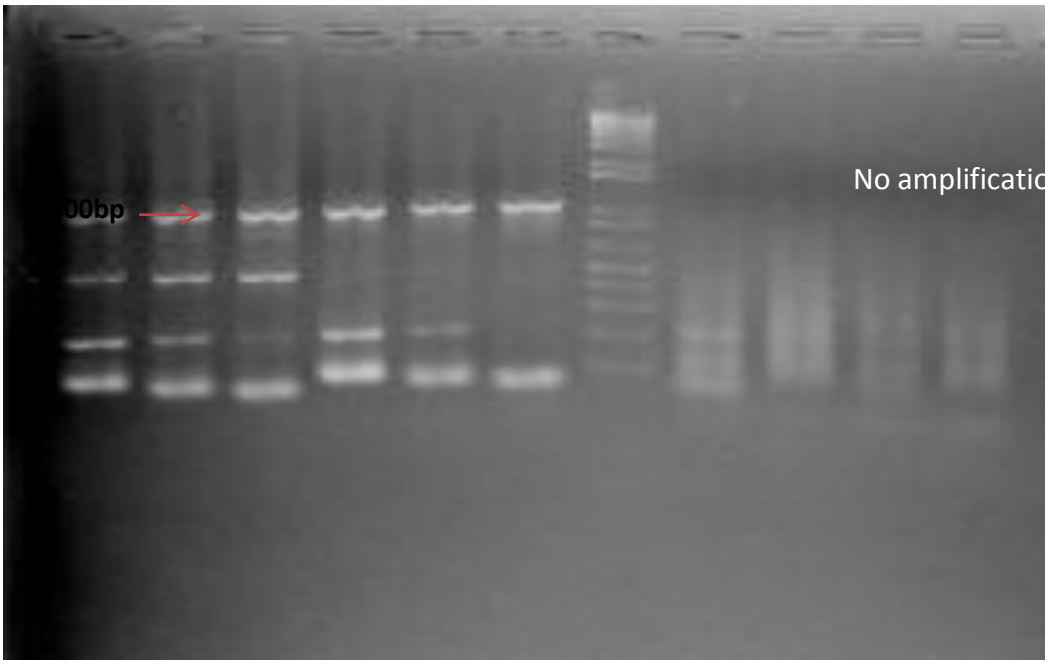
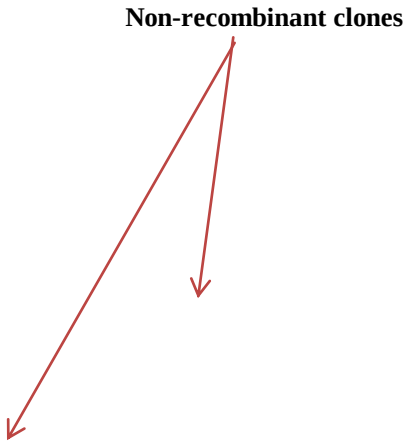


Plate 4.6 Agarose gel electrophoresis of amplified gene products of 2 isolates of FMDV outbreak in Plateau State using alternative serotype-specific primers for serotypes O and SAT1 on same samples, separated on 1.5% agarose gel and stained with gel red. Lanes: (1-3) sample 1(PL/RK/2/14); (4-6) sample 2(PL/RY/1/14); (M) 1kbp ladder; (7-8) sample 1(PL/RK/2/14); (9&10) sample 2(PL/RY/1/14).

Plate 4.7 shows the result of transformation of recombinant DNA on Luria-Bertani (LB) agar plate containing ampicillin. Numerous cell divisions, resulting in clones (bacterial colonies) were observed. The transformed bacterial cells are resistant to ampicillin and observed here as white colonies are the recombinant clones. On the other hand, transformed bacterial cells which are resistant to ampicillin and seen here as producing blue color are the non-recombinant clones, whilst the untransformed bacterial cells are sensitive to ampicillin and do not grow on the nutrient agar plate containing ampicillin.

The results of controlled digestion of recombinant DNA using restriction enzyme (EcoR1) is shown in Plate 4.8. The resulting fragments are separated by agarose gel electrophoresis. Each of the 6 clones of the tested sample produced 2 discrete fragments of about 3900bp and 1000bp size, representing the plasmid vector and the insert (FMDV) respectively. This result indicates that the DNA of the FMDV has been inserted in the desired orientation.

In Plate4.9, each of the 6 clones of the tested samples produced 2 discrete fragments of about 3900bp and 1000bp size, representing the plasmid vector and the insert (FMDV) respectively. This result indicates that the DNA of the FMDV has been inserted in the desired orientation.



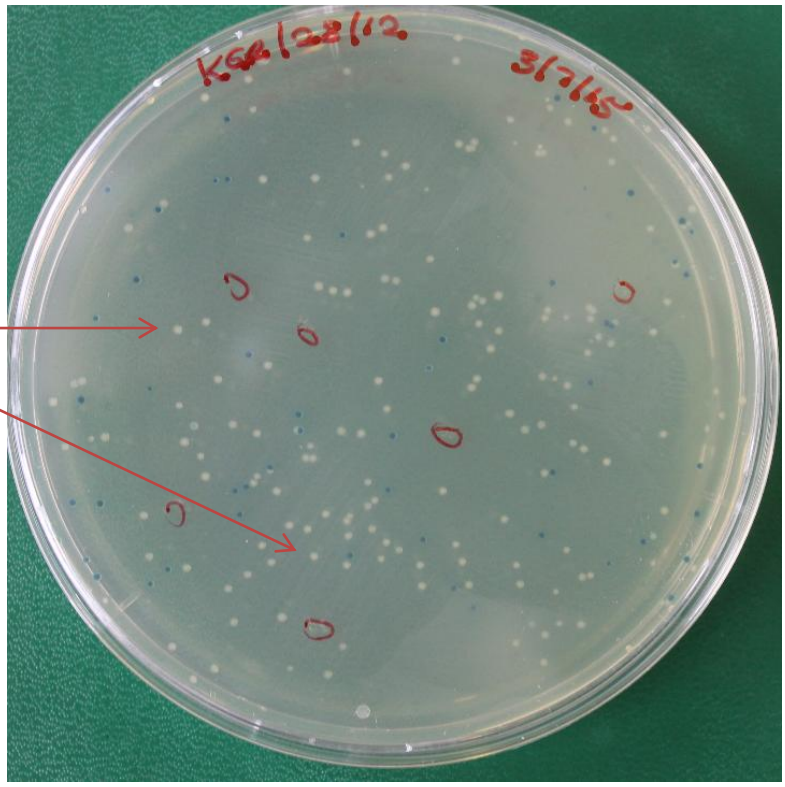


Plate 4.7 Transformation of recombinant DNA on nutrient agar plate containing ampicillin showing recombinant (white colonies) and non-recombinant (blue colonies) clones

M 1 23 4 5 6

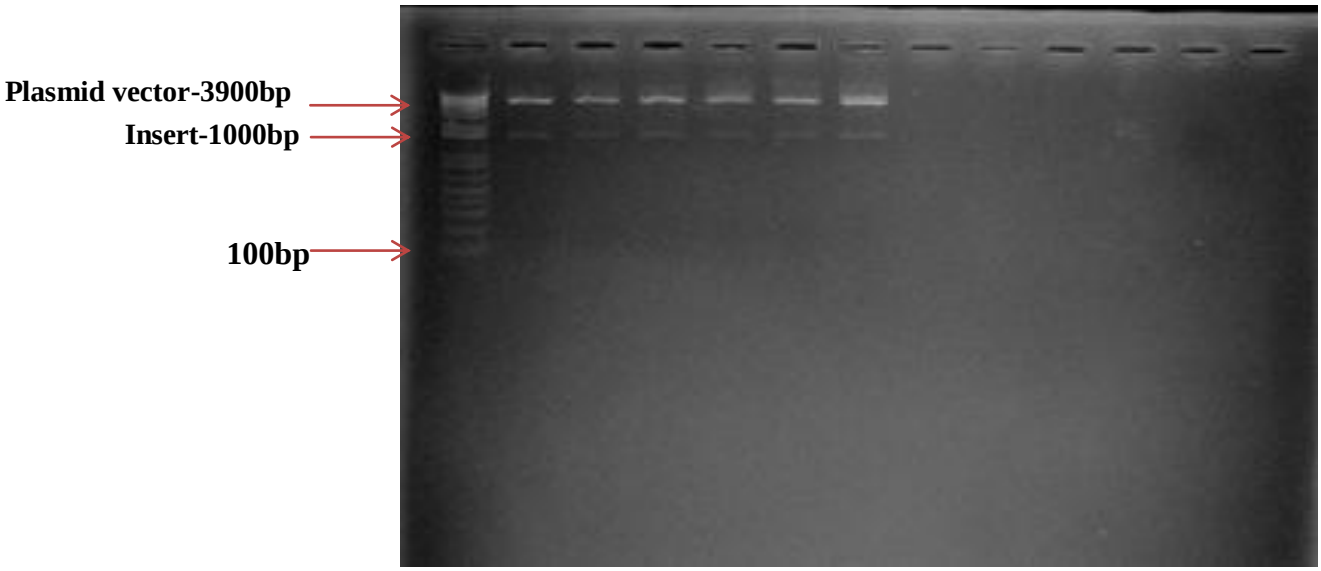


Plate 4.8 Analysis of controlled digestion of recombinant DNA by EcoR1 restriction enzyme on 1% agarose gel electrophoresis stained with gel red. Lanes: (M) 1kbp DNA ladder; (1-6) 6 clones of KGR/2/12 each with 2 discrete bands of about 3900bp and 1000bp molecular size representing plasmid vector and insert (FMDV)

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

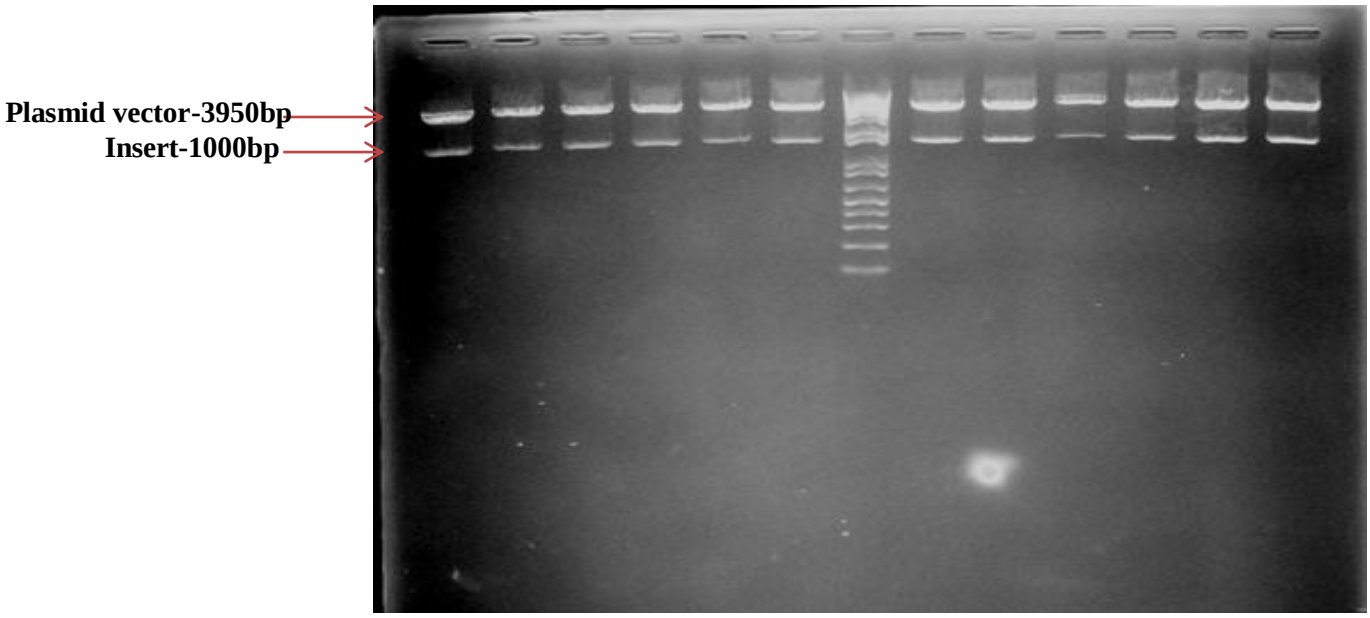


Plate 4.9 Analysis of controlled digestion of recombinant DNA by EcoR1 restriction enzyme on 1% agarose gel electrophoresis stained with gel red. Lanes: 1-6: positive clones of KGR/18/12 showing discrete bands of vector (3950bp) and insert (1000bp);(M) 100bp DNA ladder; 7-12: positive clones of KGR/22/12 also showing discrete bands of vector and insert

4.5 Phylogenetic Analyses

The phylogenetic assessment of the FMDV serotype O isolates from Kaduna and Plateau States showed that two distinct topotypes of FMDV serotype O are in circulation (Figure 4.3). The FMDV serotype O isolated in Zaria in 2013 was most closely related to three previously known FMDV serotype O from Niger republic (O/NGR/1-3/88) belonging to the West Africa (WA) topotype. However, the FMDV serotype O isolates responsible for the outbreaks in Kaduna and Plateau States in 2014 share common genetic relationship with 95.6% - 100% nt identity and are most closely related to a known serotype O strain in Sudan in 1999 (O/SUD/1/99) with 88.6 to 89.8% homology, belonging to the East Africa-3 (EA-3) topotype.

The serotype A virus isolates detected in Kaduna and Plateau States between 2009 and 2013 all fell into genotype IV (G-IV) within the AFRICA topotype. In this genotype, two distinct subclusters/variants comprising of viruses from Kaduna State (2013) and Plateau State (2009) were evident. Viruses in each subcluster were genetically identical (99%-100% nt identity) (Figure 4.4). Furthermore, virus isolates from Kaduna State had 12% nt difference from those from Plateau State. The two subclusters were most closely related to a virus strain from neighboring Cameroon in central Africa (A/CAR/15/2000) with 91.2%-91.4% homology.

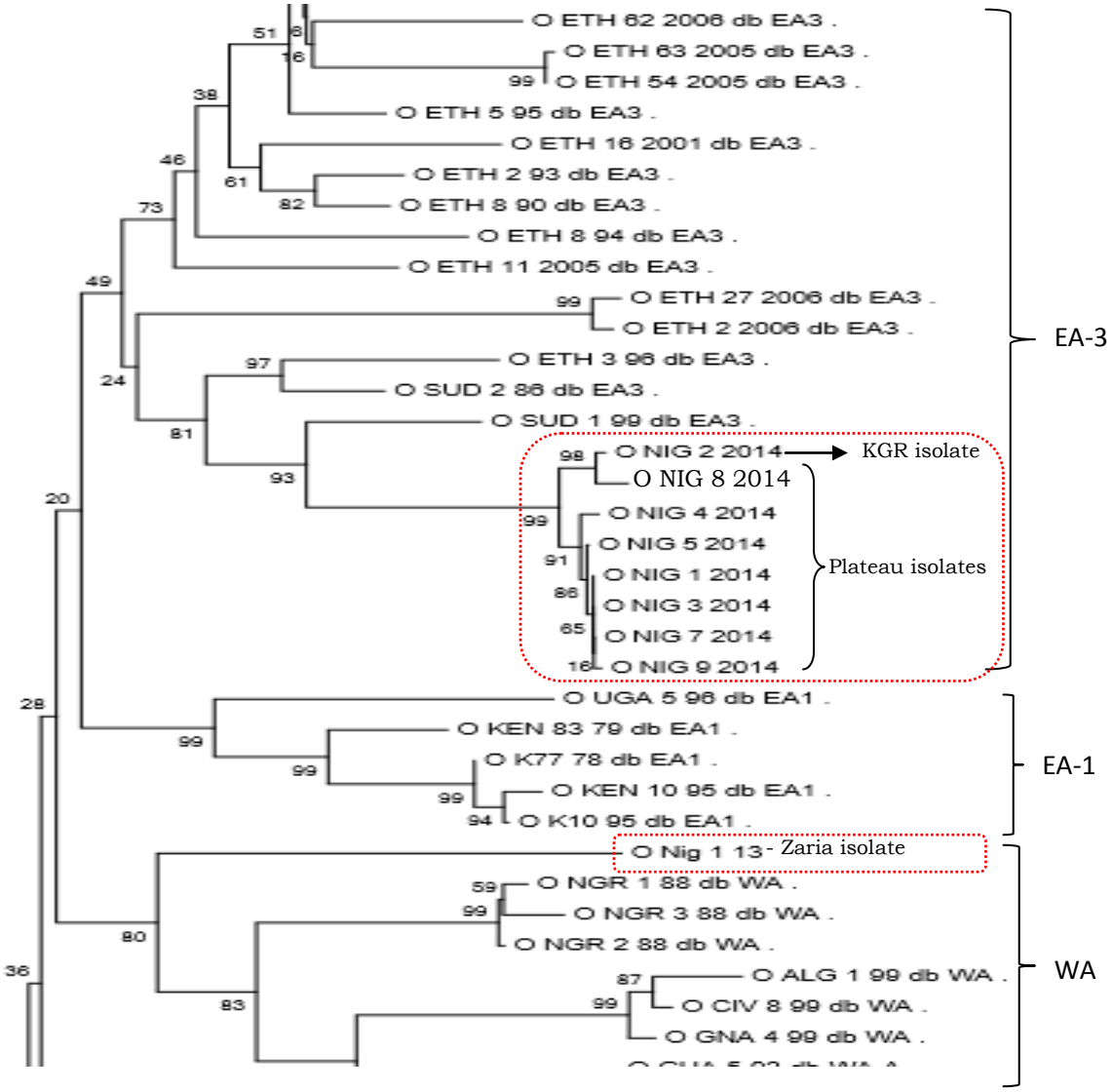


Figure 4.3: Midpoint-rooted neighbor-joining treeshowing relationships between the 2013 and 2014 FMDV serotype O isolates from Kaduna and Plateau State based on the complete VP1 sequence. Full-length VP1 sequences of additional Serotype O viruses available in GenBank were included in the analysis (EA, East Africa; WA, West Africa).

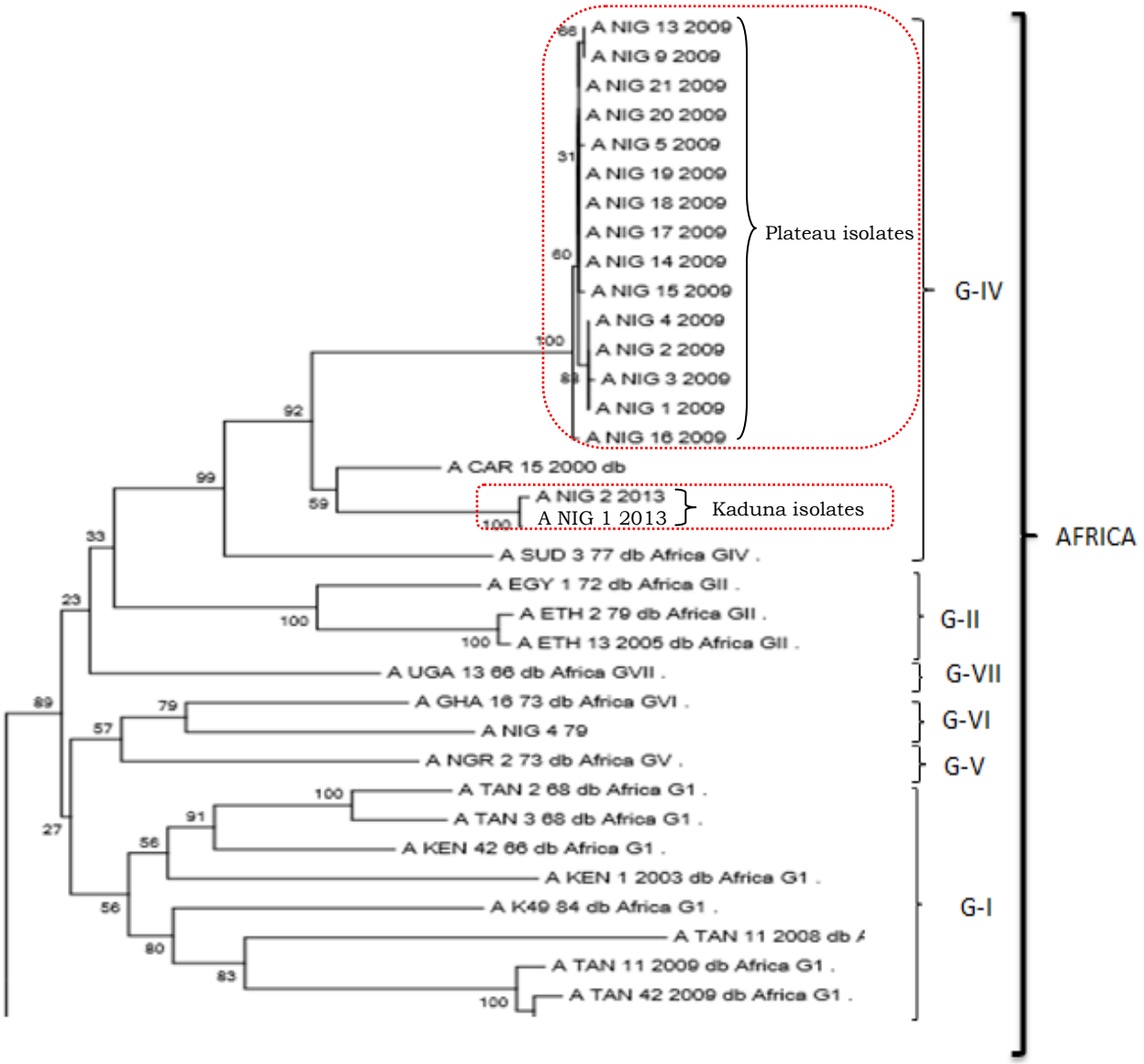


Figure 4.4:Midpoint-rooted neighbor-joining treeshowing relationships between the 2009 and 2013 type A isolates in Plateau and Kaduna States based on the complete VP1 sequence. Full-length VP1 sequences of additional Serotype A viruses available in GenBank were included in the analysis

The phylogenetic evaluation of the FMDV SAT1 isolated from Plateau State in 2015showedthat these isolates were closely related to each other (99.8% nt identity), but not closely related to any SAT1 viruses in the databaseof the GenBank examined (>20% divergence) and so it was

designated here as a new topotype (Figure 4.5). The closest genetic relation was the SAT1 isolate of 1979 in Nigeria (SAT1/NIG/2/79) with 71.6% nt identity which qualifies the present isolates to be classified as a new topotype (unnamed). There was a clear division between the 2015 SAT1 isolates with previous SAT1 strains isolated from Nigeria in the late 70s and early 80s when it was last reported.

The serotype SAT2 viruses found in Kaduna and Plateau States between 2012 and 2013 all belonged to topotype VII, with two subtypes within this topotype which were most closely related to SAT2 viruses from Libya in North Africa (Figure 4.6). These isolates except one (designated here as SAT2/NIG/3/13) share common genetic relationship (98.6-100% nt identity) and were most closely related to a previously known isolate in Nigeria in 2007 (SAT2/NIG/2/07) with 96.8-98.4% nt homology, which together clustered with a Libyan isolate of 2012 (SAT2/LIB/41/12). However, one isolate from Plateau State in 2013 (SAT2/NIG/3/13) was genetically distinct and most closely related to a 2003 isolate from Libya (SAT2/LIB/1/03)

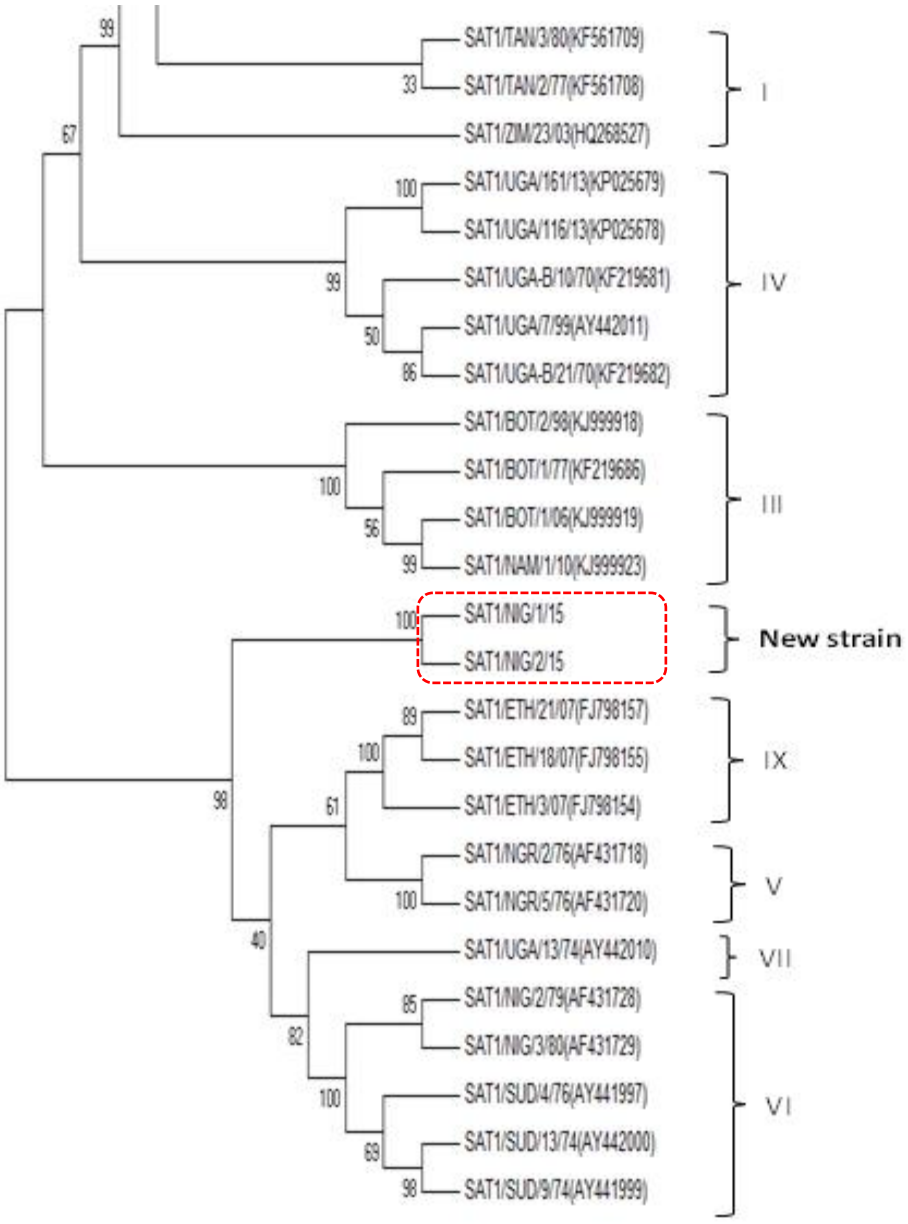


Figure 4.5: Midpoint-rooted neighbor-joining treeshowing relationships between the 2015 SAT1 isolatein Plateau based on the complete VP1 sequence. Full-length VP1 sequences of additional SAT1 viruses available in GenBank were included in the analysis

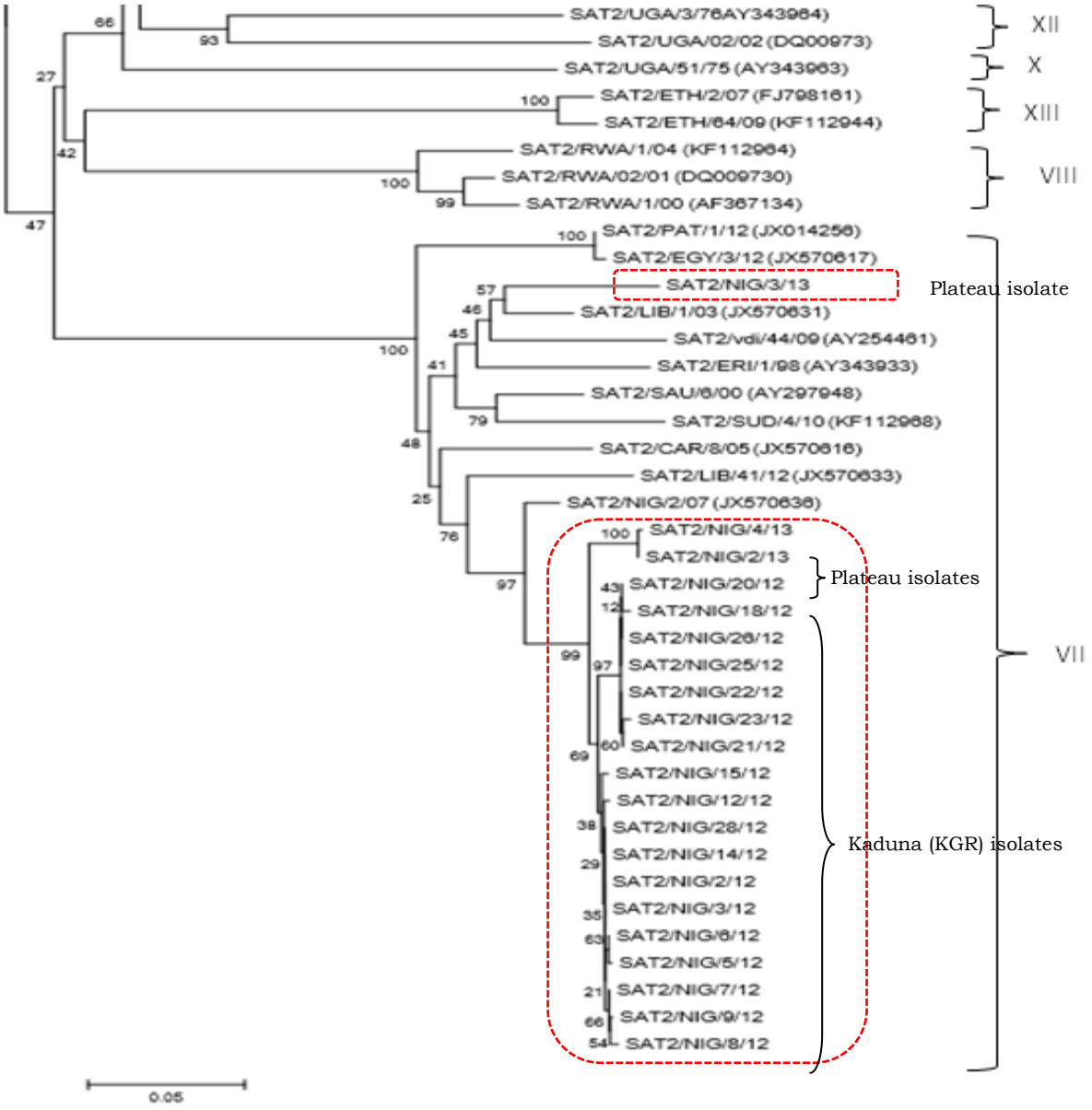


Figure 4.6: Midpoint-rooted neighbor-joining treeshowing relationships between the 2012 and 2013 FMDV serotype SAT 2 isolates from Kaduna and Plateau State based on the complete VP1 sequence. Full-length VP1 sequences of additional SAT2 viruses available in GenBank were included in the analysis

CHAPTER FIVE
5.0 DISCUSSION

Farms with suspected FMD nearby were at high risk for FMD as observed in the risk study, which is a consequence of the infectious nature of the disease. Farms that used drugs or vaccines were also likely to be FMD positive, suggesting that farmers may have treated their animals with

drugs and emergency vaccination in response to the detection of FMD-like clinical signs. Herds with >100 animals were also more likely to be FMD positive than small farms, probably because large herds have higher probability of adequate contacts, introduction of animals and consequently spread of FMD than small herds. Although not significantly associated with the outcome, cattle grazing type and contacts with wildlife (e.g. African buffalo, antelope) improved the model fitness, indicating that those factors may be assessed in future studies to elucidate whether they play any role in the epidemiology of the disease in the region. Although the role of wildlife hosts for FMD has not been proved in Western Africa, it could be possible that some susceptible wildlife may simply be a proxy for another factor, such as openness of the farm borders and feasibility of the contact between susceptible and infected animals. Large ruminants and animals kept in non-nomadic systems were more likely to be 3 ABC ELISA positive than small ruminants. Cattle are more susceptible to FMDV especially via aerosol because of their larger respiratory volume compared with sheep and goats (Donaldson, 1987). Settled herds were more FMD positive than nomadic system due to faster spread of the virus via aerosol and increased contact that may be evident at common pasture and water points they share particularly during scarcity.

Foot and mouth disease prevalence of 30.6% estimated from this studywas higher than the 27% recorded by Abegunde (1987) in abattoir samples from Plateau State, and the 13% reported by Dawe and Durojaiye (1986) in a serological survey of livestock conducted in Oyo State, Western Nigeria. These previous studies were conducted based on VIAA test as against the ELISA test used here. ELISA is said to supercede the VIAA-AGID largely due to its high sensitivity and its ability to detect antibody to the polyprotein 3ABC of FMDV which is the single most reliable indicator of infection (De Diego *et al.,* 1997; Sorensen *et al.,* 1998b).

The serotype-specific SPCE results show that FMDV serotype O appears to be the dominant serotype circulating in the studied areas in Kaduna State, followed by serotype A. These serotypes (O and A) are currently the most prevalent serotypes in Turkey, Israel and Egypt (Paton *et al.*, 2009) where trivalent vaccines of FMDV serotype O, A and Asia 1 and monovalent type-O vaccine have been regularly used to control FMD in these regions.

Multiple-serotype combinations of FMDV were observed, with high PI-value against a particular serotype and a low PI against one or more other serotypes at individual and herd levels. This may be attributable to re-occurring or repeated infections with different serotypes at various periods/points of time. Also, the possibility of cross-reaction or imperfect specificity of the serotype-specific ELISA could not be ruled out as a reason for the multiple serotypes detected. Previous studies have shown that cross-reactions are not uncommon between serotype-specific ELISAs for FMDV (Mackay *et al.*, 2001; Mwiine *et al.*, 2010). Balinda *et al.* (2009) made a similar observation of low levels of antibodies towards one or several serotypes during a serological survey in small ruminants in Uganda.They presumed that such mixed infections could be as a result of waning antibodies from previous outbreaks and may also signal imperfect specificity of the serotype-specific ELISAs in the face of repeated infections. Hamblin *et al.*(1986) postulated that these multiple serotype combinations could be an indication of some antigenic relationships between the virus serotypes. Other researchers however proposed that it could be due to a complicated serological picture involving heterotypic responses caused by priming effects from previous exposures from one or more infections and/or vaccinations (Hedger *et al.*, 1982; Mwiine *et al.*, 2010).

Furthermore, there was evidence of virus-neutralizing antibodies to five FMDV serotypes (A, O, SAT1, SAT 2 and SAT 3) in the sera from Kaduna and Plateau States. Whereas FMDV

serotypes O, A and SAT2 have been reported to have occurred in recent past in Nigeria (Knowles, 2008), no report on the existence of serotype SAT1 in Nigeria since the 1981 report, also, SAT 3 has never been isolated in the country(Tekleghiorghis *et al.,* 2014). Hence this is the first report of the probable presence of FMDV serotype SAT3 in cattle in Kaduna State and in cattle and sheep in Plateau State of Nigeria which may be associated with the extensive cross-border animal movements and inter-regional animal trades. Data from FAO in 2010 on West, Central and East Africa indicated that 2.9 million livestock were imported, 2/3 of this were small ruminants and 1/3 cattle with Nigeria being the largest importer of over 1.4 million animals in 2010, including over 1 million live cattle, sheep and goats from Niger (FAOSTAT, 2010). The serological results from this study indicate that FMDV SAT 1 and SAT 3 might have been in circulation in Nigeria.

FMDV serotype SAT 3 has the most restricted distribution of the three SAT serotypes and essentially occurs only in Southern Africa (Thomson *et al.,* 2003), and in the South-western region of Uganda (Vosloo, *et al.,* 2002). The SAT serotypes are maintained by large numbers of African buffalo (*Syncerus caffer*) found in these regions, which provides a potential source of infection for both domestic livestock and other wild, cloven-hoofed animals (Thomson *et al.,*2003; Phologaine, *et al.,* 2008). Serotype SAT 3 has never been isolated from domestic livestock in Uganda (Rweyemamu *et al.,* 2008); however, recently, positive titres for SAT 3 were found in domestic cattle in Uganda that had direct contact with African buffalo (Mwiine *et al.,* 2010). Furthermore, although serotype SAT 3 were reported in Democratic Republic of Congo (DRC) in Central Africa in 2005 (Sumption *et al.,* 2007); however, no virus genotype or species affected was documented. Serological studies on buffalo samples collected between 1994

and 2004 from East and Central Africa tested by VNT for SAT serotypes shows that 17% had SAT 3.

FMDV serotypes O, A,SAT1 and SAT 2 were identified by AgELISA from the isolated viruses in Kaduna and Plateau States. This was also confirmed by molecular methods (rRT-PCR and RT-PCR). Although FMDV SAT1 was also identified by AgELISA as mixed infection in cell culture suspension in samples collected from some parts of Plateau State, however, it could not be confirmed by molecular test. It was probably a result of cross-reaction in the AgELISA.

Ten serotype O isolates were recovered from Kaduna and Plateau State in this study andall but one of them fell into a single topotype, East Africa (EA)-3 and was genetically related to the serotype and topotype virus found in Sudan (1999) in East Africa. The one sample, from 2013 was collected from Zaria, Kaduna State formed a different serotype O topotype from the other nine, named West Africa (WA) topotype, and it was genetically related to the topotype viruses found in Niger. This has implication for control as antisera produced against one strain of a serotype may not recognize other strainsof the same serotype.

The 23 serotype A virus isolates in Kaduna and Plateau States all belonged to the FMDV serotype A genotype IV (G-IV) within the Africa topotype and were closely related to a virusisolated in 2000 in Cameroon, Central Africa, suggesting that the serotype A virus from Nigeria and Cameroon share common epidemiological link.

FMDV SAT1 is reported here after over thirty years of no reports of it's occurrence in Nigeria(WRLFMD, 2014). Limited presence of serotype SAT 1 has been reported in East Africa. Serotype SAT 1 was first isolated from cattle, sheep and goats in Ethiopia in 2007 (Ayelet *et al.,* 2009), and it was isolated from African buffalo's oro-pharyngeal probang samples in Uganda in 2007 (Ayebazibwe *et al.,* 2010). So far, there is no documented information before now on the

isolation of SAT 1 from domestic and wildlife animals in West and Central Africa for the past three decades (WRLFMD, 2014). The present SAT1 isolates from Nigeria seems to cluster differently from its most closely related strains from Nigeria. These may probably be new variants of SAT1 from previously well established topotypes of SAT1 in West and East Africa. A full genomic sequence analysis of these isolates would be required in further studies to elucidate the genetic and antigenic relatedness. The FMDV SAT 2 viruses found in this study formed two subtypes within topotype VII. One group ofthe SAT 2 isolates were identical to a previous Nigerian SAT 2 isolate of 2007 outbreak and this may be an indication of a new introduction of the same virus over time; together, the Nigerian SAT 2 isolates were most closely related to an isolate found in Libya in 2012, while the other group was genetically related to a 2003 SAT 2 isolate in Libya, thus showing the possibility of a common ancestral history of these viruses.

The phylogenetic evaluation of serotypes O, A and SAT 2 for majority of the isolates suggests strong serotype relationship amongst and between the recovered isolates in the two States studied.Kaduna and Plateau States share a common boundary towards the northern and southern ends. Clinical samples were obtained days/months/years apart within and between the States from Fulani pastoralists who normally move around and beyond the State in search of pasturage and water. Thus, it is likely that the phylogenetic differences identified between the regions within and between the two States were just an anecdotic evidence of the changes in biodiversity with time that one would expect for viruses circulating in an endemic region, rather than a reflection of true topographic boundaries for different FMD strains. However, in certain instances, the findings showed that these serotypes are contantly evolving with time and geographic location and give rise to variant viruses that are genetically unrelated.

The origin of the isolates in this study is not certain. The phylogenetic findings suggest that there is a considerable co-occurrence in virus serotypes and topotypes within West, Central, East and North Africa which is most likely linked to close contacts between acutely infected and susceptible animals which can be explained by the type of husbandry system and animal trade practised in these parts of sub-Saharan Africa. This epidemiological link demonstrates the potential of FMDV to spread over considerable distance. Nigeria livestock system is predominantly on transhumance production management system, and with a large and porous international and inter-state borders which serve as an entry point for countless migrating nomads into the country and within the States in search of feed and water resources without recourse to quarantine and control measures, serve as a great limitation to the effective implementation of movements restrictions, national veterinary quarantine services and control posts. Furthermore, the weak and often under-funded veterinary services in Nigeria pose a great limitation to disease prevention and control.

The role of wildlife in the transmission of FMDV in the study areas could not be ascertained. It was assumed that domestic livestock movements could be associated with exposure to wildlife; however, there was no evidence of an important role of wildlife in the epidemiology of FMDV in this study. Serotypes SAT (1-3) viruses have been known to be constantly evolving in the African buffalo, the true maintenance host of the SAT serotypes (Vosloo *et al.,* 1996; Bastos *et al.*, 2003; Vosloo and Thomson, 2004), which usually become subclinically infected (Vosloo *et al.*, 2007). Furthermore, the virus had been reported to persist in an individual buffalo for at least 5 years (Condy *et al.,* 1985). The fact that FMDV serotypes SAT1 and SAT 2 were found in regions without buffalo suggests that some variants of these serotypes can be maintained in domestic animals (Sangare *et al.*, 2004; Vosloo *et al.,* 2005; Hall *et al.,* 2013).

CHAPTER SIX
6.0 CONCLUSION AND RECOMMENDATIONS
6.1 Conclusion

i. The overall FMD sero-prevalence in Kaduna and Plateau States was estimated as 30.6%; Kaduna State had a 28.8% sero-prevalence and Plateau State had 34.5%.

ii. There was a serological evidence of the existence of five FMDV serotypes (A, O, SAT1, SAT2 and SAT3) in Kaduna and Plateau State of Nigeria.

iii. The observed presence of SAT 3 is the first report of the probable presence of this serotype in Nigeria.

iv. Three serotypes of FMDV (O, A and SAT 2) and four serotypes (O, A, SAT 1 and SAT 2) were isolated in Kaduna and Plateau States respectively.

v. The FMDV SAT 1 isolates in cattle from Plateau State was found to be unrelated to other topotypes in the GenBank and therefore designated a new topotype of SAT 1 in sub-Saharan Africa

vi. Phylogenetic evaluation revealed that serotypes O, A and SAT 2 found in Kaduna and Plateau States were genetically related to serotypes and topotypes occurring in West, Central, East and North Africa.

vii. The close genetic relationship of FMD viruses in Nigeria to those of other countries suggests that trade movements of animals and pastoralism from west to east and vice versa may be responsible for the spread of the virus across national borders.

6.2 Recommendations

i. There is need to strengthen border controls and as such animal quarantine stations should be established at the border and where theyexist they should be strengthened to prevent entry of FMD-infected animals into Nigeria.

ii. A complete genome study of the SAT1 isolates is required to establish their genetic relatedness to other SAT1 topotypes in Africa

iii. Vaccines that match the FMD serotype circulating in the country are to be used, hence the need for continuous surveillance in domestic animals and virus characterization.

iv. A national FMD control plan endorsed by OIE need to be in place and implemented to improve production and prevent financial losses is required to progressively control FMD in Nigeria.

v. A regional FMD control strategy should be developed and implemented with neighboring countries. Under the current situation, regionally coordinated approach under the guidance of the ECOWAS cooperation is likely to improve any FMD control strategy

REFERENCES

Abegunde, A.A. (1987). Studies on the epidemiology of foot –and- mouth disease in Nigeria.*Ph.D Thesis.Ahmadu Bello University, Zaria, Nigeria. pp.* 124-126.

Acharya, R., Fry,E., Stuart, D., Fox, G., Rowlands, D.and Brown, F. (1989). The three-dimensional structure of foot-and-mouth disease virus at 2.9 A resolution. *Nature*, 337:709-716.

Adamowicz, P.H., Legrand, B., Guerche, J. and Prunet, P. (1974).Un nouveau procede d concentration et purification de virus. Application au virus de fievre aphteuse produit sur cellules BHK 21 pour 1'obtention de vaccines hautement purifies. *Bulletin de l' Office International des Epizooties*,81: 1125-1150.

Advanced Veterinary Information System (AVIS) (2001).Foot-and-mouth disease- overview.The AVIS Multimedia suite, Telos ALEFF, London, United Kingdom (http://www.aleffgroup.com/avisfmd/accessed on 25th June, 2014.

Agol, V.I., Paul, A. V.and Wimmer, E. (1999).Paradoxes of the replication of picornaviral genomes.*Virus Research,* 62:129-147.

Ahl, R., and Rump, A. (1976). Assay of bovine interferons in cultures of the porcine cell line IB-RS-2. *Infectious Immunology,* 14:603-606.

Alexandersen, S., Forsyth, M. A., Reid, S.M. and Belsham, G. J. (2000).Development of reverse transcription-PCR (oligonucleotideprobing) Enzyme-Linked Immunosorbent Assays for diagnosis and preliminary typing of foot-and-mouth disease:a new system using simple and aqueous-phase hybridization. *Journal of Clinical Microbiology,*38: 4604-4613.

Alexandersen, S., Oleksiewicz, M.B. and Donaldson, A.I. (2001). The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time course study using TaqMan RT-PCR. *Journal of General Virology,* 82: 747-755.

Alexandersen, S., Brotherhood, I.and Donaldson, A.I. (2002a). Natural aerosol transmission of foot-and-mouth disease virus to pigs: minimal infectious dose for strain O1 Lausanne. *Epidemiology and Infection,* 128:301-312.

Alexandersen, S., Zhang, Z., Reid, S., Mutchings, G.H. and Donaldson, A.I. (2002b). Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001. *Journal of General Virology,* 83: 1915-1923.

Alexandersen, S., Zhang, Z.and Donaldson, A.I. (2002c).Aspects of the persistence of foot-and-mouth disease virus in animals-the carrier problem.*Microbes Infection,* 4:1099-1110.

Alexandersen, S., I. and Donaldson, A.I. (2002).Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs.*Epidemiology and Infection,*128:313-323.

Alexandersen, S., Quan, M., Murphy, C., Knight, J. and Zhang, Z. (2003a). Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *Journal of Comparative Pathology,* 129: 268-282.

Alexandersen, S., Z. Zhang, A.I. Donaldson, and Garland, A.J. (2003b).The pathogenesis and diagnosis of foot-and-mouth disease.*Journal of Comparative Pathology,* 129: 1-39.

Ambros, V., R.F. Pettersson, and Baltimore, D. (1978).An enzymatic activity in uninfected cells that cleaves the linkage between poliovirion RNA and the 5_terminal protein.*Cell,*15: 1439-1446.

Ambros, V. and Baltimore, D. (1980).Purification and properties of a HeLa cell enzyme able to remove the 5_ terminal protein from poliovirus RNA.*Journal of Biology and Chemistry,*255: 6739-6744.

Anderson, E.C., Anderson, J., Doughty, W. J. and Drevmo, S. (1975). The pathogenicity of bovine strains of foot and mouth disease virus for impala and wildebeest.*Journal of Wildlife Diseases,*11: 248-255.

Andino, R., Rieckhof, G. E., Achacoso, P.L.and Baltimore, D. (1993). Poliovirus RNA synthesis utilizes an RNP complex formed around the 5_ end of viral RNA. *European Molecular Biology Organization Journal,* 12: 3587-3598.

Anonymous (1993).FAO/OIE/WHO Animal Health Year Book 33.

Ansardi, D.C., Porter, D.C.and Morrow, C.D. (1992). Myristylation of poliovirus capsid precursor P1 is required for assembly of subviral particle. *Journal of Virology,* 66: 4556-4563.

Ansell, D.M., Samuel, A. R., Carpenter, W.C. and Knowles, N.K. (1994). Genetic relationships between foot-and-mouth disease type Asia 1 viruses. *Epidemiology and Infection,* 112:213-224.

Araujo, J.P., Jr. Montassier, H.J. and Pinto, A.A. (2002). Extensive antigenic and genetic variation among foot-and-mouth disease type A viruses isolated from the 1994 and 1995 foci in Sao Paulo, Brazil. *Veterinary Microbiology,* 84:15-27.

Armstrong, R., Davie, J and Hedger, R.S. (1967).Foot-and-mouth disease in man.*British Medical Journal,*4: 529-530.

Armstrong, R.M., Samuel, A.R., Carpenter, W.C., Kant, R. and Knowles, N.J. (1994). A comparative study of serological and biochemical methods for strain differentiation of foot-and-mouth disease type A viruses. *Veterinary Microbiology,* 39: 285-298.

Arnold, E., Luo, M., Vriend, G., Rossmann, M.G., Palmenberg, A.C., Parks, G.D., Nicklin, M.J.and Wimmer, W. (1987). Implications of the picornavirus capsid structure for polyprotein processing. *Proceedings of the Annual Meeting of the United States Animal Health Association,*84: 21-25.

Ayebazibwe, C., Tjornehoj, K., Mwiine, F.N., Muwanika, V.B., Adenum Okurut, A.R., Siegismund, H.R. and Alexandersen, S. (2010). Patterns, risk factors and characteristics of reported and perceived foot and mouth disease (FMD) in Uganda. *Tropical Animal Health and Production,* 42: 1547-1559.

Ayelet, G., Mahapatra, M., Gelaye, E., Egziabher, B.G., Rufeal, T., Sahle, M., Ferris, N.P., Wadsworth,J., Hutchings, G.H. and Knowles, N.J. (2009).Genetic characterization of foot and mouth disease viruses, Ethiopia, 1981-2007. *Emerging and Infectious Diseases,* 15: 1409-1417.

Bablanian, G.M., and Grubman, M.J. (1993). Characterization of the foot-and-mouth disease virus 3C protease expressed in *Escherichia coli.Virology,*197: 320-327.

Bachrach, H.L., Breese, S.S., Callis, J.J., Hess, W.R. and Patty, R.E. (1957).Inactivation of foot and mouth disease virus by pH and temperature changes and by formaldehyde.*Proceedings of the Society for Experimental Biology and Medicine,* 95: 147-152.

Bachrach, H.L. (1968). Foot-and-mouth disease.*Annual Review of Microbiology,* 22: 201-244.

Bachrach, H.L., D.M. Moore, P.D. McKercher, and Polatnick, J. (1975).Immune and antibody responses to an isolated capsid protein of foot-and-mouth disease virus.*Journal of Immunology*, 115: 1636-1641.

Bachrach, H. L. (1977). Foot-and-mouth disease virus: properties, molecular biology and immunogenicity. *Beltsville Symposium on Agricultural Research*, 1: 3-32.

Bahnemann, H.G. (1975). Binary ethylenimine as an inactivant for foot-and-mouth disease virus and its application for vaccine production.*Archives of Virology*, 47: 47-56.

Bahnemann, H.G. (1990). Inactivation of viral antigens for vaccine preparation with particular reference to the application of binary ethylenimine. *Vaccine,* 8: 299-303.

Balinda, S.N., Belsham, G.J., Masembe, C., Sangula, A.K., Siegismund, H.R. and Muwanika, V.B. (2009). Molecular characterization of SAT 2 foot and mouth disease virus from post –outbreak slaughtered animals: implication for disease control in Uganda. *Epidemiology and Infection, 138: 1204-1210.*

Banerjee, R., Echeverri, A.and Dasgupta, A. (1997). Poliovirus-encoded 2C polypeptide specifically binds to the 3_-terminal sequences of viral negative strand RNA. *Journal of Virology*, 71: 9570-9278.

Bao, H.F., Li, D., Guo, J.H., Lu, Z.J., Chen, Y.L., Liu, Z.X., Liu, X.T. and Xie, Q.G. (2008).A highly sensitive and specific multiplex RT-PCR to detect foot-and-mouth disease virus in tissue and food samples.*Archives of Virology*, 153: 205-209.

Baranowski, E., Ruiz-Jarabo, C.M., Lim, F. and Domingo, E. (2001). Foot-and-mouth disease virus lacking the VP1 G-H loop: the mutant spectrum uncovers interactions among antigenic sites for fitness gain. *Virology*, 288: 192-202.

Barasa, M., Catley, A., Machuchu, D., Laqua, H., Puot, E., Tap Kot, D.and Ikiror, D. (2008). Foot-and-Mouth Disease Vaccination in South Sudan: Benefit-Cost Analysis and Livelihoods Impact. *Transboundary and Emergency Diseases,* 55: 339-351.

Barnard, A.L., Arriens, A., Cox, S., Barnett, P., Kristensen, B., Summerfield, A. and McCullough, K.C. (2005).Immune response characteristics following emergency vaccination of pigs against foot-and-mouth disease.*Vaccine*, 23: 1037-1047.

Barnett, P.V., S.J. Cox, N. Aggarwal, H. Gerber, and McCullough, K.C. (2002).Further studies on the early protective responses of pigs following immunization with high potency foot and mouth disease vaccine.*Vaccine,*20:3197-3208.

Barnett, P. Garland, A.J.M., Kitching, R.P. and Scherrmbrucker, C. (2002).Aspects of emergency vaccination against foot and mouth disease.*Comparative Immunology and Microbiology of Infectious Disease,* 25:345-364.

Barnett, P. and Carrabin, H. (2002).A review of emergency foot and mouth disease vaccines.*Vaccine*, 20(11-12): 1505-1514.

Barteling, S.J., and Vreeswijk, J. (1991).Developments in foot-and-mouth disease vaccines.*Vaccine*,9:75-88.

Bartels, C., DeHaan, N. and McLaws, M. (2012). PCP stage 1-3. *Results, Technical Developments and Issues. Jerez la Frontera, Spain: Appliance of Science in the progressive control of FMD open session of the European Union-FMD;* 30.

Barton, D.J., B.J. O' Donnell, and Flanegan, J.B. (2001). 5_cloverleaf in poliovirus RNA is a cis-acting replication element required for negative strand synthesis. *European Molecular Biology Organizaton Journal,*20: 1439-448.

Bastos A.D.S. (1998). Detection and characterization of foot-and-mouth disease virus in sub-Saharan Africa.*Onderstepoort Journal of Veterinary Research,* 65: 37-47.

Bastos, A.D.S., Bertschinger, H.J., Coredel, C., Van Vuuren, C. de W., Net, D., Bengis, R.G., Grobler, D.G. and Thomson, G.R. (1999). Possibility of sexual transmission of foot-and-mouth disease from African buffalo to cattle.*Veterinary Record,* 145: 77-79.

Bastos, A.D.S., Boshoff, C.I., Keet, D.F., Bengis, R.G. and Thomson, G.R. (2000).Natural transmission of foot-and-mouth disease virus between African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*) in the Kruger National Park, South Africa.*Epidemiology and Infection,* 124:591-598.

Bastos, A.D.S. (2001). Molecular epidemiology and diagnosis of SAT type foot-and-mouth disease in Southern Africa. Ph.D Thesis, University of Pretoria, 67-98.

Bastos, A.D.S. and Sangare, O. (2001). Geographic distribution of SAT type 2 foot-and-mouth disease virus genotypes in Africa. *In Proceedings of Southern African Society for Veterinary Epidemiology and Preventive Medicine (SASVEPM), 10-11 May, Onderstepoort, South Africa.SASVEMPM, Pretoria,* Pp. 20-26.

Bastos, A.D.S., Haydon, D.T., Forsberg, R., Knowles, N.J., Anderson, E.C., Bengis, R.G., Nel, L.H. and Thomson, G.R. (2001).Genetic heterogeneity of SAT 1 type food-and-mouth disease viruses in Southern Africa.*Archives of Virology,* 146: 1537-1551.

Bastos, A.D.S., Haydon, D.T., Sangare, O., Boshoff, C.I., Edrich, J.L. and Thomson, G.R. (2003). The implications of viral diversity within the SAT-2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. *Journal of General Virology,* 84: 1595-1606.

Bauer, K. (1997). Foot-and-mouth disease as zoonosis.*Archives of Virology,* 13 (Suppl): 95-97.

Bautista, E.M., Ferman, G.S.and Golde, W.T. (2003). Induction of lymphopenia and inhibition of T cell function during acute infection of Swine with foot-and-mouth disease virus (FMDV). *Veterinary Immunology and Immunopathology*, 92:61-73.

Baxt, B., and Bachrach, H.L. (1980).Early interactions of foot-and-mouth disease virus with cultured cells.*Virology,* 104: 42-55.

Baxt, B. (1987). Effect of lysosomotropic compounds on early events in foot-and-mouth disease virus replication.*Virus Research,* 7: 257-271.

Baxt, B. and Mason, P.W. (1995). Foot-and-mouth disease virus undergoes restricted replication in macrophage cell cultures following Fc receptor-mediated adsorption. *Virology,* 207: 503-509.

Bayissa, B. Ayelet, G., Kyule, M., Jibril, Y. and Gelaye, E. (2011).Study of Seroprevalence, risk factors, and economic impact of foot-and-mouth disease in Borena Pastoral and agro-pastoral system, Southern Ethiopia.*Tropical Animal Health and Production,* 43 (4): 759-766.

Beard, C., Ward, G., Rieder, E., Chinsangaram, J., Grubman, M.J.and Mason, P. W. (1999). Development of DNA vaccines for foot-and-mouth disease, evaluation of vaccines encoding replicating and non-replicating nucleic acids in Swine.*Journal of Biotechnology,* 73: 243-249.

Beck, E. and Strohmaier, K. (1987).Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination.*Journal of Virology,* 61:1621-1629.

Beignon, A.S., Brown, F., Eftekhari, P., Kramer, E., Briand, J.P., Muller, S. and Partidos, C.D. (2005). A peptide vaccine administered intramuscularly together with cholera toxin elicits potent neutralizing anti-FMDV antibody responses. *Veterinary Immunology and Immunopathology,* 104: 273-280.

Belnap, D.M., B.M. McDermott, Jr., D.J. Filman, N. Cheng, B.L. Trus, H.J. Zuccola, V.R. Racaniello, J.M. Hogle, and Steven, A.C. (2000). Three-dimensional structure of poliovirus receptor bound to poliovirus. *Proceedings of the Annual Meeting of the United States Animal Health Association,* 97:73-78.

Belsham, G.J., and Brangwyn, J.K. (1990). A region of the 5_noncoding region of foot-and-mouth disease virus RNA directs efficient internal initiation of protein synthesis within cells: involvement with the role of L protease in translational control. *Journal of Virology,* 64:5389-5395.

Belsham, G.J., C.C. Abrams,A.M. King, J. Roosien, and Vlak, J. M. (1991). Myristoylation of foot-and-mouth disease virus capsid protein precursors is independent of other viral proteins and occurs in both mammalian and insect cells. *Journal of General Virology,* 72:747-751.

Belsham, G. J. (1993).Distinctive features of foot-and-mouth disease virus, a member of the picornavirus family; Aspects of virus protein synthesis, protein processing and structure.*Proceedings of Biophysiology and Molecular Biology,* 60: 241-60.

Benvenisti, L., Rogel, A., Kuznetzova, L., Bujanover, S., Becker, Y. and Stram, Y. (2001).Gene gun-mediated DNA vaccination against foot and mouth disease virus.*Vaccine,* 19: 3885-3895.

Berger, H.G., Straub, O.C., Ahl, R., Tesar, M. and Marquardt, O. (1990).Identification of foot-and-mouth disease virus replication in vaccinated cattle by antibodies to non-structural virus proteins.*Vaccine,* 8: 213-216.

Bergmann, I.E., De Mello, P.A., Neitzert, E., Beck, E. and Gomes, I. (1993). Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme-linked immunoelectrotransferblot analysis with bioengineered nonstructural viral antigens. *American Journal of Veterinary Research,* 54:825-831.

Berinstein, A., Tami, C., Taboga, O., Smitsaart, E. and Carrillo, E. (2000).Protective immunity against foot-and-mouth disease virus induced by a recombinant vaccinia virus.*Vaccine*, 18: 2231-2238.

Bhattacharya, S., Banerjee, R., Ghosh, R., Biswas, A. and Chatterjee, A. (2003).Identification of foot-and-mouth disease from a captive kangaroo in a zoological garden in India.*Veterinary Record,* 151: 667-669.

Bizimana, N. (1994). Epidemiology, surveillance and control of the principal infectious animal diseases in Africa.*Review Science and Technical office International des Epizooties,* 13:397-146.

Bolten, R., Egger, D., Gosert, R., Schaub, G., Landmann, L. and Bienz, K. (1998). Intracellular localization of poliovirus plus-and minus-strand RNA visualized by strand-specific fluorescent in situ hybridization. *Journal of Virology*, 72: 8578-8585.

Borrego, B., Camarero, J.A., Mateu, M.G. and Domingo, E. (1995). A highly divergent antigenic site of foot and mouth disease virus retains its immunodominance. *Viral Immunology,* 8: 11-18.

Borrego, B., Fernandez-Pacheco, P., Ganges, L., Domenech, N., Fernandez-Borges, N., Sobrino, F. and Rodriguez, F. (2006). DNA vaccines expressing B and T cell epitopes can protect mice from FMDV infection in the absence of specific humoral responses. *Vaccine,* 24: 3889-3899.

Brehm, K.E., Kumar, H.N., Thulke, H.H. and Haas, B. (2008). High potency vaccines induce protection against heterologous challenge with foot and mouth disease virus. *Vaccine*, 26 (13): 1681-1687.

Bronsvoort, B.M.DE C., Anderson, J., Corteyn, A., Hamblin, P., Kitching, R.P., Nfon, C., Tanya, V.N. and Morgan, K.L. (2006). Geographical and age-stratified distributions of foot –and- mouth disease virus- seropositive and probing- positive cattle herds in the Adamawa province of Cameroon. *Veterinary Record,* 159: 299-

Brooksby, J.B. (1957). The virus of foot-and-mouth disease.*Advance Virus Research,* 5: 1-37.

Brooksby, J.B. and Rogers, J. (1957). Methods used in typing the virus of Foot-and-mouth disease at Pirbright, 1950-55. *In: Methods of typing and cultivation of foot-and-mouth disease virus: Project No. 208. Paris: European Productivity Agency of the Organization of European Cooperation (OEEC).*

Brooksby, J.B. (1982). Portraits of viruses: Foot-and-mouth disease virus. *Intervirology,* 18:1-23.

Brown, F., and Cartwright, B. (1961).Dissociation of foot-and-mouth disease virus into its nucleic acid and protein components.*Nature,* 192: 1163-1164.

Brown, F. (1992). New approaches to vaccination against foot-and-mouth disease.*Vaccine,* 10: 1022-1026.

Brown, C.C., Meyer, R.F., Olander, H.J., House, C.and Mebus, C.A. (1992).A pathogenesis study of foot-and-mouth disease in cattle, using in situ hybridization.*Canadian Journal of Veterinary Research,* 56: 189-193.

Brown, C.C., Olander, H.J.and Meyer, R.F. (1995).Pathogenesis of foot-and-mouth disease *in Swine, studied by in-situ hybridization. Journal of Comparative Pathology*, 113:51-58.

Brown, C.C., Piccone, M.E, Mason, P.W, McKenna, T.S. and Grubman, M.J. (1996).Pathogenesis of Wildtype and leaderless foot-and-mouth disease virus in cattle.*Journal of Virology,* 70, 5638-5641.

Brown, C.C., Chinsangaram, J.and Grubman, M.J. (2000). Type I interferon production in cattle infected with 2 strains of foot-and-mouth disease virus, as determined by in situ hybridization. *Canadian Journal of Veterinary Research,* 64: 130-133.

Bruckner, G.K., Vosloo, W., Du Plessis, B.J., Kloeck, P.E., Connoway, L., Ekron, M.D., Weaver, D.B., Dickason, C.J., Schreuder, F.J., Marais, T. and Mogajane, M.E. (2002). Foot and Mouth disease: the experience of South Africa. *Review Science and technical Office International des Epizooties,* 21: 751-764.

Bulman, G. and Terraza, M.I. (1976).Effect of foot-and-mouth disease on milk production at a model farm in Cochabamba, Bolivia.*Revista de Medicina Veterinary Argentina,*5-10.

Bunch, T., Rieder, E. and Mason, P. (1994). Sequence of the S fragment of foot-and-mouth disease virus type A12.*Virus Genes,*8: 173-175.

Burrows, R. (1966).The infectivity assay of foot-and-mouth disease virus in pigs, *Journal of Hygiene (London),* 64: 419-429.

Brurrows, R. (1968a). Excretion of foot-and-mouth disease virus prior to the development of lesions.*Veterinary Record,* 83: 387-388.

Burrows, R. (1968b).The persistence of foot-and-mouth disease virus in sheep.*Journal of Hygiene (London),* 66: 633-640.

Burrows, R., Mann, J.A., Garland, A.J., Greig, A. and Goodridge, D. (1981).The pathogenesis of natural and simulated natural foot-and-mouth disease infection in cattle.*Journal of Comparative Pathology,* 91: 599-609.

Callahan, J.D., Brown, F., Osorio, F.A., Sur, J.H., Kramer, E., Long, G.W., Lubroth, J., Ellis, S.J., Shoulars, K.S., Gaffney, K.L., Rock, D.L. and Nelson, W.M. (2002). Use of a portable real-time reverse transcriptase-polymerasae chain reaction assay for rapid detection of foot-and-mouth disease virus.*Journal of the American Veterinary Medical Association,* 220, 1636-1642.

Callens, M. and De Clercq, K. (1997). Differentiation of the Seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. *Journal of Virological Methods,* 67: 35-44.

Callens, M., De Clercq, K., Gruia, M. and Danes, M. (1998).Subclinical FMD infection in contact sheep: detection of FMDV by RT-PCR-ELISA and transmission of the virus to sentinel pigs. *Veterinary Quarterly 20, Suppl.* 2: S37-40.

Capozzo, A.V., O. H. Periolo, B. Robiolo, C. Seki, J.L. La Torre, and Grigera, P.R. (1997). Total and isotype humoral responses in cattle vaccinated with foot and mouth disease virus (FMDV) immunogen produced either in bovine tongue tissue or in BHK-21 cell suspension cultures. *Vaccine*, 15: 624-630.

Carrillo, E.C., C. Giachetti, and Campos, R.H. (1984). Effect of lysosomotropic agents on the foot-and-mouth disease virus replication.*Virology,* 135: 542-545.

Carrillo, E. C., C. Giachetti, and Campos, R. (1985). Early steps in FMDV replication further analysis on the effects of chloroquine. *Virology*, 147: 118-125.

Carrillo, E. C.; Tulman, E. R.; Delhon, G.; Lu, Z.; Carreno, A.; Vagnozzi, A.; Kutish, G. F. and Rock, D. L. (2005). Comparative genomics of foot and mouth disease virus.*Journal of Virology*, 10: 6487-6504.

Center for food Security and Public Health (CFSPH) (2007).Foot-and-mouth disease.Iowa State University, College of Veterinary Medicine. Ames, IOWA. http://www.cfsph.iastate.edu/cab/factsheet/fmd/accessed on 25th June, 2014.

Chen, H., Peng, Y., Zhang, Y. and Liu, X. (2012). Detection of foot-and-mouth disease serotype O by ELISA using a monoclonal antibody. *Hybridoma (Larchmt),* 31: 462-464.

Cheng, I.C., Liang, S.M., Tu, W.J., Chen, C.M., Lai, S.Y., Cheng, Y.C., Lee, F., Huang, T.S., Jong, M.H. (2006). Study on the porcinophilic foot-and-mouth disease virus I. Production and characterization of monoclonal antibodies against VP1. *Journal of Veterinary Medical Science,* 68.859-864.

Childerstone, A.J., Cedillo-Baron, L., Foster-Cuevas, M.and Parkhouse, R.M. (1999).Demonstration of bovine CD8_T-cell responses to foot-and-mouth disease virus.*Journal of General Virology*, 80:663-669.

Chinsangaram, J., Piccone, M. E.and Grubman, M.J. (1999). Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. *Journal of Virology*, 73: 9891-9898.

Chinsangaram, J., Koster, M.and Grubman, M.J. (2001). Inhibition of L-deleted foot-and-mouth disease virus replication by alpha/beta interferon involves double-stranded RNA-dependent protein kinase. *Journal of Virology,* 75: 5498-5503.

Chinsangaram, J., Moraes, M.P., Koster, M.and Grubman, M.J. (2003). Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease. *Journal of Virology*, 77: 1621-1625.

Chow, M., Newman, J.F., Filman, D., Hogle, J.M., Rowlands, D.J.and Brown, F. (1987).Myristylation of picornavirus capsid protein VP4 and its structural significance.*Nature,*327: 482-486.

Clavijo, A., Viera-Pereira, P. J. and Bergmann, I. (2003).Use of reverse transcription polymerase chain reaction (RTPCR) for rapid diagnosis of foot and mouth disease in South America.*Veterinary Research Communication*,27: 63-71.

Collen, T., and Doel, T.R. (1990).Heterotypic recognition of foot-and-mouth disease virus by cattle lymphocytes.*Journal of General Virology,* 71: 309-315.

Collen, T., Pullen, L.and Doel, T.R. (1989).Tcell-dependent induction of antibody against foot-and-mouth disease virus in a mouse model.*Journal of General Virology,* 70: 395-403.

Condy, J.B., Hedger, R.S., Hamblin, C.and Barnett, I. T. (1985).The duration of the foot-and-mouth disease virus carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd.*Comparative Immunology and Microbiology of Infectious Diseases,* 8: 259-265.

Correa Melo, E., Saraiva. V. and Astodillo, V. (2002). Review of the status of foot and mouth disease in countries of South America and approaches to control and eradication.*Review Science and Technical office International des Epizooties,* 21 (3): 429-436.

Costa Giomi, M.P., Bergmann, I. E., Scodeller, E.A., Auge de Mello, P., Gomez, I.and La Torre, J.L. (1984). Heterogeneity of the polyribocytidylic acid tract in aphthovirus: biochemical and biological studies of viruses carrying polyribocytidylic acid tracts of different lengths. *Journal of Virology,* 51: 799-805.

Cottam, E.M., Wadsworth, J., Shaw, A.E., Rowlands, R.J., Goatley, L., Maan, S., Maan, N.S., Mertens, P.P., Ebert, K., Li, Y., Ryan, E.D., Juleff, N., Ferris, N.P., Wilesmith, J.W., Haydon, D.T., King, D.P., Paton, D.J., and Knowles, N.J. (2008). Transmission pathways of foot-and-mouth disease virus in the United Kingdom in 2007.*PLoS Pathogen,* 4:e1000050.

Cottral, G.E., Patty, R.E., Gailiunas, P. and Scott, F.W. (1965). Sensitivity of cell cultures, cattle, mice, and guinea-pigs for detection of nineteen foot-and-mouth pathogenesis of FMD 29 disease virus. *Bulletin de l' Office International des Epizooties,* 63: 1607-1625.

Cottral, G.E. and Bachrach, H.L. (1968).Foot-and-mouth disease viremia.*Proceedings of the Annual Meeting of the United States Animal Health Association,* 72: 383-399.

Cottral, G.E. (1969). Persistence of foot-and-mouth disease virus in animals, their products and the environment.*Bulletin de l' Office International des Epizooties.* 70: 549-568.

Cowan, K.M., and Graves, J.H. (1966). A third antigenic component associated with foot-and-mouth disease infection. *Virology*,30: 528-540.

Crowther, J.R., S. Farias, W.C. Carpenter, and Samuel, A.R. (1993). Identification of a fifth neutralizable site on type O foot-and-mouth disease virus following characterization of single and quintuple monoclonal antibody escape mutants. *Journal of General Virology,* 74: 1547-1553.

Curry, S., Abrams, C.C., Fry, E., Crowther, J.C., Belsham, G.J., Stuart, D.I.and King, A.M. (1995). Viral RNA modulates the acid sensitivity of foot-and-mouth disease virus capsids. *Journal of Virology,* 69: 430-438.

David, M., Torres, A., Mebus, C., Carrillo, B.J., Schudel, A., Fondevilla, N., Blanco Viera J. and Marcovecchio, F.E. (1993).Further studies on foot and mouth virus in Illama.*Proceedings of the Annual Meeting of United States Animal Health Association,* 97: 280-285.

Davie, J. (1962). The classification of subtype variants of the virus of foot-and-mouth disease.*Rapp. A¯la Xe Conference de la Commission permanente de la fi¯vre aphteuse, O.I.E., 12 Rue de Prony, Paris 17e, Pp.* 34.

Davie, J. (1964). A complement fixation technique for the quantitative measurement of antigenic differences between strains of foot and mouth disease virus.*Journal of Hygiene (Camb.),* 62: 407-411.

Dawe, P.S. and Pinto, A.A. (1978). Antibody responses to type-specific and virus infection-associated antigens in cattle vaccinated with inactivated polyvalent foot and mouth disease virus in North Malawi. *British Veterinary Journal,* 134: 504-510.

Dawe, P.S. and Durojaiye, O.A. (1986).A preliminary survey of antibody to VIA antigen of foot and mouth disease virus in Nigerian Livestock.*Review Science and Technical office International des Epizooties,* 5(3): 711-714.

Dawe, P.S., Flanagan, F.O., Madekurozwa, R.L., Sorensen, K.J., Foggin, E.C., Ferris, N.P.and Knowles, N.J. (1994a).Natural transmission of foot-and-mouth disease virus from African buffalo (*Syncerus caffer*) to cattle in a wildlife area of Zimbabwe.*Veterinary Record,* 134: 230-232.

Dawe, P.S., Sorensen, K., Ferris, N.P., Barnett, I.T., Armstrong, R.M.and Knowles, N. J. (1994b). Experimental transmission of foot-and-mouth disease virus from carrier African buffalo (*Syncerus caffer*) to cattle in Zimbabwe.*Veterinary Record,* 134:211-215.

De Castro, M.P. (1964). Behavior of the foot and mouth disease virus in cell cultures: susceptibility of IBRS-2 cell line. *Arquivos do Instituto Biologico (Sao Paulo),* 31: 63-78.

De Castro, M.P. and Pisani, R.C.B. (1964).The chromosome complement of the IBRS-2 swine cell line susceptible to the foot and mouth disease virus.*Arquivos do Instituto Biologico (Sao Paulo),* 31: 155-166.

De Clercq, K., Goris, N., Barnett, P.V. and MacKay, D.K. (2008). FMD Vaccines: Reflections on Quality Aspects for Applicability in European Disease Control Policy. *Transboundary and Emerging Diseases,* 55: 46-56.

De Diego, M., Brocchi, E., Mackay, D.and F. De Simone. (1997). The nonstructural polyprotein 3ABC of foot-and-mouth disease virus as a diagnostic antigen in ELISA to differentiate infected from vaccinated cattle. *Archives of Virology,* 142:2021-2022.

Dekker, A. (2008). Vaccination: overcoming the constraints to achieving effective immunity rates. *In report of the Open Session of the Standing Technical Committee of the EuFMD commission. The global control of FMD-tools, ideas and ideals, Erice, Italy, 14-17 October, 2008, FAO, Rome, Pp.* 89-93.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbois, C. (1999). Livestock to 2020.The Next Foot Revolution.Food, Agriculture and the Environment Discussion Paper 28.*IFRI, Washington DC, USA. Pp.* 72.

Di Nardo, A., Knowles, N.J. and Paton, D.J. (2011).Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia.*Review Science and technical Office International des Epizooties,* 30: 63-85.

Dmitrieva, T.M., Norkina, K.B.and Agol, V.I. (1991).Encephalomyocarditis virus RNA polymerase preparations, with and without RNA helicase activity.*Journal of Virology,* 65: 2714-2717.

Doel, T.R. (1999). Optimization of the immune response to foot-and-mouth disease vaccines.*Vaccines,*17: 1767-1771.

Doel, T.R. (2003). FMD vaccines.*Virus Research,* 91: 81-99.

Doel, T.R. (2005). Natural and vaccine induced immunity to FMD. *Current Topics in Microbiology and Immunology,* 288: 103-131.

Domingo, E., Davila, M.and Ortin, J. (1980).Nucleotide sequence heterogeneity of the RNA from a natural population of foot-and-mouth- disease virus.*Gene,*11: 333-346.

Domingo, E., Diez, J., Martinez, M.A., Hernandez, J., Holguin, A., Borrego, B.and Mateu, M.G. (1993). New observations on antigenic diversification of RNA viruses. Antigenic variation is not dependent on immune selection. *Journal of General Virology,* 74: 2039-2045.

Domingo, E., Escarmis, C., Baranowski, E., Ruiz-Jarabo, C.M., Carrillo, E., Nunez, J.I.and Sobrino, F. (2003). Evolution of foot-and-mouth disease virus.*Virus Research,* 91:47-63.

Donaldson, A.I., Herniman, K.A., Parker, J. and Sellers, R.F. (1970).Further investigations on the airborne excretion of foot-and-mouth disease virus.*Journal of Hygiene (London),* 68: 557-654.

Donaldson, A.I. and Ferris, N.P. (1980). Sites of release of airborne foot-and-mouth disease virus from infected pigs. *Research in Veterinary Science,* 29: 315-319.

Donaldson, A.I., Gloster, J., Harvey, L.D., and Deans, D.H. (1982).Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981.*Veterinary Record,* 110: 53-57.

Donaldson, A.I., Ferris, N.P. and Wells, G.A. (1984).Experimental foot-and-mouth disease in fattening pigs, sow, and piglets in relation to outbreaks in the field.*Veterinary Record,* 115, 509-512.

Donaldson, A.I. (1987). Foot-and-mouth disease: the principal features. *Irish Veterinary Journal* 41: 325-327.

Donaldson, A.I., Gibson, C.F., Oliver, R., Hamblin, C. and Kitching, R.P. (1987). Infection of cattle by airborne foot-and-mouth disease virus: minimal doses with O1 and SAT 2 strains. *Research in Veterinary Science,* 43: 339-346.

Donaldson, A.I. (1997). Risks of spreading foot and mouth disease through milk and dairy products.*Review Science and technical Office International des Epizooties,* 16: 117-124.

Donaldson, A.I., and Sellers, R.F. (2000).Foot-and-mouth disease, In W.B. Martin and I.D Aitken (ed.), *Diseases of sheep.Blackwell Science, Oxford, United Kingdom. Pp.* 254-258.

Donaldson, A.I. and Alexandersen, S. (2001). The relative resistance of pigs to infection by natural aerosols of foot-and-mouth disease virus.*Veterinary Record,* 148: 600-602.

Donaldson, A. I., Alexandersen, S.,Sorensen, J.H. and Mikkelsen, T. (2001). The relative risks of the uncontrollable (airborne) spread of foot-and-mouth disease by different species. *Veterinary Record,* 148: 602-604.

Donaldson, A. I. and Alexandersen, S. (2002). Predicting the Spread of foot and mouth disease by airborne virus.*Review Science and technical Office International des Epizooties,* 21:569-575.

Dorsch-Hasler, K., Yogo, Y.and Wimmer, E. (1975).Replication of picornaviruses. I. Evidence from in vitro RNA synthesis that poly (A) of the poliovirus genome is genetically coded. *Journal of Virology,* 16: 1512-1517.

Drake, J.W., and Holland, J.J. (1999). Mutation rates among RNA viruses. *Proceedings of the Annual Meeting of the United States Animal Health Association,*96: 13910-13913.

Dukes, J.P., King, D.P. and Alexandersen, S. (2006). Novel reverse-transcription loop-mediated isothermal amplification for rapid detection of foot-and-mouth disease virus.*Archives of Virology,* 151: 1093-1106.

Dunn. C.S. and Donaldson, A.I. (1997). Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. *Veterinary Record,* 141: 174-175.

Durojaiye, A.O. (1981). Incidence of foot-and-mouth disease in Oyo State of Nigeria 1967-1981.*Nigerian Veterinary Journal,* 10 (1): 7-13.

Dus Santos, M.J., Carrillo, C., Ardila, F. Rios, R.D., Franzone, P., Piccone, M.E., Wigdorovitz, A. and Borca, M.V. (2005). Development of transgenic alfalfa plants containing the foot and mouth disease virus structural polyprotein gene P1 and its utilization as an experimental immunogen. *Vaccine,* 23: 1838-1843.

Egger, D., R. Gosert, and Bienz, K. (2002).Role of cellular structures in viral RNA replication, *In* B.L. Semler and E. Wimmer (ed.), Molecular biology of picornaviruses.ASM Press, Washington, D.C. 146. Pp. 247-253.

Ehizibolo, D. O (2010).Serological survey of foot and mouth disease virus in ruminants in Plateau State, Nigeria. MSc Thesis. Ahmadu Bello University, Zaria.Pp 73-86

Eigen, M. (1971).Self-organization of matter and the evolution of biological macromolecules.*Naturwissenschaften,* 58: 465-523.

Ellard, F.M., Drew, J., Blakemore, W.E., Stuart, D.I.and King, A.M. (1999).Evidence for the role of His-142 of protein 1C in the acid-induced disassembly of foot-and-mouth disease virus capsids.*Journal of General Virology,* 80: 1911-1981.

Escarmis, C., Dopazo, J., Davila, M., Palma, E.L.and Domingo, E. (1995). Large deletions in the 5_-untranslated region of foot-and-mouth disease virus of serotype C. *Virus Research,* 35: 155-67.

Eskildsen, M. (1969).Experimental pulmonary infection of cattle with foot-and-mouth disease virus.*Nordisk Veterinaer Medicin.* 21: 86-91.

Evans, D.J., and Almond, J.W. (1998).Cell receptors for picornaviruses as determinants of cell tropism and pathogenesis.*Trends in Microbiology,* 6: 198-202.

FAO (Food and Agricultural Organization of the United Nations), (1984).Emerging diseases of livestock.Vol 1.The diseases and their diagnosis (Geering, W.A., ed.), Rome. Italy, Pp. 43-51.

FAO (Food and Agricultural Organization of the United Nations) (2011). Progressive control pathway, for foot-and-mouth disease control (PCP-FMD). Rome, Italy: *Food and Agriculture Organization of the United Nations,* Pp. 5-10.

FAOSTAT (2011). Food and agricultural organization statistical database. Available at www.FAOSTAT.fao.org (accessed October 20, 2013).

Fares, M.A., Moya, A., Escarmis, C., Baranowski, E., Domingo, E.and Barrio, E. (2001). Evidence for positive selection in the capsid protein-coding region of the foot-and-mouth disease virus (FMDV) subjected to experimental passage regimens. *Molecular Biology and Evolution,* 18: 10-21.

Feigelstock, D., Mateu, M.G., Piccone, M. E., De Simone, F., Brocchi, E., Domingo, E.and Palma, E. L. (1992). Extensive antigenic diversification of foot-and-mouth disease virus by amino acid substitutions outside the major antigenic site.*Journal of General Virology,* 73: 3307-3311.

Fernandez, A.A., Mello, P., Auge de Gomes, I. and Rosenberg, F. (1975a). The use of virus-infection-associated antigen (VIA) in the detection of cattle exposed to FMDV.*Boletin del Centro Panamericano de Fiebre Aftosa,* 17-22.

Fernandez, T., Angeli Quinton, P., Godofredo, M.B., Ferreira, M. E. V., Sondahl, M.S. and Fernandez, A.A. (1975b).Serological survey of foot and mouth disease in sheep in the central valley of Cochabamba. Bolivia. *Boletin del Centro Panamerixcano de Fiebre Aftosa,* 21-22.

Ferris, N.P. and Dawson, M. (1988).Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular disease.*Veterinary Microbiology,* 16: 201-209.

Ferris, N. P.; Powell, H. and Donaldson, A. I. (1988).Use of pre-coated immunoplates and freeze-dried reagents for diagnosis of foot and mouth disease and swine vesicular disease by enzyme-linked immunosorbent assay (ELIZA). *Journal of Virological Methods,* 19: 197-206.

Ferris, N.P., Kitching, R.P., Oxtoby, J.M., Philpot, R.M. and Rendle, R. (1990).Use ofinactivated foot and mouth disease virus antigen in liquid-phase blocking ELISA.*Journal of Virological Methods,* 29: 33-41.

Ferris, N.P. and Donaldson, A.I. (1992). The World Reference Laboratory for foot and Mouth Disease: a review of thirty-three years of activity (1958-1991). *Review Science and Technical office International des Epizooties, 11: (3): 657-684.*

Ferris, N.P., Grazioli, S., Hutchings, G.H. and Brocchi, E. (2011). Validation of a recombinant integrin αvβ6/monoclonal antibody based antigen ELISA for the diagnosis of foot-and-mouth disease. *Journal of Virological Methods,* 175: 253-260.

Fisher, J.R., (1894). Foot-and-mouth disease in Australia.*Australian Veterinary. Journal,* 16:58-161.

Forman, A.J. and Garland, A.J.M. (2002). Foot and mouth disease: the future of vaccine banks. *Review Science and Technical office International des Epizooties*, 21(3): 601-612.

Forman, S., Le Gall, F., Belton, D., Evans. B., Francois, J.L., Murray, G., Sheesley, D. (2009). Moving towards the global control of foot and mouth disease: an opportunity for donors.*Review Science and technical Office International des Epizooties*, 28 (3): 883-896.

Forss, S., Strebel, K., Beck, E.and Schaller, H. (1984). Nucleotide sequence and genome organization of foot-and-mouth disease virus.*Nucleic Acids Research,* 12: 6587-6601.

Francis, M.J., Hastings, G.Z., Brown, F., McDermed, J., Lu, Y.A.and Tam, J.P. (1991).Immunological evaluation of the multiple antigen peptide (MAP) system using the major immunogenic site of foot-and-mouth disease virus.*Immunology,* 73: 249-254.

Frenkel, H.S. (1947). La culture de virus de la fievre aphteuse sur l'epithelium de la langue des bovides. *Bulletin del Office International des Epizooties,*28: 155-162.

Fry, E.E., Stuart, D.I. and Rowlands, J.D. (2005).The structure of food and mouth disease virus.*CurrentTopics in Microbiology and Immunology,*288:71-101.

Gall, F.L. and Leboucq, N. (2004). The role of animal disease control in poverty reduction, food safety, market access and food security in Africa. *Office International des Epizooties* (*OIE). www.oie.int/doc/en_document.php?3363503*. Accessed 5th April, 2015.

Gamarnik, A.V. and Andino, K. (1998). Switch from translation to RNA replication in a positive-stranded RNA virus. *Genes Development,* 12: 2293-2304.

Garcia-Valcarcel, M., Doel, T., Collen, T., Ryan, M.and Parkhouse, R.M. (1996).Recognition of foot-and-mouth disease virus and its capsid protein VP1 by bovine peripheral T lymphocytes.*Journal of General Virology*, 77: 727-735.

Garland, A.J., (1974). The inhibitory activity of secretions in cattle against FMDV.*Ph.D Thesis, London School of Tropical Hygiene and Medicine, University of London, Pp.* 1-262.

Garland, A.J. M. and Donaldson, A.I. (1990).Foot-and-mouth disease.*Surveillance,* 17: 6-8.

Garland, A.J.M. (1997). The availability of vaccines for emergency vaccination in Europe.*In Report of the 32nd Session of the European Commission for the control of Foot-and-Mouth Disease (EUFMD), 2-4 April, Rome. EUFMD, Rome, Appendix,*8, 89-111.

Garland, A.J.M. and De Clercq, K. (2011). Cattle, sheep and pigs vaccinated against foot and mouth disease: does trade in these animals and their products present a risk of transmitting the disease? In the spread of pathogens through international trade in animals and animal products (S. MacDiarmid, ed.). *Review Science and technical Office International des Epizooties,* 30(1): 189-206.

Geering, W.A. (1967). Foot-and-mouth disease in sheep.*Australian Veterinary Journal*, 43: 485-489.

Gibbens, J.C. and Wilesmith, J.W. (2002). Temporal and geographical distribution of cases of foot and mouth disease during the early weeks of the 2001 epidemic in Great Britain. *Veterinary Research,* 151: 407-412.

Gibson, C.F., Donaldson, A. I.and Ferris, N.P. (1984). Response of sheep vaccinated with large doses of vaccine to challenge by airborne foot-and-mouth disease virus. *Vaccine,*2: 157-61.

Gibson, C.F. and Donaldson, A.I. (1986).Exposure of sheep to natural aerosols of foot-and-mouth disease virus.*Research in Veterinary Science,* 41: 45-49.

Giridharan, P., Hemadri, D., Tosh, C., Sanyal, A. and Bandyopadhyay, S.K. (2005).Development and evaluation of a multiplex PCR for differentiation of foot-and-mouth disease virus strains native to India.*Journal of Virological Methods,* 126: 1-11.

Gloster, J., Blackall, J., Sellers, R.F. and Donaldson, A.I. (1981).Long distance transport of foot-and-mouth disease virus over the sea.*Veterinary Record,* 110: 47-52.

Gloster, J., Sellers, R.F. and Donaldson, A.I. (1982).Long distance transport of foot-and-mouth disease virus over the sea.*Veterinary Record, 110, 47-52.*

Golding, S. M., Hedger, R. S., Talbot, P. and Watson, J. (1976).Radial immunodiffusion and seroneutralization techniques for the assay of antibodies to swine vesicular disease.*Research in Veterinary Science,*20: 142-147.

Golini, F., Semler, B.L., Dorner, A.J.and Wimmer, E. (1980). Protein-linked RNA of poliovirus is competent to form an initiation complex of translation in vitro. *Nature,*287: 600-603.

Gonzalez, M.J., Saiz, J.C., Laor, O. and Moore, D.M. (1991).Antigenic stability of foot and mouth disease virus variants on serial passage in cell culture.*Journal of Virology,* 65: 3949-3953.

Grubman, M.J. (1980). The 5_end of foot-and-mouth disease virion RNA contains a protein covalently linked to the nucleotide pUp. *Archives of Virology,* 63: 311-315.

Grubman, M.J., Robertson, B.H., Morgan, D.O., Moore, D.M.and Dowbenko, D. (1984). Biochemical map of polypeptides specified by foot-and-mouth disease virus genome RNA programmed cell-free translation system and in infected cells. *Journal of Virology,* 56:120-126.

Grubman, M.J., Morgan, D.O. Kendall, J.and Baxt, B. (1985). Capsid intermediates assembled in a foot-and-mouth disease virus genome RNA programmed cell-free translation system and in infected cells. *Journal of Virology,* 56: 120-126.

Grubman, M.J., Lewis, S.A.and Morgan, D.O. (1993). Protection of swine against foot-and-mouth disease with viral capsid proteins expressed in heterologous systems. *Vaccine,*11: 825-829.

Grubman, M.J., Zellner, M., Bablanian, G., Mason, P.W.and Piccone, M.E. (1995).Identification of the active-site residues of the 3C proteinase of foot-and-mouth disease virus.*Virology,* 213: 581-589.

Grubman, M.J. and Baxt, B. (2004).Foot-and-mouth disease.*Clinical Microbiology Review,* 17: 465-493.

Gurumurthy, C.B., Sanyal, A., Venkataramanan, R., Tosh, C., George, M. and Hemadri, D. (2002). Genetic diversity in the VP1 gene of foot-and-mouth disease virus serotype Asia 1. *Archives of Virology*, 147: 85-102.

Hall, M.D., Knowles, N.J., Wadsworth, J., Rambaut, A. and Woolhouse, M.E.J. (2013). Reconstructing geographical movements and host species transitions of foot and mouth disease virus serotype SAT 2. *mBio,* 4: 1-10.

Hamblin, C., Armstrong, R.M. and Hedger, R.S. (1984).A rapid enzyme-linked immunosorbent assay for the detection of foot and mouth disease virus in epithelial tissues.*Veterinary Microbiology,* 9: 435-443.

Hamblin, C., Barnett, I.T. and Crowther, J.R. (1986). A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouth disease virus. II. Application. *Journal of Immunological Methods*, 93: 123-129.

Hamblin, C., Kitching, R.P., Donaldson, A.I., Crowther, J.R. and Barnett, I.T. (1987). Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouthdisease virus. III. Evaluation of antibodies after infection and vaccination. *Epidemiology and Infection.* 99: 733-744.

Hamond, J. (2011). FMD Vaccine: Practical Applications from an International Perspective-FMDV vaccine to Live. An event organized by NFUS, Moredun and Scottish Government, 15 March, 2011.

Harber, J.J., Bradley, J., Andersen, C.W. and Wimmer, E. (1991). Catalysis of poliovirus VPO maturation cleavage is not mediated by serine 10 of VP2. *Journal of Virology,* 65: 326-334.

Hargreaves, S.K., Foggin, C.M., Anderson, E.C., Bastos, A.D.S., Thomson, G.R., Ferris, N.P. and Knowles, N.J. (2003).An investigation into the source and spread of foot-and-mouth disease virus from a wildlife conservancy in Zimbabwe.*Review Science and Technical office International des Epizooties,* 22 (3): 223-225.

Haydon, D., Lea, S., Fry, L., Knowles, N., Samuel, A.R., Stuart, D.and Woolhouse, M.E. (1998). Characterizing sequence variation in the VP1 capsid proteins of foot and-mouth disease virus (serotype 0) with respect to virion structure.*Journal of Molecular Evolution,* 46: 465-475.

Haydon, D.T., Samuel, A.R.and Knowles, N.J. (2001).The generation and persistence of genetic variation in foot-and-mouth disease virus.*Preventive Veterinary Medicine,* 51:111-124.

He, Y., Bowman, V.D., Mueller, S., Bator, C.M.,Bella, J., Peng, X., Baker, T.S., Wimmer, E., Kuhn, R.J.and Rossmann, M.G. (2000). Interaction of the poliovirus receptor with

poliovirus.*Proceedings of the Annual Meeting of the United States Animal Health Association*,97: 79-84.

Hedger, R.S., Forman, A.J. and Woodford, M.H. (1973).Food-and-mouth disease in East African buffalo.*Bullin of Epizootic Diseases of Africa,* 21: 99-101.

Hedger, R.S. (1976).Foot-and-mouth disease in wildlife with particular reference to the African buffalo (*Syncerus caffer).In Wildlife disease (L.A. Page, ed.). New York Plenum Publishing, Pp.* 235-244.

Hedger, R. S. (1981): Foot-and-Mouth diseases. *In infectious diseases of wild animals, 2nd ed., Edited by Davis J.W., Karstad L.H. & Trainer D.O. Ames, IA: Iowa State University Press. Pp.* 87-96.

Hedger, R.S., Barnett, I.T.R., Gradwell, D.V. and Travassos Dias, P. (1982). Serological tests for foot-and-mouth disease in bovine serum samples. Problems of interpretation. *Review Science and Technical office International des Epizooties,* 1: 387-393.

Hellen, C.U., Witherell,G.W., Schmid, M., Shin, S.H., Pestova, T.V., Gil, A.and Wimmer, E. (1993). A cytoplasmic 57-kDa protein that is required for translation of picornavirus RNA by internal ribosomal entry is identical to the nuclear pyrimidine tract-binding protein. *Proceedings of the Annual Meeting of the United States Animal Health Association*,90: 7642-7646.

Hemadri, D., Tosh, C., Sanyal, A. and Venkataramanan, R. (2002). Emergence of a new strain of type O foot-and-mouth disease virus: its phylogenetic and evolutionary relationship and the PanAsia pandemic strain. *Virus Genes,* 25:23-34.

Henderson, W.M. (1948). Further consideration of some of the factors concerned in intracutaneous injection of cattle.*Journal of Pathology and Bacteriology, 60: 137-139.*

Henderson, W.M. (1952). A comparison of different routes of inoculation of cattle for detection of the virus of foot-and-mouth disease.*Journal of Hygiene (London).* 50: 182-194.

Herold, J., and Andino, R. (2001). Poliovirus RNA replication requires genome circularization through a protein-protein bridge. *Molecular Cell,* 7: 581-591.

Hughes, G.J., Mioulet, V., Kitching, R.P., Woolhouse, M.E., Alexandersen, S. and Donaldson, A.I. (2002). Foot-and-mouth disease infection in sheep: implication for diagnosis and control. *Veterinary Record, 150:* 724-727.

Hyde, J.L., Blackwelll, J.H.and Callis, J.J. (1975). Effect of pasteurization and evaporation on foot-and-mouth disease virus in whole milk from infected cows. *Canadian Journal of Comparative Medicine,* 39: 305-309.

Hyslop, N.S. (1965). Secretion of foot-and-mouth disease virus and antibody in the saliva of infected and immunized cattle.*Journal of Comparative Pathology.* 75: 111-117.

Hyslop.N.S. (1970).The epizootiology and epidemiology of foot and mouth disease.*Advances in Veterinary Science and Comparative Medicine,* 14: 261-307.

Imler, J.L. (1995). Adenovirus vectors as recombinant viral vaccines. *Vaccine,*13:1143-1151.

Jackson, T., Ellard, F.M., Ghazaleh, R.A., Brookes, S.M., Blakemore, W.E.,Corteyn, A.H., Stuart, D.I., Newman, J.W.and King, A.M. (1996). Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparin sulfate. *Journal of Virology,* 70: 5282-5287.

Jackson, T., King,A.M.Stuart, D.I.and Fry, E. (2003).Structure and receptor binding.*Virus Research,* 91: 33-46.

Jamal, S.M., Ferrari, G., Ahmed, S.M., Ferrari, G., Ahmed, S., Normann, P., Belsham, G.J. (2011). Genetic diversity of foot and mouth disease serotype O viruses from Pakistan and Afghanistan, 1997-2009. *Infection, Genetics and Evolution,* 11: 1229-1238.

Jamal, S.M. and Belsham, G.J. (2013). Foot-and-mouth disease: Past, present and future. *Veterinary Research,* 44: 116.

James, A.D. and Ellis, P.R. (1978). Benefit-cost analysis in foot-and-mouth disease control programmes. *British Veterinary Journal,* 134 (1), 47-52.

James, A.D. and Rushton, J. (2002).The economics of foot and mouth disease. In foot and Mouth disease: Facing the new dilemmas (G.R. Thomson, ed.). *Review Science and Technical Office International des Epizooties,* 21 (3), 637-644.

Jang, S.K., Krausslich, H.G., Nicklin, M.J., Duke, G.M., Palmenberg, A.C.and Wimmer, E. (1988). A. segment of the 5_nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *Journal of Virology,* 62: 2636-2643.

Kaarianen, L., and Ranki, M. (1984). Inhibition of cell functions by RNA virus infections. *Annual Review of Microbiology,* 38: 91-109.

Kaduna State Government (KDSG) (2008). Kaduna State Achievements. In: Data on Estimated Annual Animal Population and Fish Production Investment Opportunities in Kaduna State Pp 16-18.

Kaminski, A., Hunt, S.L., Patton, J.G.and Jackson, R.J. (1995).Direct evidence that polypyrimidine tract binding protein (PTB) is essential for internal initiation of translation of encephalomyocarditis virus RNA.*RNA* 1: 924-938.

Keet, D.F., Hunter, P., Bengis, R.G., Bastos, A.D. and Thomson, G.R. (1996).The 1992 foot-and-mouth disease epizootic in the Kruger National Park.*Journal of South African Veterinary Association,* 67: 83-87.

Khounsy, S., Conlan, J.V., Gleeson, L.J., Westbury, H.A., Colling, A., Paton, D.J., Ferris, N.P., Valarcher, J.F., Wadsworth, J., Knowles, N.J. and Blacksell, S.D. (2009). Molecular epidemiology of foot and mouth disease viruses from South East Asia 1998-2006: the Lao perspective. *Veterinary Microbiology,* 137: 178-183.

King, A.M.Q., McCahon, D., Saunders, K., Newman, J.W.and Slade, W.R. (1985).Multiple sites of recombination with the RNA genome of foot-and-mouth disease virus.*Virus Research,* 3: 373-384.

King, A.M.Q. (1988). Preferred sites of recombination in poliovirus RNA: an analysis of 40 intertypic crossover sequences. *Nucleic Acids Research,* 16: 11705-11723.

King, D.P., Ferris, N.P., Shaw, A.E., Reid, S.M., Hutchings, G.H., Giuffre, A.C., Robida, J.M., Callahnan, J.D., Nelson, W.M., and Beckham, T.R. (2006). Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two independent real-time reverse transcription polymerase chain reaction assays. *Journal of Veterinary Diagnostic Investigations,* 18: 93-97.

Kit, M., Kit, S., Little, S.P., Di Marchi, R.D.and Gale, C. (1991). Bovine herpesvirus-1 (infectious bovine rhinotracheitis virus)-based viral vector which expresses foot-and-mouth disease epitopes. *Vaccine,*9: 564-572.

Kitching, R.P. and Donaldson, A.I. (1987).Collection and transportation of specimens for vesicular virus investigation.*Review Science and Technical office International des Epizooties,* 6: 263-272.

Kitching, R.P. (1992). Foot-and-mouth disease, In A.H. Andrews, R.W. Blowey, H. Boyd, and R.G. Eddy (ed.), Bovine medicine: diseases and husbandry of cattle. Blackwell Science Inc., Oxford, United Kingdom. Pp. 537-543.

Kitching, R.P. (1994). Foot-and-mouth disease in the Middle East.*Foot and Mouth Disease Newsletter,* 1: 2-4.

Kitching, R.P. (1998). A recent history of foot-and-mouth disease.*Journal of comparative pathology,* 118, 89-108.

Kitching, R.P. (2002a). Future research on foot and mouth disease. In foot and mouth disease: facing the new dilemmas (Thomson, G.R., ed.). *Review Science and Technical Office International des Epizooties,* 21(3): 885-889.

Kitching R.P. (2002b). Clinical variation in foot and mouth disease: cattle. *Review Science and Technical Office International des Epizooties,* 21: 499-504.

Kitching, R.P. and Alexandersen, S. (2002). Clinical variation in foot and mouth disease: pigs. *Review Science and Technical office International des Epizooties,* 21: 513-518.

Kitching, R.P. and Hughes, G.J. (2002). Clinical variation in foot and mouth disease: sheep and goats. *Review Science and Technical Office International des Epizooties,* 21: 505-512.

Kleid, D.G., Yansura, D., Small, B., Dowbenko, D., Moore, D.M., Grubman, M.J., McKercher, P.D., Morgan, D.O., Robertson, B.H.and Bachrach, H.L. (1981). Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and Swine. *Science,*214: 1125-1129.

Klein, J. (2009). Understanding the molecular epidemiology of foot-and-mouth disease virus.*Infection, Genetics and Evolution,* 9: 153-161.

Kleina, L.G., and Grubman, M.J. (1992).Antiviral effects of a thiol protease inhibitor on foot-and-mouth disease virus.*Journal of Virology,* 66: 7168-7175.

Klump, W., Marquardt, O.and Hofschneider, P.H. (1984). Biologically active protease of foot-and-mouth disease virus is expressed from cloned viral cDNA in Escherichia coli. *Proceedings of the Annual Meeting of the United States Animal Health Association,*81: 3351-3355.

Knipe, T., Reider, E., Baxt, B. Ward, G.and Mason, P.W. (1997). Characterization of Synthetic foot-and-mouth disease virus provirions separates acid-mediated disassembly from infectivity. *Journal of Virology,* 71: 2851-2856.

Knowles N.J. and Samuel A.R. (1995). Polymerase Chain reaction amplification and cycle sequence of the ID (VP1) gene of foot-and-mouth viruses. *Report of the session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease held jointly with the FMD sub-group of the Scientific Veterinary Committee of the Commission of the European Community Modeling, Vienna, Austria, 19-22 September, 1994. Appendix 8 Pp* 45-53.

Knowles, N.J., Ansell, D.M. and Samuel, A.R. (1998).Molecular comparison of recent foot-and-mouth disease type A viruses from West Africa with historical and reference virus strains.*Paper presented to the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease (EUFMD), 14-18 September, Pirbright, United Kingdom. EUFMD, Rome,* 1-6.

Knowles, N.J. and Samuel, A.R. (1998).Molecular techniques in foot-and-mouth disease epidemiology.*In: Towards Livestock Disease Diagnosis and Control in the 21st Century (Proceeding of an International Symposium on Diagnosis and Control of Livestock Diseases jointly organized by IAEA and the FAO, held in Vienna 7-11 April 1997) Pp* 185-201.

Knowles, N.J. and Davies, P.R. (2000).Origin of Recent Outbreaks of Foot-and-Mouth Disease in North Africa, the Middle East and Europe.*Report of the session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Borovets, Bulgaria, 5-8 September, 2000 (Appendix 3). FAO, Rome, Pp.* 39-45.

Knowles, N.J., Davies, P.R., Henry, T., O_Donnell, V., Pacheco, J.M. and Mason, P.W. (2001a). Emergence in Asia of foot-and-mouth disease viruses with altered host range: characterization of alterations in the 3A protein. *Journal of Virology,* 75: 1551-1556.

Knowles, N.J., Samuel, A.R., Davies, P.R., Kitching, R.P. and Donaldson, A.I. (2001b). Outbreak of foot-and-mouth disease virus serotype O in the UK caused by a pandemic strain. *Veterinary Record,* 148: 258-259.

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

Knowles, N.J., Samuel, A.R., Davies, P.R., Midgley, R.J., Valarcher, J.F. (2005). Pandemic strain of foot-and-mouth disease virus serotype O. *Emerging Infectious Diseases,* 11: 1887-1893.

Knowles, N.J., Ebert, K. and Wadswoth, J. (2008).Molecular epidemiology report on foot and mouth disease virus O and SAT 2 from Nigeria in 2007-2008.*Molecular epidemiology report form IAH-P-EP-MEG-FOR-005-1, FAO, WRLFMD.*

Ko¨nig, G., Blanco, C., Knowles, N.J., Palma, E.L., Maradei, E. and Piccone, M.E. (2001). Phylogenetic analysis of foot-and-mouth disease viruses isolated in Argentina. *Virus Genes,* 23: 175-182.

Korn, G. (1957). Experimental studies of the demonstration of virus during the incubation period of foot-and-mouth disease and of its pathogenesis. *Archives of Experimental Veterinary Medicine,* 11: 637-649.

Kuhn, R., N. Luz, and Beck, E. (1990).Functional analysis of the internal translation initiation site of foot-and-mouth disease virus.*Journal of Virology,* 64:4625-4631.

Lee, W.M., Monroe, S.S.and Rueckert, R.R. (1993). Role of maturation cleavage in infectivity of picornaviruses: activation of an infectosome. *Journal of Virology,* 67: 2110-2122.

Leforban, Y. (1995). Availability of vaccines for emergency vaccination in Europe.*In Report of the 31st Session of the European Commission for the control of Foot-and-Mouth Disease (EUFMD), 5-7 April, Rome. EUFMD, Rome, Appendix 9,* 60-65.

Leforban, Y. and Gerbier, G. (2002). Review of the status of foot and mouth disease and approach to control/eradication in Europe and Central Asia. *Review Science and Technical Office International des Epizootics,* 21 (3): 437-449.

Letshwenyo, M., Mapitse, N. and Hyera, J.M. (2006).Foot-and-mouth disease in a kudu (*Tragelaphus strepsiceros*) in Botswana.*Veterinary Record,* 159: 252-253.

Lewis, S. A., Morgan, D. O. and Grubman, M. J. (1991).Expression, processing and assembly of foot-and-mouth disease virus capsid structures in heterologous systems: induction of a neutralizing antibody response in guinea pigs.*Journal of Virology*,65: 6572 - 6580.

Li, G., Chen, W., Yan, W., Zhao, K., Liu, M., Zhang, I., Fei, L., Xu, Q., Sheng, Z., Lu, Y. and Zheng, Z. (2004). Comparison of immune responses against foot-and-mouth disease virus induced by fusion proteins using swine IgG heavy chain constant region or beta-galactosidase as a carrier of immunogenic epitopes. *Virology,* 328: 274-281.

Li, Y., Aggarwal, N., Takamatsu, H.H., Sterling, C.M., Voyce, C. and Barnett, P.V. (2006).Enhancing immune responses against a plasmid DNA vaccine encoding a FMDV empty capsid from serotypeO.*Vaccine,* 24: 4602-4606.

Libeau, J. (1960). Foot and Mouth Disease in Africa South of the Sahara-the present situation, *Bulletin of Epizootic Diseases of Africa,* 8: 152-158.

Lopez de Quinto, S., Saiz, M., de la Morena, D., Sobrino, F.and Martinez-Salas, E. (2002). IRES-driven translation is stimulated separately by the FMDV 3_-NCR and poly (A) sequences. *Nucleic Acids Research,* 30:4398-4405.

Lukaszewski, R.A., and Brooks, T.L. (2000). Pegylated alpha interferon is an effective treatment for virulent Venezuelan equine encephalitis virus and has profound effects on the host immune response to infection. *Journal of Virology*, 74: 5006-5015.

Luz, N., and Beck, E. (1990). A cellular 57 kDa protein binds to two regions of the internal translation initiation site of foot-and-mouth disease virus. *Federation of European Biochemical Societies Letters,*269: 311-314.

Mackay, D.K.J., Forsyth, M.A., Davies, P.R., Berlinzani, A., Belsham, G.J., Flint, M. and Ryan, M.D. (1997).Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA.*Vaccine,* 16: 446-459.

Mackay, D. K., Bulut, A. N., Rendle, T., Davidson, F. and Ferris, N. P. (2001).A solid phase competition ELISA for measuring antibody to foot-and-mouth disease virus.*Journal of Virological Methods,* 97:33-48.

MAFF (Ministry of Agricuture, Fisheries and Food) (1986).Foot-and-mouth disease. Ageing of lesions. Her majesty's stationary Office, London, UK.

Marc, D., Girard, M.and van der Werf, S. (1991). A Glyl to Ala substitution in poliovirus capsid protein VP0 blocks its myristoylation and prevents viral assembly. *Journal of General Virology*, 72: 1151-1157.

Marquardt, O. and Adam, K.H. (1989). Sequences of capsid protein VP1 of two type A foot-and-mouth disease viruses. *Virus Genes,* 2: 283-291.

Marquardt, O. and Freiberg, B. (2000). Antigenic variation among foot and-mouth disease virus type A field isolates of 1997-1999 from Iran. *Veterinary Microbiology*, 74: 377-386.

Marquardt, O., Rahman, M.M., Freiberg, J., Mateu, M.G., Sobrino, F., Domingo, E. and Knowles, N.J. (1992).Evolution of the capsid protein genes of foot-and-mouth disease virus.Antigenic variation without accumulation of amino acid substitutions over six decades.*Journal of Virology,* 66: 3557-3565.

Marquardt, O., Straub, O. C., Ahl, R. and Haas, B. (1995).Detection of foot and mouth disease virus in nasal swabs of asymptomatic cattle by RT-PCR within 24h.*Journal of Virological Methods,* 53: 255-261.

Marquardt, O., Rahman, M.M. and Freiberg, B. (2000). Genetic and antigenic variance of foot-and-mouth disease virus type Asia 1.*Archives of Virology,* 145: 149-157.

Martinez, M.A., Herna'ndez, J., Piccone, M.E., Palma, E.L., Domingo, E., Knowles, N.J. and Mateu, M.G. (1991).Two mechanisms of antigenic diversification of foot-and-mouth disease virus.*Virology,* 184: 695-706.

Martinez-Salas, E., Ramos, R., Lafuente, E.and Lopez de Quinto, S. (2001).Functional interactions in internal translation initiation directed by viral and cellular IRES elements.*Journal of General Virology,* 82: 973-984.

Mason, P.W., Baxt, B., Brown, F., Harber, J., Murdin, A.and Wimmer, E. (1993). Antibody-complexed foot-and-mouth disease virus, but not poliovirus, can infect normally unsusceptible cells via the Fc receptor. *Virology,*192: 568-577.

Mason, P.W., Bezborodova, S.V.and Henry, T.M. (2002).Identification and characterization of a *cis*-acting replication element (*cre*) adjacent to the internal ribosome entry site of foot-and-mouth disease virus.*Journal of Virology,* 76: 9686-9694.

Mason, P.W., Grubman, M.J. and Baxt, B. (2003).Molecular basis of pathogenesis of FMDV.*Virus Research,* 91:9-32.

Mateu, M.G., Hernandez, J., Martinez, M.A., Feigelstock, D., Lea, S., Perez, J.J., Giralt, E., Stuart, D., Palma, L.and Domingo, E. (1994). Antigenic heterogeneity of a foot-and-mouth disease virus serotype in the field is mediated by very limited sequence variation at several antigenic sites. *Journal of Virology,* 68:1407-1417.

Mateu, M.G., Camarero, J.A., Giralt, E., Andreu, D.and Domingo, E. (1995).Direct evaluation of the immunodominance of a major antigenic site of foot-and-mouth disease virus in a natural host.*Virology,* 206:298-306.

Mayr, G.A., O'Donnell, V., Chinsangaram, J., Mason, P.W.and Grubman, M.J. (2001). Immune responses and protection against foot-and-mouth disease virus (FMDV) challenge is swine vaccinated with adenovirus-FMDV constructs. *Vaccine,* 19:2152-2162.

McCahon, D., King A. M., Roe, D. S., Slade, W.R., Newman, J.W. and Cleary, A.M. (1985).Isolation and biochemical characterization of inter-typic recombinants of foot-and-mouth disease virus.*Virus Research,* 3: 87-100.

McCullough, K.C., Parkinson, D. and Crowther, J.R. (1988). Opsonization enhanced phagocytosis of foot-and-mouth disease virus. *Immunology,* 65:187-191.

McCullough, J.C., De Simone, F., Brocchi, E., Capucci, L., Crowther, J.R.and Kihm, U. (1992).Protective immune response against foot-and-mouth disease.*Journal of Virology,* 66:1835-1840.

McKenna, T.S., Lubroth, J.,Rieder, E., Baxt, B.and Mason, P.W. (1995). Receptor binding site-deleted foot-and-mouth disease (FMD) virus protects cattle from FMD. *Journal of Virology,* 69:5797-5790.

McVicar, J.W. and Sutmoller, P. (1970). Foot-and-mouth disease: the agar gel immunodiffusion precipiting test for antibody to virus-infection-associated (VIA) antigen as a tool for epizootiologic surveys. *American Journal of Epidemiology,* 92:273-278.

McVicar, J. W. and Sutmoller, P. (1976).Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation.*Journal of Hygiene (London),* 76: 467-481.

Melchers, W.J., Hoenderop, J.G., Bruins H.J., Slot, C.W. Pleij, E.V. Pilipenko, Agol, V.I.and Galama, J.M. (1997). Kissing of the two predominant hairpin loops in the coxsackie B

virus 3-untranslated region is the essential structural feature of the origin of replicating required for negative-strand RNA synthesis.*Journal of Virology,* 71:686-696.

Meyer, R.F., Brown, C.C., House, C., House, J.A. and Molitor, T.W. (1991).Rapid and sensitive detection of foot-and-mouth disease virus in tissues by enzymatic RNA amplification of the polymerase gene.*Journal of Virological Methods,* 161-172.

Meyer, R.F., Babcock, G.D., Newman, J.F., Burrage, T.G., Toohey, K., Lubroth, J. and Brown, F. (1997). Baculovirus expressed 2C of foot-and-mouth disease virus has the potential for differentiating convalescent from vaccinated animals. *Journal of Virological Methods,* 65:33-43.

Mitchell, R. (2001). Foot and mouth disease epidemic disposal measures-assessment and monitoring of possible risks to public health.*Eurosurveillance,* 5 (23): 1739.

Moraes, M.P., Mayr, G.A., Mason, P. W.and Grubman, M.J. (2002).Early protection against homologous challenge after a single dose of replication defective human adenovirus type 5 expressing capsid proteins of foot-and mouth disease virus (FMDV) strain A24.*Vaccine,* 20:1631-1639.

Moraes, M.P., Chinsangaram, J., Brum, M.C.S., and Grubman, M.J. (2003). Immediate protection of swine from foot-and-mouth disease: a combination of adenoviruses expressing interferon alpha and a foot-and-mouth disease virus subunit vaccine. *Vaccine,* 22:268-276.

Moraes, M.P., Los Santus, de T., Koster, M., Turecek, T., Wang, H., Andreyev, V.G. and Grubman, M.J. (2007).Enhanced antiviral activity against foot-and-month disease virus by a combination of type I and II porcine interferons.*Journal of Virology,* 81: 7124-7135.

Morioka, K., Fukai, K., Yoshida, K., Yamazoe, R., Onozato, H., Ohashi, S., Tsuda, T. and Sakamoto, K. (2009). Foot-and-mouth disease virus antigen detection enzyme-linked immunosorbent assay using multi-serotype-reactive monoclonal antibodies. *Journal of Clinical Microbiology,* 47: 3663-3668.

Moscufo, N., J. Simons, and Chow, M. (1991). Myristoylation is important at multiple stages in poliovirus assembly. *Journal of Virology,* 65:2372-2380.

Muthuchelvan, D., Venkataramanan, R., Hemadri, D., Sanyal, A. and Tosh, C. (2001).Sequence analysis of recent Indian isolates of foot and mouth disease virus serotypes O, A and Asia 1 from clinical materials.*Acta Virology,* 45: 159-167.

Mulcahy, G., Gale, C., Robertson, P., Iyisan, S., Dimarchi, R.D.and Doel, T.R. (1990). Isotype responses of infected, virus-vaccinated and peptide vaccinated cattle to foot-and-mouth disease virus. *Vaccine,* 8:249-256.

Mwiine, F.N., Ayebazibwe, C., Olaho-Mukani, W., Alexandersen, S., Balinda, S.N., Masembe, C., Ademun Okurut, A.R., Christensen, L.S., Sorensen, K.J. and Tjornehoj, K. (2010). Serotype specificity of antibodiesagainst foot and mouth disease virus in selected districts in Uganda. *Transboudary and Emerging Disaeses,* 57: 365-374.

Namatovu, A., Wekesa, S., Tjorneho, K., Dhikusooka, M., Muwanika, V., Siegsmund, H. and Ayebazibwe, C. (2013). Laboratory capacity for diagnosis of foot-and-mouth disease in Eastern Africa: Implications for the progressive control pathway. *Veterinary Research,* 9:19.

Nargi, F., Kramer, E., Mezencio, J., Zamparo, J., Whetstone, C., Van Regenmortel, M.H., Braiand, J. P., Muller, S.and Brown, F. (1999). Protection of swine from foot-and-mouth disease with one dose of an all-D retro peptide.*Vaccine,* 17: 2888-2893.

Nawathe, D.R. and Goni, M. (1976).Foot and Mouth disease in Nigeria.*Bulletin of Animal Health and Production in Africa,* 24:1-4.

Nayak, B., Pattnaik, B., Tosh, C., Sanyal, A., Hemadri, D., Patil, S.S. and Venkataramanan, R. (2001). Genetic and antigenic analysis of type A foot-and-mouth disease viruses isolated in India during 1987-1006. *Acta Virology,* 45:13-21.

Newton, S.E., Carroll, A. R., Campbell, R.O., Clarke, B.E.and Rowlands, D.J. (1985).The sequence of foot-and-mouth disease virus RNA to the 5-side of the poly (C) tract.*Gene,* 40-331-336.

Niepmann, M., Petersen, A., Meyer, K.and Beck, E. (1997).Functional involvement of polypyrimidine tract-binding protein in translation initiation complexes with the internal ribosome entry site of foot-and-mouth disease virus.*Journal of Virology,* 71:8330-9339.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000).Loop-mediated isothermal amplification of DNA.*Nucleic Acids Research,* 28: E63.

Novak, J.E. and Kirkegaard, K. (1991). Improved method for detecting poliovirus negative strands used to demonstrate specificity of positive strand encapsidation and the ratio of positive to negative strands in infected cells. *Journal of Virology*, 65:3384-3387.

Nugent, C.I., Johnson, K.L., Sarnow, and Kirkegaard, K. (1999).Functional coupling between replication and packaging of poliovirus replicon RNA.*Journal of Virology,* 73:427-435.

Obi, T.U. and Newman, B. (1988). The detection of antibodies against foot and mouth disease virus infection associated antigen by the enzyme-linked immunosorbent assay in Nigerian sheep and goat sera. *Tropical Veternarian,*6: 71-6.

Oleksiewicz, M.B., Donaldson, A.I.and Alexandersen, S. (2001). Development of a novel real-time RT-PCR assay for quantitation of foot-and-mouth disease virus in diverse porcine tissues.*Journal ofVirologicalMethods,* 92:23-35.

OIE (World Organization for Animal Health) (2012).Foot-and-mouth disease.*In Manual of diagnostic tests and vaccines for terrestrial animals (Mammals, birds and bees).Volume 1, 7th edition. Paris, France: pp.*190-217.

OIE(World Organization for Animal Health)(2013).Official Disease Status/FMD. [http://www.oie.int/en/animal.health-in-the-world/official-disease-status/fmd/en-fmd-carte/]. Accessed 12th March, 2014.

Ortiz-Pelaez, A., Pfeiffer, D.U., Soares-Magalhaes, R.J. and Guitian, F.J. (2006). Use of social network analysis to characterize the pattern of animal movements in the initial phases of the 2001 foot and mouth disease (FMD) epidemic in the UK. *Preventive Veterinary Medicine,* 76: 40-55.

Owolodun, B.Y. (1971). Foot and Mouth disease and virus type distribution in Nigeria. *Bulletin of Epizootic Diseases of Africa*, 152-158.

Parker, J. (1971).Presence and inactivation of foot-and-mouth disease virus in animal faeces.*Veterinary Record,* 88: 659-662.

Paton, D., Ferris, N., Valarcher, J., Knowles, N. and Stathan, B. (2005). FMD WRL report 2005. *Report of the FAO Executive Committee of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Den Haag, the Netherlands, 29-30 November, pp.* 60-65.

Paton, D., Sumption, K.J. and Charleston, B. (2009). Options for control of foot and mouth disease: knowledge, capability and policy. *Philosophical Transactions of the Royal Society B: Biological Science,* 364: 2657-2667.

Paul, A.V. (2002). Possible unifying mechanism of picornavirus genome replication.*In B.L. Semler and E. Wimmer (ed.), Molecular biology of picornaviruses. ASM Press, Washington, D.C. pp.* 227-246.

Pelletier, J., and Sonenberg, N. (1988). Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature,* 334:320-325.

Pereira, H.G. (1977). Subtyping of FMDV.*Proceedings of the International symposium on foot-and-mouth disease,* 5-8 October 1976, Lyon. *Developments in Biological Standardization,* 35: 167-174.

Perry, P.B., Kalpravidh, W., Coleman, P.G., Horst, H.S., McDermott, J.J., Randolph, T.F. and Gleeson, L.J. (1999). The economic impact of foot-and-mouth disease and its control in South-East Asia: a preliminary assessment with special reference to Thailand. *In* The economics of animal disease control (B.D. Perry, ed.). *Review Science and technical Office International des Epizooties,* 18 (3), 478-497.

Perry, B.D., Gleeson, L.J., Khounsey, S., Bounma, P. and Blacksell, S.D. (2002). The dynamics and impact of foot and mouith disease in smallholder farming systems in South-East Asia: a case study in Laos. *Review Science and technical Office International des Epizooties,* 21: 663-673.

Pfister, T., and Wimmer, E. (1999).Characterization of the nucleoside triphosphatase activity of poliovirus protein 2C reveals a cmechanism by which guanidine inhibits poliovirus replication.*Journal of Biological Chemistry,*274:6992-7001.

Pfister, T., Jones, K. W.and Wimmer, E. (2000). A cysteine-rich motif in poliovirus protein 2C (ATPase) is involved in RNA replication and binds zinc in vitro. *Journal of Virology,* 74:334-343.

Phologaine, B.S., Dwarka, R.M., Haydon, D.T., Gerber, I.J. and Vosloo, W. (2008). Molecular characterization of SAT 2 foot and mouth disease virus isolate obtained from cattle during a four-month period in 2001 in Limpopo Province, South Africa. *Onderstepoort Journal of Veterinary Research,* 75: 267-277.

Piaba, G.A., Anderson, J., Paton, D.J., Soldan, A.W., Alexandersen, S., Corteyn, M.,Wilsden, G., Hamblin, P., Mackay, D.K. and Donaldson, A.I. (2004). Validation of a foot-and-mouth disease antibody screening solid-phase competitive ELISA (SPCE). *Journal of Virological Methods,* 115: 145-158.

Piccone, M.E., Rieder, E., Mason, P.W. and Grubman, M.J. (1995). The foot-and-mouth disease virus leader proteinase gene is not required for viral replication. *Journal of Virology,*69:5376-5382.

Piccone, M.E., Lopez, J.W., Blanco, C., Samuel, A.R., Ko nig, G., Viera, P.J., Sepulueda, L.M., Davies, P.R., Maradei, E., Palma, E.L., and Knowles, N.J., (2000). Genetic relationships between recent and historical isolates of foot-and-mouth disease viruses from South America. 200.*XIth Meeting of the European Study Group on the Molecular Biology of Picornaviruses, Baia delle Zagare, Mattinata. Italy, 25-31 May 2000. Abstract D15.*

Pilipenko, E.V., Maslova, S. V., Sinyakov, A.N.and Agol, V. I. (1992). Towards identification of cis-acting elements involved in the replication of enterovirus and rhinovirus RNAs: a proposal for the existence of tRNA-like terminal structures. *Nucleic Acids Research,*20:1739-1745.

Pilipenko, E.V., Pestova, T.V., Kolupaeva, V.G., Khitrina, E.V., Poperechnaya, A.A., Agol, V. I.and Hellen, C.U. (2000). A cell cycle-dependent protein serves as a template-specific translation initiation factor. *Genes Development,* 14:2028-2045.

Polatnick, J. (1980). Isolation of a foot-and-mouth disease polyuridylic acid polymerase and its inhibition by antibody.*Journal of Virology,*33:774-779.

Prempeh, H., Smith, R. and Muller, B. (2001). Foot and mouth disease: the human consequences, *British Medical Journal,* 322: 565-566.

Purdue University Cooperative Extension Service (PUCES) (2001). Frequently asked questions about foot and mouth disease.I-888-EXT-INFO.www.agcom.purdue.edu/AgCom/pubs/menu. (Accessed 10/11/2014).

Ray, D.K., Bhattacharyya, U.K., Chowdhury, B., Dasgupta, P.and Bhattacharyya, A.K. (1989). Studies on a severe outbreak of foot-and-mouth disease in regularly vaccinated cross-exotic dairy cattle in West Bengal (India). *Indian Journal of Animal Health,* 28:50-55.

Reid, S. M., Hutchings, G. H., Ferris, N. P. and De Clercq, K. (1999).Diagnosis of foot and-mouth disease by RT-PCR: evaluation of primers for serotypic characterization of viral RNA in clinical specimens. *Journal of Virological Methods,* 83: 113-123.

Reid, S. M., Ferris, N.P., Hutchings, G.H., De, Clercq, K., Newman, B.J., Knowles, N.J. and Samuel, A.R. (2001a). Diagnosis of foot-and-mouth disease by RT-PCR: use of phylogenetic data to evaluate primers for the typing of viral RNA in clinical samples.*Archives of Virology,* 146: 2421-2434.

Reid, S.M., Ferris, N.P., Bruning, A., Hutchings, G.H., Kowalska, Z. and Akerblom, L. (2001b). Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen.*Journal ofVirological Methods,* 96: 189-202.

Reid, S.M., Ferris, N.P., Hutchings, G.H., Zhang, Z., Belsham, G.J. and Alexandersen, S., (2002). Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay. *Journal ofVirological Methods,* 105: 67-80.

Reid, S.M., Grierson, S.S., Ferris, N.P., Hutchings, G.H. and Alexandersen, S. (2003).Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *Journal ofVirological Methods,* 107: 129-139.

Rieder, E., Bunch, T., Brown, F.and Mason, P.W. (1993). Genetically engineered foot-and-mouth disease viruses with poly(C) tracts of two nucleotides are virulent in mice. *Journal of Virology,* 67:5139-5145.

Rieder, E., Berinstein, A., Baxt, B., Kang, A.and Mason, P.W. (1996). Propagation of an attenuated virus by design: engineering a novel receptor for a noninfectious foot-and-mouth disease virus. *Proceedings of the Annual Meeting of the United States Animal Health Association,*93:10428-10433.

Rigden, R.C., Carrasco, C.P., Summerfield, A. and McCullough, K.C. (2002). Macrophage phagocytosis of foot and mouth disease virus may create infectious carriers. *Immunology,* 106: 537-548.

Rigden, R.C., Carrasco, C.P., Barnett, P.V., Summerfield, A.and McCullough, K.C. (2003).Innate immune responses following emergency vaccination against foor-and-mouth disease virus in pigs.*Vaccine,*21:1466-1477.

Robertson, B.H., Morgan, D.O., Moore,D.M., Grubman, M.J., Card, J., Fischer,T., Weddell, G., Dowbenko,D.and Yansura, D. (1983). Identification of amino acid and nucleotide sequence of the foot-and-mouth disease virus RNA polymerase.*Virology,*126:614-623.

Robertson, B.H., Grubman, M.J., Weddell, G.N., Moore, D.M., Welsh, J.D., Fischer, T., Dowbenko, D.J., Yansura, D.G. Small, B.and Kleid, D.G. (1985). Nucleotide and amino

acid sequence coding for polypeptides of foot-and-mouth disease virus type A12. *Journal ofVirology,* 54:651-660.

Rodriguez, A., Martinez-Salas, R., Dopazo, J., Davila, M., Saiz, J.C. and Sobrino, F. (1992).Primer design for specific diagnosis by PCR of highly variable RNA viruses typing of foot-and-mouth disease virus.*Virology,* 189:363-367.

Rodriguez,L.L., Barrera, J., Kramer, E., Lubroth, J., Brown, F. and Golde, W.T. (2003). A synthetic peptide containing the consensus sequence of the G-H loop region of foot and mouth disease virus type O VP1 and a promiscuous T-helper epitope induces peptide-specific antibodies but fails to protect cattle against viral challenge. *Vaccine,* 21: 3751-3756.

Roeder, P.L. and Le Blanc Smith, P.M. (1987). Detection and typing of foot-and-mouth disease virus by enzyme linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. *Research in Veterinary Science,* 43: 225-232.

Roehl, H.H., and Semier, B.L. (1995). Poliovirus infection enhances the formation of two ribonucleoprotein complexes at the 3_end of viral negative-strand RNA. *Journal of Virology,* 69:2954-2961.

Rohll, J.B., Moon, D.H., Evans, D.J.and Almond, J.W. (1995). The 3_untranslated region of picornavirus RNA: features required for efficient genome replication. *Journal of Virology,* 69:7835-7844.

Rueckert, R.R. (1985). Picornaviruses and their replication.*In Virology (Eds Fields, B.N., D.M. Knipe, and P.H. Howley) Raven Press, New York, P.* 705.

Rueckert, R.R. (1996). Picornaviruses: the viruses and their replication. *In Fields, B.N. D.M. Knipe, and P.H. Howley (eds),Fields Virology, 3rd ed. Lippincott- Raven, Philadelphia, Pa. Pp.* 609-654.

Rueckert, R.R., and Wimmer, E. (1984).Systematic nomenclature of picornavirus proteins.*Journal of Virology,* 50: 957-959.

Rushton, J. (2009). The economics of animal health production.*CABI Publishing,Wallingford, UK. P.* 364.

Russell, W. C. (2000). Update on adenovirus and its vectors. *Journal of General Virology,* 81:2573-2604.

Rweyemamu, M., Roeder, P., Mackay, D., Sumption, K., Brownlie, J., Leforban, Y., Valarcher, J.F., Knowles, N.J. and Saraiva, V. (2008). Epidemiological patterns of foot-and-mouth disease worldwide. *Transboundary and Emerging Diseases,* 55:57-72.

Ryan, J. (2001). Availability of vaccines for emergency vaccination in Europe.*In Report of the 34th Session of the European Commission for the control of Foot-and-Mouth Disease (EUFMD), 21-23 March, Rome. EUFMD, Rome, Appendix 12,* 117-127.

Ryan, M.D., King, A.M.and Thomas, G.P. (1991). Cleavage of foot-and-mouth disease virus polyprotein is mediated by residues located within a 19 amino acid sequence. *Journal of General Virology,* 72:2727-2732.

Saiz, J.C., and Domingo, E. (1996).Virulence as a positive trait in viral persistence.*Journal of Virology,* 70:6410-6413.

Saiz, J.C., Rodriguez, A., Gonzalez, M., Alonso, F.and Sobrino, F. (1992). Hererotypic lymphoproliferative response in pigs vaccinated with foot-and-mouth disease virus. Involvement of isolated capsid proteins.*Journal of General Virology,* 73:2601-2607.

Sakamoto, K., Kanno, T., Yamakawa, M., Yoshida, K., Yamazoe, R. and Murakami, Y. (2000).Isolation of foot-and-mouth disease virus from Japanese black cattle in Miyazaki Prefecture, Japan, 2000.*Journal of Veterinary Medical Science,* 64:91-94.

Sakamoto, K. and Yoshida, K. (2002).Recent outbreaks of foot and mouth disease in countries of East Asia.*Review Science and Technical office International des Epizooties.* 21(3): 459-463.

Salt, J.S. (1993).The carrier state in foot and mouth disease - an immunological review.*British Veterinary Journal,* 149:207-223.

Salt, J.S., Mulcahy, G.and Kitching, R.P. (1996a).Isotype-specific antibody responses to foot-and-mouth disease virus in sera and secretions of "carrier" and "non-carrier" cattle.*Epidemiology and Infection,* 117:349-360.

Salt, J.S., Samuel, A.R. and Kitching, R.P. (1996b).Antigenic analysis of type O foot-and-mouth disease virus in the persistently infected bovine.*Archives of Virology,* 141:1407-1421.

Samuel, A.R. and Knowles, N.J. (1988). Serological and biochemical study of an isolate of foot-and-mouth disease virus type A from Bavaria in 1984. *Review Science and Technical office International des Epizooties,* 7:365-373.

Samuel, A.R., Knowles, N.J., Kitching, R.P. and Hafez, S.M. (1997).Molecular Analysis of foot-and-mouth disease type O viruses isolated in Saudi Arabia between 1983 and 1995.*Epidemiology and Infection,* 119:389.

Samuel, A.R., and Knowles, N.J. (2001a). Foot-and-mouth disease virus: cause of the recent crisis for the UK livestock industry. *Trends in Genetics,* 17:421-424.

Samuel, A.R. and Knowles, N.J. (2001b). Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). *Journal of General Virology.* 82: 609-621.

Sangar, D.V., Rowlands, D.J., Harris, T.J.and Brown, F. (1977). Protein covalently linked to foot-and-mouth disease virus RNA. *Nature,* 268:648-650.

Sangar, D.V., Newton,S.E., Rowlands, D.J.and Clarke, B.E. (1987). All foot and mouth disease virus serotypes initiate protein synthesis at two separate AUGs. *Nucleic Acids Research,* 15:3305-3315.

Sangare, O., Bastos, A.D.S., Marquardt, O., Venter, E.H., Vosloo, W. and Thomson, G.R. (2001).Molecular epidemiology of serotype O foot-and-mouth disease virus with emphasis on West and South Africa.*Virus Genes,* 22 (3): 345-351.

Sangare, O., Bastos A.D.S., Venter, E.H. and Vosloo, W. (2003).Retrospective genetic analysis of SAT 1 type foot-and-mouth disease outbreaks in West Africa 1975-1981.*Veterinary Microbiology,* 93: 279-289.

Sangare, O., Bastos, A.D.S., Venter, E.H. and Vosloo, W. (2004). A first molecular epidemiology study of SAT 2 type foot-and-moth disease viruses in West Africa. *Epidemiology and Infections,* 132:525-532.

Sanson, R.L. (1994). The epidemiology of foot-and-mouth disease: Implications for New Zealand. *New Zealand Veterinary Journal,* 42: 41-53.

Santodonato, L., Ferrantini, M., Palombo, F.,Aurisicchio, L., Delmastro, P.,La Monica, N.,Di Marco, S., Ciliberto, G.M., Du, X., Taylor, M.W.and Belardelli, F. (2001). Antitumor activity of recombinant adenoviral vectors expressing murine IFN-alpha in mice injected with metastatic IFN-resistant tumor cells. *Cancer Gene Therapy,* 8:63-72.

Sard D. M. (1978): Clinical aspects of foot and mouth disease. *Veterinary Record,*102: 186 –187.

Scherbakov, A., Timina, A. and Borisov, V. (2008). Phylogenetic study of FMDV isolates collected in Russia, CIS-countries and Mongolia in 2000-2007. *In the global control of FMD-tools, ideas and ideals open session of the Research Group of the Standing Technical Committee on FMD, Erice, Italy: EuFMD/FAO, Rome, Italy: 2008.*

Schneider-Schaulies, J. (2000). Cellular receptors for viruses: links to tropism and pathogenesis. *Journal of General Virology,* 81:1413-1429.

Scott, F.W., Cottral, G.E. and Gailiunas, P. (1966).Persistence of foot-and-mouth disease virus in external lesions and saliva of experimentally infected cattle.*American Journal of Veterinary Research,* 27: 1531-1536.

Segarra, A.E. and Rawson, J.M. (2001). Foot-and-mouth Disease: A Threat to U.S. Agriculture, *In CRS Report for Congress. USA: Congressional Research Service. The Library of Congress:* 6.

Sekiguchi, K., Franke, A.J.and Baxt, B. (1982). Competition for cellular receptor sites among selected aphthoviruses. *Archives of Virology,* 74:53-64.

Sellers, R.F. and Parker, J. (1969).Airborne excretion of foot-and-mouth disease virus.*Journal of Hygiene (London),* 67: 671-677.

Sellers, R.F., Burrows, R., Garland, A.J., Greig, A. and Parker, J. (1969).Exposure of vaccinated bulls and steers to airborne infection with foot-and-mouth disease.*Veterinary Record,* 85: 198-199.

Sellers, R.F. (1971).Quantitative aspects of the spread of foot and mouth disease.*Veterinary Bulletin,* 41: 431-439.

Sellers, R.F. and Forman, A.J. (1973).The Hampshire epidemic of foot-and-mouth disease, 1967. *Journal of Hygiene (London),* 71: 15-34.

Shao, H.J., Chen, L. and Su, Y.B. (2005). DNA fragment encoding human IL-1beta 163-171 peptide enhances the immune responses elicited in mice by DNA vaccine against foot-and-mouth disease. *Veterinary Research Communication,* 29: 35-46.

Shin, J.H., Sohn, H.J., Choi, K.S., Kwon, B.J., Choi, C.U., Kim, J.H., Hwang, E.K., Park, J.H., Kim, J.Y., Choi, S.H. and Kim, O.K. (2003). Identification and isolation of foot-and-mouth disease virus from primary suspect cases in Korea in 2000. *Journal of Veterinary Medical Science,* 65: 1-7.

Skern, T., Fita, I.and Guarne, A. (1998). A structural model of picornavirus leader proteinases based on papain and bleomycin hydrolase. *Journal of General Virology,* 79:301-307.

Smitsaart, E., Espinoza, A.M., Sanguinetti, R., Filippi, J., Ham, A. and Bellinzoni, R. (2004). Addition of saponin to double oil emulsion FMD vaccines enhances specific antibody responses in cattle and pigs. *Report of the Session of the FAO Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Crete, Greece, 12-15 October, pp.* 344-351.

Snowdon, W.A. (1966). Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. *Nature,* 210: 1079-1080.

Sobrino, F., Davila, M., Ortin, J.and Domingo, E. (1983). Multiple genetic variants arise in the course of replication of foot-and-mouth disease virus in cell culture. *Virology,* 128:310-318.

Sobrino, F., Saiz, M., Jimenez-Clavero, M.A., Nunez, J.I., Rosas, M.F., Baranowski, E. and Ley, V. (2001). Foot-and-mouth disease virus: a long known virus, but a current threat. *Veterinary Research,* 32:1-30.

Sorensen, K.J., Madsen, K.G., Madsen, E.S., Salt, J.S., Nquindi, J. & Mackay, D.K.J. (1998a). Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Archives of Virology,* 143:1461-1476.

Sorensen, K.J., Hansen, C.M., Madsen, E.S. and Madsen, K.G. (1998b). Blocking ELISAs using the FMDV nonstructural proteins 3D, 3AB, and 3ABC produced in the baculovirus expression system. *Veterinary Quarterly,* 20:S17-S20.

Sorensen, J.H., Mackay, D.K., Jensen, C.O.and Donaldson, A.I. (2000).An integrated model to predict the atmospheric spread of foot-and-mouth disease virus.*Epidemiology and Infection,* 124:577-590.

Sorensen, J., Jensen, C.O., Mikkelsen, T., Mackay, D.K. and Donaldson, A.I. (2001).Modelling the atmospheric dispersion of foot-and-mouth disease virus for emergency preparedness. *Physics Chemistry Earth,* 26: 93-97.

Strohmaier, K., Franze, R.and Adam, K.H. (1982). Location and characterization of the antigenic portion of the FMDV immunizing protein.*General Virology,* 59: 295-306.

Summerfield, A., Guzylack-Piriou, L., Saurer, L. and McCullough, K.C. (2004). Novel immunological approaches for emergency FMD vaccines inducing mucosal immunity. *Report of the Session of the FAO Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Crete, Greece, 12-15 October, p.* 358.

Sumption, K.J., Pinto, J., Lubroth, J., Morzaria, S., Murray, T. and Rocque, S. (2007). Foot and mouth disease: situation worldwide and major epidemiological events in 2005-2006. EMPRES Focus On Bulletin 1.

Sumption, K., Rweyemamu, M. and Wint, W. (2008). Incidence and distribution of foot and mouth disease in Asia, Africa and South America; combining expert opinion, official disease information and livestock populations to assist risk assessment. *Transboundary and Emerging Disease,* 55: 5-13.

Sumption, K., Domenech, J. and Ferrari, G. (2012).Progressive control of FMD on a global scale.*Veterinary Record,* 170: 637-639.

Sun, M., Qian, K., Su, N., Chang, H., Liu, J. and Shen, G. (2003). Foot-and-mouth disease virus VP1 protein fused with cholera toxin B subunit expressed in *Chlamydomonas reinhardtii* chloroplast. *Biotechnology Letters,* 25: 1087-1092.

Sutmoller, P., McVicar, J.W.and Cottral, G.E. (1968).The epizootiological importance of foot-and-mouth disease carriers. I. Experimentally produced foot-and-mouth disease carriers in susceptible and immune cattle. *Archiv fu¨r die Gesamte Virusforschung,*23:227-235.

Sutmoller, P. and McVicar, J.W. (1973). Foot-and-mouth disease: growth of virus after conjunctival inoculation of cattle. Brief report.*Archiv fu¨r die Gesamte Virusforschung,*43: 284-287.

Sutmoller, P., and McVicar, J.W. (1976). Pathogenesis of foot-and-Mouth disease: the lung as an additional portal of entry of the virus. *Journal of Hygiene (London),* 77:235-243.

Sutmoller, P. and Casas Olascoaga, R. (2002). Unapparent foot and mouth disease infection (Sub-clinical infections and carriers): Implication for control. *Review Science and Technical office International des Epizooties,* 21(3): 519-529.

Sutmoller, P., Barteling, S.S., Olascoaga, R.C. and Sumption.K.J. (2003).Control and eradication of foot-and-mouth disease.*Virus research,* 91 (1), 101-104.

Swofford, D.L.(1998). PAUP*.Phylogenetic analysis using parsimony, version 4. Sunderland, MA: Sinauer Associates.

Tam, S., Clavijo, A., Engelhard, E. K. and Thurmond, M.C. (2009). Fluorescence-based Multiplex realtime RT-PCR arrays for the detection and serotype determination of foot-and-mouth disease virus. *Journal ofVirological Methods,* 161:183-191.

Taylor, M.N. and Tufan, M. (1996).Detailed investigations, using farmer interviews, to assess the losses caused by FMD outbreaks in Turkey.*Report of Turkish-German Animal health Information Project (GTZ), Ministry of Agriculture and Rural Affairs (MARA), Republic of Turkey.*

Tekleghiorghis, T., Moormann, R.J.M., Weerdmeester, K. and Dekker, A. (2014). Serological eveidence indicates that foot and mouth disease virus serotype O, C and SAT1 are most dominant in Eritrea. *Transboundary and Emerging Diseases,* 61: e83-e88.

Thevasagayam, S.J. (1996). Molecular and serological characterization of Southern African Territory (SAT) serotypes of foot-and-mouth disease virus (FMDV). *Ph.D. thesis, Univeristy of Hertfordshire, UK, P.*160.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmogin, F.and D.G. Higgins, (1997). The CLUSTER_X windows interface flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids Research*, 24, 4876-4882

Thompson, D., Muriel, P., Russell, D., Osborne, P., Bromley, A., Rowland, M., Creigh- Tyte, S. and Brown, C. (2002). Economic costs of the foot-and-mouth disease outbreak in the United Kingdom in 2001. *Review Science and technical Office International des Epizooties,* 21: 675-697.

Thomson, G.R., Vosloo, W., Esterhuysen, J.J. and Bengis, R.G. (1992). Maintenance of foot and mouth disease viruses in buffalo (*Syncerus caffer,* Sparrman, 1779) in Southern Africa.*Review Science and technical Office International des Epizooties,* 11: 1079-1107.

Thomson, G.R. (1994). Foot-and-mouth disease.*In Infectious diseases of livestock with special reference to Southern African.1st ed.Vol 2 (eds. Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C.).Oxford University Press, Oxford, England Pp.* 823-852.

Thomson, G.R. (1995). Overview of foot and mouth disease in Southern Africa.*Review Science and Technical office International des Epizooties,* 14: 503-520.

Thomson, G.R. (1997). The role of carrier animals in the transmission of food and mouth disease.*In comprehensive Report on Technical Items presented to the International Committee or to Regional Commissions, 1996. Office International des Episzooties, Paris,* 87-103.

Thomson, G.,R., Bengis, R.G., and Brown, C. (2001). Picornaviruses.*In infectious disease of wild animals, 3rd ED. (Williams E.S. and Barker, I.K, eds) Iowa University Press. Ames*, 119-130.

Thomson, G.R., Vosloo, W. and Bastos, A.D. (2003).Foot and Mouth disease in Wildlife.*Virus Research*, 91: 145-161.

Toja, M., Escarmis, C.and Doming, E. (1999).Genomic nucleotide sequence of a foot-and-mouth disease virus clone and its persistent derivatives.Implications for the evolution of viral quasispecies during a persistent infection.*Virus Research*, 64:161-171.

Toma, B., Dufour, B., Sanan, M., Benet, J. J., Moutou, F., Lauza, A. and Ellis, P. (1999).Sampling methods. In Applied veterinary epidemiology and the control of diseases in populations. (Alexandra, S. translation) Jouve, Paris, pp 103-108

Tosh, C., Sanyal, A., Hemadri, D. and Venkataramanan, R. (2002a). Phylogenetic analysis of serotype A foot-and-mouth disease virus isolated in India between 1977 and 2000. *Archives of Virology*. 147:193-513.

Tosh, C., Hemadri, D.and Sanyal, A. (2002b). Evidence of recombination in the capsid coding region of type A foot-and-mouth disease virus. *Journal of General Virology*, 83:2455-2460.

Tulasiram, P., Tyagi, M., Srinivas, K., Prabhudas, K., Natarajan, C. and Suryanarayana, V. (1997). Antigenic variation in foot and mouth disease virus type Asia 1 isolates circulated during 1993-1995 in India. *Virus Genes*, 15: 247-253.

United State Department of Agriculture (USDA) (2008).Fact sheet on food and mouth disease.www.aphis.usda.gov/oa/fmd. Accessed 10/11/2014.

Vakharia, V.N., Devaney, M.A., Moore, D.M., Dunn, J.J.and Grubman, M.J. (1987). Preteolytic Processing of foot-and-mouth disease virus polyproteins expressed in a cell-free system from clone-derived transcripts. *Journal of Virology*, 61:3199-3207.

Valdazo-Gonzalez, B., Knowles, N.J., Wadsworth, J., King, D.P., Hammond, J.M., Ozyoruk, F., Firat-Sarac, M., Parlak, U., Polyhronova, L. and Georgiev, G.K. (2011).Foot-and-mouth disease in Bulgaria.*Veterinary Record*, 168:247.

Valdazo-Gonzalez, B., Knowles, N.J., Hammond, J. and King, D.P. (2012). Genome sequences of SAT 2 foot-and-mouth disease viruses from Egypt and Palestinian Autonomous Territories (Gaza Strip). *Journal of Virology*, 86: 8901-8902.

Valdazo-Gonzalez, B., Poligronova, L., Alexandrov, T., Normann, P., Knowles, N., Hammond, J., Georgiev, G., Ozyoruk, F., Sumption, K., Belsham, G.J. and King, D.P. (2012). Reconstruction of the transmission history of RNA virus outbreaks using full genome sequences: foot-and-mouth disease virus in Bulgaria in 2011. *PLoS One*, 7: e4950.

Vallee, H. and Carre, H. (1922).Sur la pluralite du virus aphteux.*Comptes Rendus Hebdomadaires des Seances de l' Academie des Sciences*,174: 1498-1500.

Van Bekkum, J.G., Frenkel, H.S., Frederiks, H.J. and Frenkel, S. (1959). Observation on the carrier state of cattle exposed to foot and mouth disease virus.*Tijdschrift voor Diergeneeskunde*,84: 1159-1164.

Vangrysperre, W. and De Clercq, K. (1996). Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. *Archives of Virology*,141: 331-344.

Van Rensburg, H. G. and Mason, P.W. (2002). Construction and evaluation of a recombinant foot-and-mouth disease virus: implications for inactivated vaccine production. *Annals of the New York Academy of Sciences*, 969: 83-87.

Vilcek, J. and Sen, G.C. (1996).Interferons and other cytokines. *In Fields, B.N., Knipe, D.M. and Howley, P.H. (eds). Fields virology.3rd ed. Lippincott-Ravens, Philadephia, Pa. pp.* 375-399.

Vosloo, W., Knowles, N.J. and Thomson, G.R. (1992). Genetic relationships between Southern African SAT 2 isolates of foot-and-mouth disease virus. *Epidemiology and Infection*, 109: 547-558.

Vosloo, W., Kirkbride, E., Bengis, R.G., Keet, D.F. and Thomson, G.R. (1995). Genome variation in the SAT types of foot-and-mouth disease viruses prevalent in buffalo (*Syncerus caffer*) in the Kruger National Park and other regions of Southern Africa, 1986-1993. *Epidemiology and Infection*, 114: 203-218.

Vosloo, W., Bastos, A.D.S., Kirkbride, E., Esterhuysen, J.J., Janse Van Renburg, D., Bengis, R.G., Keet, D.F., and Thomson, G.R. (1996).Persistent infection of African buffalo (*Syncerus caffer)* with SAT type foot-and-mouth disease viruses; rate of fixation of mutations, antigenic change and interspecies transmission.*Journal of General Virology*, 77 (7): 1457-1467.

Vosloo, W., Bastos, A.D.S., Michel, A. and Thomson, G.R. (2001).Tracing movement of African buffalo in Southern Africa.*Review Science and Technical Office International des Epizooties*, 20: 630-639.

Vosloo, W., Bastos, A.D.S., Sangare, O., Hargreaves, S.K. and Thomson, G.R. (2002). Review of the status and control of foot and mouth disease in Sub-Saharan Africa. *Review Science and Technical Office International des Epizooties*, 21(3): 437-449.

Vosloo, W. and Thomson, G.R. (2004). Natural habitats in which foot and mouth disease viruses are maintained. *In: Domingo, E. and Sobrino, F. (eds). Foot and mouth disease: Current perspectives,CRC press, London. pp.* 383-410.

Vosloo, W., Bastos, A.D.S., Sahle, M. Sangare, O. and Dwarka, R.M. (2005). Virus topotypes and the role of wildlife in foot and mouth disease in Africa. *In: Osofsky, S.A. (ed.), Conservation and Development Interventions at the Wildlife/Livestock Interface: Implication for Wildlife, Livestock and Human Health, Chapter 10, IUCN Publications Services Unit, Cambridge, UK. pp.* 67-73.

Vosloo, W., de Klerk, L.M., Boshoff, C.J., Botha, B., Dwarka, R.M., Keet, D. and Haydon, D.T. (2007). Characterization of a SAT 1 outbreak of foot and mouth disease in captive African buffalo (*Syncerus caffer*): Clinical symptoms, genetic characterization and phylogenetic comparison of outbreak isolates. *Veterinary Microbiology*, 120: 226-240.

Waldmann, O. and Trautwein, K. (1926). Experimentalle untersuchungen urber die pluralitet desmaul-und klauenseuche virus. *Berliner und Munchener Tierarztliche Wochenschrift*, 42: 569-571.

Waldmann, D., Kobe, K.and Pyl, G. (1937). Die active Immunisierung des Rindes gegen Maul-und Klaueseuche Mittels Formolimpfstoff. *Zentrabatt fur Bakteriollgie, Parasitenkunde, Infektionskhrankheiten und Hygiene,*138:401-412.

Wang, C.Y., Chang, T.Y., Walfield, A.M., Ye, J., Shen, M., Chen, S.P., Li, M.C., Lin, Y.L., Jong, M.H., Yang, P.C., Chyr, N., Kramer, E. and Brown, F. (2002).Effective synthetic peptide vaccine for foot-and-mouth disease in swine.*Vaccine,* 20: 2603-2610.

Wang, J. H., Liang, C.M., Peng, J.M., Shieh, J.J., Jong, M.H., Lin, Y.L., Sieber, M. and Liang, S.M. (2003).Induction of immunity in swine by purified recombinant VP1 of foot-and-mouth disease virus.*Vaccine,* 21: 3721-3729.

Ward, G., Rieder, E.and Mason, P.W. (1997). Plasmid DNA encoding replicating foot-and-mouth disease virus genomes induces antiviral immune responses in Swine. *Journal of Virology*, 71:7442-7447.

Wernery, U. and Kaaden, O.R. (2004). Foot-and-mouth disease in camelids: a review. *Veterinary Journal*, 168: 134-142.

Wigdorovitz, A., Perez Filgueira, D.M., Robertson, N., Carrillo, C., Sadir, A.M., Morris, T.J.and Borca, M.V. (1999). Protection of mice against challenge with foot and mouth disease virus (FMDV) by immunization with foliar extracts from plants infected with recombinant tobacco mosaic virus expressing the FMDV structural protein VP1. *Virology* 264:85-91.

Wilson, V., Taylor, P.and Desselberger, U. (1988). Crossover regions in foot-and-mouth disease virus (FMDV) recombinants correspond to regions of high local secondary structure. *Archives of Virology,* 102:131-139.

Wimmer, E., Hellen, C.U.and Cao, X. (1993).Genetics of poliovirus.*Annual Review of Genetics*, 27:353-436.

Wong, H.T., Cheng, S.C., Chan, E.W., Sheng, Z.T.,Yan, W.Y., Zheng, Z.X., and Xie, Y. (2000). Plasmids encoding foot-and-mouth disease virus VP1 epitopes elicited immune responses in mice and swine and protected swine against viral infection. *Virology,*278:27-35.

Wong, H.T., Cheng, S.C., Sin, F.W., Chan, E.W., Sheng, Z.T.and Xie, Y. (2002). A DNA vaccine against foot-and-mouth disease elicits an immune response in swine which is enhanced by co-administration with interleukin-2. *Vaccine, 20:2641*-2647.

WRLFMD (2014). Molecular epidemiology/genotyping, OIE/FAO FMD reference laboratory network reports. www.wrlfmd.org/fmd_genotyping/index.html.Accessed 22/12/14.

Wu, Q., Brum, M.C.S., Caron, L., Koster, M.and Grubman, M.J. (2003). Adenovirus-mediated type 1 interferon expression delays and reduces disease signs in cattle challenged with food-and-mouth disease virus. *Journal of Interferon and Cytokine Research,*232:359-368.

Yadin, H. and Cloudia, K. (1995).Small ruminants as FMD virus carriers.*Report of the session of the research group of the Standing Technical Committee on Control of Food and mouth Disease; Vladimir, Russia: Rome: FAO. Pp.*26-32.

Yoon, S.H., Lee, K.N., Park, J.H. and Kim, H. (2011). Molecular epidemiology of foot-and-mouth disease virus serotypes A and O with emphasis on Korean isolates: temporal and spatial dynamics. *Archives of Virology,* 156: 817-826.

Zhang, Z. and Kitching, P. (2000).A sensitive method for the detection of foot and-mouth disease virus by in situ hybridization using biotin-labelled oligodeoxynucleotides and tyramide signal amplification.*Journal of Virological Methods,* 88: 187-192.

Zhang, Z.D., and Kitching, R.P. (2001).The localization of persistent foot and mouth disease virus in the epithelial cells of the soft palate and pharynx.*Journal of Camparative Pathology,* 124:89-94.

Zhang, Z.D., Hutching, G., Kitching, P.and Alexandersen, S. (2002). The effects of gamma interferon on replication of foot-and-mouth disease virus in persistently infected bovine cells. *Archives of Virology,* 147:2157-2167.

Zhang, Z., Bashiruddin, J. B., Doel, C., Horsington, J., Durand, S. and Alexandersen, S. (2006).Cytokine and Toll-like receptor mRNAs in the nasal-associated lymphoid tissues of cattle during foot-and-mouth disease virus infection.*Journal of Comparative Pathology,* 134: 56-62.

APPENDICES

Appendix I: Questionnaire

Form Number…….
Local Government……..
Village…….
Full address……

Herd information	Product system	Movement	Grazing
Species – Herd Size- Breed – Age-	Dairy – Meat- Dual -	Settled – Nomadic- Trade -	Common – Enclosed – Zero -

FMD … Outb

No of affected/ deaths –
Date of last FMD Vaccination –
Source / Types of Vaccine Used –

* Has there been FMD Suspicion near his location in the last 2 years. Yes……. No……
  If yes, Where …….
* Does the farmer consider FMD an important problem?
  - In the past –
  - At present –
  - In future –
* When and where herds went on transhumance …….
* Any contact between herds during grazing Yes…..... No……
  If Yes,
  How many contacts in a day ……..

*Any contact with wildlife when in transhumance or at pasture? Yes……., No…….
If Yes, Which types of wildlife? Buffalo……., Antelope……, Others……

* Where were animals kept at night and are there contact between herds at night
* Any Purchases made from the markets Yes…, No …….
  If Yes, when?
  Last month ……, Last year……, others……

* Will you buy cattle from the market if you know there is FMD in the area?
  Yes……., No ……
* Will you sell your animals to the market if you know it is suffering from FMD?
  Yes……., No …….
* Other livestock kept and whether they were mixed with the cattle
  ……..
* Where herds were watered and the number of contacts with other herds at watering points.

* Do you lend your bull to your neighbor Yes…, No …….
* Do you borrow bull from your neighbor Yes …, No ……
* What should be done to control FMD?
  a. I don't know
  b. Quarantine herd with clinical cases
  c. Use of drugs /vaccines
  d. Use of infected tissues to infect animals
  e. Let there be control on livestock movements
* What do you do when your animals contact FMD?
  a. Report to a veterinarian
  b. Separate sick animals from the rest of the herd
  c. Treat animalswith antibiotics
  d. Treat with traditional medicine
  e. Treat with both antibiotic and traditional medicine.

Appendix II: Spearman-Karber Calculation

Karber Formula for the estimation of virus titre for *Calculation* of the serum titre that *neutralizes 100TCID50* of each virus

The quantity of virus in a specified suspension volume (e.g., 0.5ml) that will infect 50% of a number (n) of cell culture microplate wells is termed the Tissue Culture Infectious Dose 50 (TCID50).

In a test to measure the 50% endpoint dilution

Virus dilution proportion (p) of infected cultures:

10^{-1} 12/12 = 1
10^{-2} 12/12 = 1
10^{-3} 12/12 = 1
10^{-4} 12/12 = 1
10^{-5} 9/12 = 0.75
10^{-6} 4.5/12= 0.375
10^{-7} 0/12 = 0

The TCID50 is given by the simple equation: 100 TCID50 = X^a + (Sp/n + 0.5).

X^a = the last dilution index for which all n cultures are infected (p = 1)

Sp = summation of p between the last dilution for which all n cultures are infected (p = 1) and the first dilution for which all n cultures are uninfected (P - 0)

n = number of cell culture microplate per row (12)

Therefore:

100 TCID50 = 10^{-4} + (13.5/12 + 0.5)
= $10^{-5.625}$
100 TCID50 = $10^{-3.625}$