STUDIES OF BOVINE FOOT AND MOUTH DISEASE IN KWARA STATE-NIGERIA

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DECLARATION

I declare that the work in this dissertation entitled **"Studies of Bovine Foot and Mouth Disease in Kwara state, Nigeria"** was conducted by me in the Department of Veterinary Microbiology, Faculty of Veterinary Medicine under the supervision of Prof. H.M Kazeem (FCVSN), Dr. M.A. Raji and Prof. N.D.G. Ibrahim.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

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Name of student Signature Date

CERTIFICATION

This dissertation entitled "STUDIES OF BOVINE FOOT AND MOUTH DISEASE IN KWARA STATE, NIGERIA" by Olatunde Hamza OLABODE meets the regulations governing the award of the degree of Doctor of Philosophy in Veterinary Microbiology of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to God Almighty, my dad and mum Mallam H. O. Olabode and Mrs. S. A. Olabode, respectively, and my lovely kids Oladayo, Oladimeji, Folake, and Bolanle.

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ABSTRACT

Studies of Bovine Foot and Mouth Disease in Kwara state-Nigeria were conducted to establish Foot and Mouth Disease Virus (FMDV) serotypes, occurrence, dynamics, associated hematological variations as well as knowledge, attitude and practice (KAP) of Fulani herdsmen. A purposive study was conducted in twelve (12) cattle herds from 15 outbreaks over a period of 4 months (December, 2010 – March, 2011) in Ilesha Baruba. In the second phase, serological survey of FMD antibodies was carried out on sampled cattle with unknown FMD vaccination history in cattle markets and slaughter slabs in five local government areas (Offa, Bode Sadu, Ilesha Baruba, Ilorin, Ajasse) over a period of 3 months (August-September-October, 2011). Outbreak blood, epithelial tissues, and sera samples obtained randomly were stored and processed in accordance with standard methods. FMD Participatory appraisal was conducted during sampling and all outbreak locations were geo-referenced in accordance with standard methods. Sera were subjected to NS-ELISA and SPC-ELISA tests, while epithelial tissues were subjected to RNA extraction, Real-Time Polymerase Chain Reaction (rRT-PCR) and sequencing of positive amplicons in accordance with standard protocols. Geo-spatial and participatory appraisal analysis was conducted on obtained data. The blood was subjected to standard hematological analysis. Out of 450 cattle screened 75.11% (338) were positive for FMD antibodies. Overall sero-positivity was highest amongst cows (78.4%), White Fulani (Bunaji) breed (85.3%), young cattle (85.2%) and Ilesha Baruba district (84.4%) which showed FMD association $p<0.05$ by Chi square analysis. FMD serotypes amongst trade cattle were A, O, SAT1, and SAT2. However, multiple serotype of O and A, A and SAT2 as well as O and SAT2 were also observed. Vaccination evidence was observed in some sero-positive trade cattle. NS-ELISA sero-diagnosis in outbreak herd representatives

showed FMD evidence in 71.11% (64) cattle with overall incidence of 19.8% amongst cows (75.5%), White Fulani (Bunaji) breed (73.2%), young cattle (74.4%). The occurrence rates of serotype O, A and multiple serotype of O and A and O and SAT 2 in FMD outbreaks were 83% (53), 7.8% (5), 4.7% (3) and 1.6% (1) respectively, while vaccine strains O and SAT 2 occurred at the rates of 1.6% (1) each. No FMD virus RNA extract was obtained from blood samples. The blood beta-actin ranged from Ct=30 to 34. Real-time RT-PCR assay showed six (6) positives (Ct values =15.28 to 26.42) and one negative. Direct product sequencing confirmed SAT2 serotype was closely related to SAT2 isolate of LIB/7/2003 sequence. RT-PCR product cloned in pGEM-T easy vector for sequencing confirmed serotype A isolate closely related with A/NIG/21/2009 isolate, though multiple NIG/2009 sequences were also identified. Obtained sequence isolates were named A/NIG/1/2011 and SAT2/NIG/3/2011. Geo-spatial distribution of FMD outbreaks and serotypes indicated inter-connectivity of towns, road network, rivers and forest as possible features that enhanced virus spread. Cattle outbreak locations in 500m-1km buffer zones further confirmed road network, rivers and clustered (NNI Ratio=0.095054) herds and outbreaks in Ilesha Baruba district as potential facilitators of virus spread. Appraisal responses were independent and statistically significant by chi square ($P<0.05$) except responses on source of water, season of occurrence and type of medication. Hematological picture observed outbreak sero-positive cattle showed anemia characterized by anisocytosis and poikilocytosis, leucopenia and leucocytosis with no association ($P>0.05$) with FMD. The disease serotype endemicity and susceptibility in all sex, ages and breeds of both unvaccinated and vaccinated trade cattle especially amongst white Fulani, young cows within Nigerian local markets and Fulani herds were highlighted. In conclusion, findings of this study confirmed FMD predicament amongst Fulani livestock pastoralists which

requires proactive intervention plan while hematological variations also provided preliminary information on some diagnostic parameters associated with natural FMD outbreak. Thus, established circulating FMDV serotypes responsible for this sero-positivity should be used to design control strategy to limit FMD economic losses in livestock industry. In addition, continuous geo-spatial surveillance and quarantine of cattle within herds and inter-state and national borders should be encouraged.

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ABBREVIATIONS

FMD	Foot and Mouth Disease
FMDV	Foot and Mouth disease Virus
Cre	*cis*-acting replicative element
ISH	in situ hybridization
QA/QC	Quality Assurance/Quality control system
CFT	Complement Fixation Test
DNA/RNA	Dexo-ribonucleic acid /Ribonucleic acid
NRL	National Reference Laboratories
BSE	Bovine spongiform encephalopathy
GMP	Good manufacturing practice
BEI	Binary ethylene-imine
NSP	Non structural proteins
SP	Structural proteins
VIA	Virus infection antigens
VIAA	Virus infection associated Ag assay
ORF	Open reading frame
CPE	Cytopathic effect
TAD	Trans-boundary animal disease
EUFMD	European Commission for the Control of Foot-and-Mouth Disease
SAT	Southern African Territories
RGD	Receptor-binding recognition sequence (Arg-Gly-Asp sequence)
PCBP	poly (rC) binding protein
IRES	internal ribosome entry site

EMCV	Encephalomyocarditis virus
PTBP	Polypyrimidine tract binding protein
EU	European Union
CBPP	Contagious Bovine Pleuro-pneumonia
$TCID_{50}$	Tissues culture infective dose
LAMP	Loop-mediated amplification
RT-LAMP	Reverse Transcription Loop-mediated Isothermal Amplification
EMEA	European Medicines Agency
OECD	Organization for Economic Cooperation and Development countries
GLEWS	Global Early Warning System
BSE	Bovine spongiform encephalopathy
RRA	Rapid rural appraisal
PRA	Participatory rural appraisal
PE	Participatory epidemiology
EVK	Existing veterinary knowledge
GIS	Geographic information system
SRTM	Shuttle radar topography mission
NASRDA	National Space Research Development Agency
NCAD	National Centre for Animal Diseases
NVRI	National Veterinary Research Institute
WRL	World Reference Laboratories
ELISA	Enzyme-linked immunosorbent assay (ELISA)
Ag-ELISA	Antigen Enzyme-linked immunosorbent assay (ELISA)
mAbs	Monoclonal antibodies

µl	Microliter
PI	Percentage of inhibition
OD	Optical density
Ct value	Crossing threshold that is the first PCR where significant increase in fluorescence above background level or above a defined threshold level.
FAM	6-carboxyfluorescein
TEST	6-Tetra chlorofluorescein
NFQ-MGB	non-fluorescent quencher minor groove binder
SPC	Synthetic positive control (for the described FMDV RRT-PCR)
PCR	Polymerase Chain Reaction
RT-PCR	Reverse transcription polymerase chain reaction
rRT-PCR	Real Time Polymerase Chain Reaction
X^2	chi square

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Foot and mouth disease (FMD), with synonyms as aphthous fever or infectious aphthous stomatitis, is a highly contagious viral disease of both domestic and wild cloven hoofed animals, characterized by vesicle formation in the buccal mucosa (tongue, lips cheeks gums, dental pads, palate), gastrointestinal tract (particularly the rumen) and skin between and above the claws of the feet, teats and udder (Kitching, 2002a). Although mortality is usually low, the disease decreases livestock productivity and affected countries are excluded from participation in international trade of animals and animal products (Domingo *et al*., 2002). In Africa, cattle and buffalo (*Syncerus caffer*) are usually the common hosts of FMD viruses. Other susceptible domesticated species include pigs, sheep, goats as well as species of cloven-hoofed wildlife such as deer, antelope and wild pigs (FAO, 1984).

The disease is caused by FMD virus (FMDV) belonging to the family *Picornaviridae* and consists of a single-stranded, positive-sense RNA genome of approximately 8,500 bases, which encodes a large polyprotein, that eventually cleaves into structural proteins and nonstructural proteins (Shao *et al*., 2010). There are four structural proteins which form an icosahedral capsid (Rueckert and Wimmer, 1984).

The virion is 140S particle consisting of 60 copies each of four structural proteins (VP1 [1D], VP2 [1B], VP3 [1C], and VP4 [1A]) encoded in the P1 and P2 regions of viral genome that encode three viral nonstructural (NS) proteins, 2A, 2B, and 2C, while the P3 region encodes NS proteins 3A, three copies of VPg, 3Cpro, and 3Dpol (Grubman and Baxt, 2004).

FMDV genome structural protein is highly variable however non-structural protein (3Dpol RNA polymerase) genomic region is highly conserved amongst different sero- and subtypes of FMDV (George *et al*., 2001). There are 7 (seven) distinct serotypes which include Type A, O, C (European types), South African types (SAT_1, SAT_2, SAT_3) and Asiatic Type. Within the serotypes, many strains have been identified by biochemical and immunological tests (Shao *et al*., 2010). Thus, a set of common primers against FMD virus RNA polymerase genes has been designed and used for rapid detection of all the seven serotypes (Shao *et al*., 2010).

Global epidemiological studies indicated FMD serotypes were not uniformly distributed in the regions of the world where the disease occurred (Rweyemamu *et al*, 2008a). The cumulative incidence of FMD serotypes showed that six of the seven serotypes (O, A, C, SAT-1, SAT-2, SAT-3) have occurred in Africa, while Asia still contends with four sero-types (O, A, C, Asia-1), and South America with only three (O, A, C). Periodically there have been incursions of SAT-1 and SAT-2 from Africa into the Middle East (Rweyemamu *et al*, 2008a).

The epidemiology of FMD on the African continent is influenced by two different patterns, which include a cycle that wildlife plays a role in maintaining and spread of the disease to other susceptible domestic animals and wild ungulates as well as the cycle maintained

within domestic animals independent of the wildlife (Vosloo *et al*., 2002). Available evidence indicated that although other domestic and wild species of animals may become infected, infections are maintained only a few months in absence of cattle or African buffalo, as wildlife outside Africa has not been shown to maintain FMD viruses (OIE, 2008). However, wild animals such as buffalos, antelopes and duikers have been incriminated in outbreaks in some parts of the world (Libeau, 1960).

Persistent inapparent infection is a common sequel to clinical and sub-clinical infections of susceptible and vaccinated ruminants by FMD Virus, hence, the so-called carrier state. A carrier of FMDV is defined as an animal from which virus can be recovered for more than 28 days post infection (Alexandersen *et al*., 2002; Kitching, 2002b). The duration of persistent FMDV infection can be upto $3^1/_2$ years in cattle (Sutmoller and Casasolascoaga, 2002) as this duration vary depending on species and individual animal (Sutmoller and Gaggero, 1965). Field evidence has implicated carrier cattle in number of outbreaks (Alexandersen *et al*., 2002).

FMD virus replicates at the site of entry, either in the mucosa and lymphoid tissue of upper respiratory tract or dermal and sub dermal tissue (Kitching, 1992). The virus enters the blood circulation as free virus or associated with mononuclear cells and is distributed around the body to glandular tissues and predilection sites in the stratum spinosum, where replication occurs. The stratum spinosum cells undergo ballooning degeneration and as the cells rupture and vesicular fluid accumulates, vesicles develop which coalesce to form the aphthae and bullae that characterize FMD (Kitching, 1992). These lesions may be seen in the squamous epithelium of the buccal mucosa, rumen, reticulum, and omasum.

In young animals, viruses invade myocardiac cells and macroscopic grey areas may be observed particularly in the left ventricular wall as stripes (tiger heart) and hyaline degeneration of skeletal muscle cells (Kitching, 1992). Moderate leucopenia charaterised by lymphopenia and diverse levels of monocytes were also observed in keratinized epithelium of tongue and foot in cattle and buffaloes infected with FMD virus (Asia 1) (Mohan *et al*., 2008).

FMD diagnostic methods include virus isolation, demonstration of FMD viral antigen or nucleic acid in tissue or vesicular fluid samples and detection of virus–specific antibody (OIE, 2008). The antibodies to viral nonstructural proteins (NSPs) can be used as indicators of infection, irrespective of vaccination status (Sorensen *et al.,* 1998). Complement fixation (CF) has been replaced in most laboratories by the enzyme-linked immunosorbent assay (ELISA), as it is more specific, sensitive and usually not affected by cattle serum anti-complement factors (Roeder and Le Blanc Smith (1987). When sample is inadequate or diagnosis remains uncertain, sample materials can be inoculated on to susceptible cell cultures or into 2 to 7-day old unweaned mice to amplify any live virus that may be present **(**Clarke and Spier, 1980). The cultures should preferably be of primary bovine (calf) thyroid, but pig, lamb or calf kidney cells, or cell lines of comparable sensitivity may be used. Once cytopathic effect (CPE) is completed in cultures, the fluids can be used in CF tests, ELISAs or in reverse transcription polymerase chain reaction (RT-PCR) (Scheibner *et al.,* 2000) and similar tests can be performed on homogenized suspensions of dissected musculo-skeletal tissues of any dead mice (OIE, 2008).

Nucleic acid recognition tests, such as reverse transcription-polymerase chain reaction (RT-PCR) are being used increasingly as rapid and sensitive diagnostic methods (Reid et al., 2001b). Real time reverse transcription-polymerase chain reaction (rRT-PCR) assays have been developed for FMDV detection in a variety of sample matrices (Moonen *et al*., 2003) and recognition of all the 7 FMDV serotypes (Shaw, *et al*., 2004). These assays characteristically possess high analytical sensitivity, specificity and capability to detect diverse FMDV strains (pan-reactivity) as an additional requirement for any routine FMD diagnostic assay compared with virus isolation (VI) and antigen–enzyme-linked immunosorbent assay (Ag-ELISA) combined (Reid *et al*., 2001c).

In some cases, this approach allowed identification of infected animals even before development of clinical signs or positive virus isolation as well as identification of positive cattle at the end of the course of infection when virus isolation may be negative (Callahan, *et al*., 2002). Electron microscopic examination of sample lesions could be used to differentiate FMD from disease caused by other viruses (OIE, 2008).

In serological tests, demonstration of specific antibodies to structural proteins in non-vaccinated animals with vesicular condition is sufficient for a positive diagnosis (Sorensen *et al.,* 1998). This is particularly useful in mild cases or where epithelial tissue cannot be collected (Hamblin *et al*., 1986a). Tests for antibodies to FMD virus NSPs are useful in providing evidence of previous or current viral replication in the host, irrespective of vaccination status (Sorensen *et al*., 2005). NSPs, unlike structural proteins, are highly conserved and therefore not serotype specific hence detection of these antibodies is not serotype restricted. Virus neutralisation (VN) tests and ELISAs for antibodies to structural proteins are used as serotype-specific serological tests (Hamblin *et al*., 1986b).

VN tests depend on tissue cultures and therefore more prone to variability than ELISAs as they are slower and subject to contamination (Golding *et al*., 1976). ELISAs for antibodies are faster and independent on cell cultures. The ELISA can be performed with inactivated antigens, requiring less restrictive biocontainment facilities. Hence, indirect sandwich ELISA test procedure is preferred for detection FMD viral antigen and viral serotype identification (Ferris and Dawson, 1988).

FMD is a single most devastating animal disease constraint to international animal products trade, which needs to be controlled to safe-guard livestock population in industrialized countries as well as the livelihood and income generation of developing countries where the disease continues to be endemic (Rweyemamu *et al.*, 2008b). The internationally accepted and advocated control methods include combination of animal movement restrictions and slaughter without vaccination "No vaccination policy" in outbreak herds (Grubman and Baxt, 2004). Quarantine and vaccination using inactivated vaccine is also being practiced (Barnett *et al*., 2008). Control efforts based on FMD risk reduction in the context of progressive market access of livestock commodities from developing countries was also advocated by Rweyemamu *et al.*, 2008b).

In Nigeria, the disease is enzootic causing annual outbreaks especially around December-March during stressful weather charaterised by little available grazing pasture, resulting in serious economic losses especially in cattle (Abdulkadir, 1989). The first reported and typed outbreak was in early 1950 amongst herds in the North-Eastern Nigeria (Nawathe and Goni, 1976) with subsequent outbreak reports from other parts of the country caused by Serotype A and SAT 2 (Durojaiye, 1981).

In an update status of FMD by Nwanta and Ojemiren (1999), serotypes A, O, SAT 1 and SAT 2 have been associated with disease outbreaks in Northern Nigeria. Chukwuedo *et al*., (2008) demonstrated antibodies to SAT1 and SAT 2 serotypes, while Knowles *et al*., (2008) reported serotype O and SAT2 from outbreaks in North-Eastern Nigeria. Hence, occurrence and distribution pattern of these known serotypes still remain unexplored due to poor understanding of FMD epidemiology in Nigeria.

The role of persistent carrier cattle, wild rodents and other game animals in FMD outbreaks remains largely uninvestigated in Nigeria. Studies by Durojaiye, (1981) indicated that disease morbidity was generally regarded as high (50-100%) with low mortality in indigenous adult breeds but may be highly fatal in calves. Evidence from various reports revealed that most outbreaks originated from northern Nigeria and spread to western parts through trade cattle activities (Owolodun, 1971). The inaccurate reported endemicity of the disease (Nawathe and Goni, 1976) is associated with non-reporting of outbreaks (Durojaiye, 1981) due to inefficient disease reporting system in the country (Chukwuedo *et al*., 2003) and this has made planning for disease prevention and control very difficult (Chukwuedo *et al*., 2008).

FMD control programme is not available except attempts to restrict movement of affected herds by veterinarians while traditional practice amongst pastoralists involved the process where by saliva of diseased animal is applied onto the oral mucosa of healthy animals. This result in “freeing the herd sooner” because the animals go through the disease at the same time, this immunity conferred is short lived and serotype specific as the animal can be immediately infected by another type (Abdulkadir, 1989).

Effective quarantine procedure at various international and interstate control post and control of animal movement are the most feasible methods of control (Basto *et al*., 2003a). However, these methods were saddled with difficulties such as inadequate veterinary policing of interstate and national borders due to insufficient veterinary staff (Durojaiye, 1981).

1.2 Statement of Research Problem

Foot-and-mouth disease is enzootic in Nigeria (Abdulkadir, 1989; Chukwuedo *et al.*, 2008) and results in serious impact on social and economic consequences dur to reduced milk and meat production and loss of market value of affected cattle (Sangare *et al.*, 2004a).

FMD outbreaks data are scanty and grossly under-report, the true disease situation in Nigeria is associated with in adequate disease reporting system (Chukwuedo *et al*., 2003). Despite disease endemicity with annual outbreaks, sero-typing and molecular studies have not been adequately utilized to establish epidemiological profile of the disease in Nigeria.

Furthermore, FMD surveillance is rarely conducted with limited data available to allow for correction of disparity between prevalence based on outbreaks report data and prevalence based on serological detection of recovered animal (Sumption *et al*., 2008).

Persistent or in-apparent infections (carrier state) in non-vaccinated as well as in vaccinated ruminants have been associated with outbreaks (Alexandersen *et al*., 2002). Recovery of virus from carrier animals is usually small but may be present in cattle for years (Salt, 1993; Salt, 1998). Thus possible carrier state in our indigenous cattle compounded by the absence of an indigenous vaccine could be likely militating factors for disease endemicity.

1.3 Justification of the Study

FMD epidemiology in Nigeria has not been fully investigated (Chukwuedo *et al*., 2003). This characteristic paucity of reported FMD information in Kwara state, where high concentration of cattle herds (both grazing cattle and transit trade cattle) exist (RIM, 1992), necciestated this study. As a result, a standardized and quantitative indication of bovine FMD burden in this gateway state is required to provide assessor with sufficient data and status of FMD in the study area.

The absence of national control prograrmmes has contributed to the increasing number of outbreaks with considerable morbidity and mortality especially in calves (Durojaiye, 1981) resulting in high economic losses in the country's livestock industry.

In addition, establishing and quantifying spatial distribution of serotypes will contribute to our understanding of FMD epidemiology across eco-climate zone.

FMD control would improve the livelihoods of livestock dependent communities in FMD endemic settings as well as improve and increase demand for effective national veterinary services and disease surveillance. Thus leading to the attainment of MDGs goals (1 and 8) alleviating poverty and hunger as well as promoting partnership for international development respectively.

Finally, this outbreak and serotype investigation would genetically identify the causative FMDV using serological and molecular techniques, which could help to proffer biological strategies including the possibility of development of a local vaccine to control FMDV spread within the study area and other parts of Nigeria as well as West African sub region.

1.4 Aim of the Study

To determine the presence of Bovine FMD and investigate FMDV serotypes in Kwara state.

1.5 Objectives of the Study

1. To detect Non-structural protein antibodies to FMDV amonsgt trade cattle in livestock market and slaughter houses in five local government areas of Kwara State using NS-ELISA, and $_{C}$ELISA

2. To determine the nature of association between age, sex, breed and location of cattle with FMD antibodies

3. To detect serotype specific antibodies of FMDV responsible for outbreaks in Ilesha Baruba LGA and livestock market from 5 LGA_s of Kwara state using Solid Phase Competition ELISA.

4. To detect FMD virus antigens from outbreaks using whole blood and epithelial tissues by Real-Time Polymerase chain reaction (rRT-PCR).

5. To sequence VP1/1D gene of FMD virus PCR amplicons derived from positive samples

6. To establish geo-spartial distribution of FMD outbreak locations and serotypes detected in Ilesha baruba district during the study.

7. To access the Fulani herdsmen (pastoralist), cattle traders and butchers' knowledge, mode of identification and management of FMD using semi structured interviews.

8. To determine the nature of association between sex, age, breed and hematological changes with FMD incidence in outbreak cattle herds in Ilesha baruba district during the study period.

1.6 Research Questions

R_{q1} – Can FMD antibody be detected in trade cattle sera?

R_{q2} – Can FMD gene be detected and amplified in blood and epitheial tissues collected from the outbreak cattle?

R_{q3} – Is there a difference between FMD serotypes in trade cattle and outbreak cattle found in the study area.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background of Foot-and-Mouth Disease

Food and Agriculture Organization of the United Nations (UN-FAO) and World Organization for Animal Health [Office International des Epizooties (OIE)] described Foot-and-mouth disease (FMD) as a transboundary animal disease (TAD) (FAO-OIE, 2004). TADs have three key characteristics which include economic, trade and/or food security importance for a considerable number of countries, easy spread and attainment of epidemic proportions in affected countries and finally disease control or management, including exclusion, required cooperation between several countries (Rweyemamu *et al.,* 2008b).

The first written description of FMD probably occurred in 1514, when Fracastorius described a similar cattle disease in Italy (Fracastorius, 1546) as reviewed (Gurbman and Baxt, 2004). Almost 400 years later, in 1897, Loeffler and Frosch demonstrated that a filterable agent caused FMD. This demonstrated the first filterable agent that caused animal disease and ushered in the era of virology (Loeffler and Frosch, 1897; Blancou, 2002). Subsequently it was tagged as a member of List A diseases (foot and mouth disease [FMD] (OIE, 2008), which is highly infectious in cloven-hoofed animals and wild ruminants causing severe economic losses to infected countries and regions (Murphy *et al*., 1999; Leforban, 1999). FMD assumed endemic status in many areas of the world and only 59 countries are considered free by the Office International des Epizooties (OIE, 2007c). Trade restrictions imposed to non-FMD-free regions have helped most European Union (EU) member countries to maintain their FMD-free status since the first eradication of disease in 1992 (Martı´nez-Lo´pez *et al*., 2008).

FAO and OIE identified FMD as principal animal disease of global concern in all consultations carried out during preparation of the Global Framework for the Progressive Control of TADs' [GF-TADs] joint proposal (FAO-OIE, 2004). The both organizations advocated progressive control of TADs at source in developing countries (Rweyemamu *et al.,* 2008b) as a contribution towards International Public Good, as this GF-TADs programme proposed effective prevention and progressive control of FMD and other major TADs enhanced achievement of the UN Millennium Development Goals.

The idea of a European cooperative work in FMD control was launched at an FAO meeting in London in 1949 and was further discussed at subsequent meetings held jointly by FAO and OIE in Paris in 1950, by OIE in Berne in 1951, and by the OEEC Working Party on Animal Health in Paris in May 1952. The member countries undertook to combat FMD with a view to ultimately eradicating the disease. To achieve this objective, members committed to apply, in addition to suitable quarantine and sanitary measures, one or more of the following methods; Slaughter policy; Slaughter together with vaccination; maintenance of a totally immune cattle population by vaccination and vaccination in zones surrounding outbreaks. Europe achieved an unparalleled success in disease elimination from an area of the world where highly dense and productive livestock populations and intensive animal trade within area that favoured virus circulation and persistence for centuries (FAO, 2004a).

Prevention of infection entry into Europe remained a priority, as well as development of regular testing and updating of emergency response plans. However the lessons learnt over 50 years and supported by 2001 events, clearly indicated that actions are needed to reduce

risk of infection and exiting maintained reservoirs entering other populations at risk (FAO, 2004b).

The FMD outbreaks in disease-free countries particularly Taiwan in 1997 and the United Kingdom in 2001, have significantly increased FMD public awareness especially on the possible use of FMD virus as a bioterrorism agent. These events have directed efforts of the scientific community to reexamine the knowledge of FMD viral agent that causes the disease and current methods of disease control (Gurbman and Baxt, 2004).

2.1.1 Developments and progress since 2001: lessons learnt:

Issues considered after the 2001 epidemic was the diagnostic capacity of both FMD national reference laboratories (NRLs) and FAO/OIE world reference laboratory. In addition to the virological and serological test procedures which were conducted during outbreaks, requirement for national serosurveillance to prove FMD-freedom post-outbreak led to serological testing on a previously unimaginable scale. The latter was greatly assisted by application of computerised, automated systems and newer, faster test methodologies (Solid phase competitive ELISA developed at Pirbright and the ELISA developed at CIDC, Lelystad). The UK further designated regional laboratories as centres where serological screening tests could be performed thus greatly increasing their serodiagnostic capacity and relieved pressure on the high security containment laboratories at Pirbright (De Clercq *et al*., 2008).

2.1.2 Ensuring preparedness of member countries in the event of future FMD outbreaks:

State veterinary services were encouraged to finalise contingency plans and advised to include contingency planning for laboratory diagnosis where special consideration was

given to the serodiagnostic capacity of NRL. The biosecurity standards required for FMD-serodiagnostic laboratories, supply of serodiagnostic reagents in crisis situation and the logistics of specimen transportation and handling in the laboratory were discussed by the EUFMD Research Group workshop for NRLs held in Cordoba in April 2004 (FAO, 2004). The adoption by the European Union (EU) of a non-vaccination policy for FMD control, allowed greater movement of livestock and their products within a single market (Directive 90/423/EEC) and increased trade with other FMD-free regions (Davies, 1993) which created avenue for the European domestic livestock population to be highly susceptible to FMD within and around the EU (Kitching, 1998) and served as potential source for FMD epidemic threat (Barnett and Cox, 1999).

2.1.3 The Nigerian experience

The earliest recorded history of FMD in Nigeria dated back to 1924 when colonial government set up the Nigeria's embryonic veterinary service (Anon, 1925). Before that period, FMD probably existed in Nigeria but was not documented. FMD was detected and diagnosed clinically among trade cattle in Borno Province in 1929 (Anon, 1929). Subsequently, the disease spread widely throughout the country amongst trade cattle along several trade routes in northern-Nigeria with little or no control measure being instituted (Anon, 1943). This resulted in sporadic outbreaks in young stocks especially during dry season in the forties, (1946, 1947 and 1948) (Abegunde, 1987).

In 1957, outbreak reports revealed serotype O was introduced by trade cattle from highland of Mambilla Plateau from the Cameroon spreading disease to the Benue province (Anon, 1959). In 1960, the disease maintained mild epizootic status in north eastern part of Nigeria amongst trade cattle spreading along cattle routes with serotype A causing

outbreaks and the introduction of SAT 2 from animals from Chad which was subsequently isolated from severe outbreaks in the region.

Review of FMD situation in Africa reported outbreaks in Northern Nigeria was associated with serotype O and none in the Eastern and Western parts of the country (Libeau, 1960). During the early-mid sixties, as the collection and dispatch of field samples to World Reference Laboratory, Pirbright improved, outbreak viral isolation indicated serotype A in Adamawa Province, serotype O in Bauchi Province, SAT1 in Bauchi, Borno, and Zaria Provinces while SAT2 was isolated in Zaria Province (Anon, 1965). This changed the sporadic FMD outbreaks reports in the northern states to enzootic disease status occurring annually (Ezeokoli *et al*., 1989) between December and March usually associated with harmattan periods in the North (Abdulkadir, 1989). There are also subsidiary outbreaks especially during the rainy season around July, August and September as most outbreaks were associated with cattle concentrations along river banks (Nwanta and Ojemiren, 1999).

Reviews of FMD outbreaks (Owolodun, 1971; Nawathe and Goni, 1976 and Asagba, 1982) documented the scanty available FMD information in Nigeria. These reviews indicated FMD virus distribution and outbreak occurrence was limited to Northern-Nigeria amongst transit trade cattle being transported into sourthern parts of the country. However, investigations revealed outbreaks in the Western (Anon, 1974) and South-Eastern Nigeria (Anon, 1975). Serotype A and SAT 2 were known to be active in these outbreaks (Nawathe and Goni, 1976; Durojaiye, 1981). Serotype O, SAT1 and SAT2 were associated with outbreak reports while serological survey using Virus infection antigens (VIA) and 146S whole virion antigen and indirect ELISA revealed overall incidence 9.8% for outbreaks and 55% prevalence for surviellance (Abegunde, 1987).

In an update status of FMD by Nwanta and Ojemiren, (1999), serotypes A, O, SAT 1 and SAT 2 were reported as been responsible for disease outbreaks in Northern Nigeria. Chuwuedo *et al*., (2008) demonstrated antibodies to SAT1 and SAT 2 serotypes in trade cattle, while Knowles *et al.,* (2008) reported serotype O and SAT2 from outbreaks. Recent review confirmed serotypes, O, A, SAT1 and SAT2 have being circulation in the country in the last 54 years (1955-2009) (Olabode *et al*., 2012). Ishola *et al.,* (2011) reported a prevalence of 56.3% in Jos south LGA in Plateau state. Other serological surveys conducted between 2009 and 2011 in six Border States and two other states along major cattle trek routes using ELISA showed higher seroprevalence was recorded in cattle sampled from Yobe State (82%), followed by those from Plateau (80%), Ogun (77.77%), Taraba (73.50%), Adamawa (68%), Borno (67%), Sokoto (63%) and Bauchi (27.84%), for sheep and goats (Lazarus *et al.,* 2012).

Since the advent of FMD in Nigeria several authors [Durojaiye, (1981), Dawe and Durojaiye (1986); Abegunde, (1987); Obi and Newman, (1988); Nwanta and Ojemiren, (1999); Chuwuedo *et al*., (2008); Knowles *et al.,* (2008)] have conducted different research on FMD outbreak diagnosis, epidemiology and seroprevalence, very little attention has been paid to control of the disease probably due to minimal mortality and mild disease course in traditional pastoral Fulani herds. The lack of formal vaccination programme against foot and mouth disease have continued to cause considerable morbidity and economic loss to the country's livestock industry especially as small ruminants were found positive to ELISA-VIA, thus the need to incoprate them in the epidemiology of FMD in countries like Nigeria where communual herding of cattle and sheep is practiced (Obi and Newman, 1988).

Suggested control of the disease based on quarantine and trade cattle movement restrictions were un-realistic and poorly attempted. An intial vaccination trial to control the disease with a polyvalent attenuated vaccine gave inconclusive results (Kane, 1964). Later another trial using homologus polyvalent vaccines produced from local strains of FMD virus (Mowat *et al.,* 1975) was conducted. Although this prearation gave encouraging results, it was not available commercially. Limited scope of vaccination was conducted using imported vaccines in some farms (Anon, 1977) which did not achieve desired effect under the Nigerian conditions (Mowat *et al*., 1975).

Nawathe and Majiyagbe (1981) further reported non satisfactory results in Nigerian cattle vaccinated with commercial polyvalent vaccines formulated from non Nigerian serotype A, SAT1 and SAT2 viruses. Also limited studies have been carried out on the suitability of Nigerian field strains for vaccine formulation for use in Nigeria by Preston *et al*., (1982) and showed suitable vaccine could be made from Nigerian strains. Nicholls *et al*., (1983) reported satisfactory protection of Nigerian cattle using inactivated vaccines formulated with Nigerian strains with revaccination requirement in every six months. This vaccine was not however used on a large scale for FMD control in Nigeria as outbreaks continued to occur (Abegunde, 1987). Currently, no indigenous Nigerian strain vaccines are available (Olabode *et al.,* 2012) with limited use of imported FMD vaccines amonsgt few pastoralists due to high cost.

2.2 Etiology of Foot and Mouth Disease

Foot-and-mouth disease virus (FMDV) is a non-enveloped, positive-sense (direct messenger RNA) single-stranded RNA molecule of the genus *Aphthovirus* and family *Picornaviridae* (King, 2000).

FMDV particles are made up of naked icosahedrons encapsidating 60 copies each of four structural proteins VP1- 4, in the viral genome. The protein coding region has a continuous open reading frame of 6915 or 6999 nucleotides in length depending on which of two functional in-frame start codons 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, secondly, Structural proteins (SP) P1: P1A, 1B, 1C and 1D equivalent to VP4, VP2, VP3 and VP1, respectively, thirdly, NSP P2: (P2A, 2B and 2C); and lastlty, NSP P3: P3A, P3B or VPg**,** P3C, and P3D (Knipe *et al*., 1997).

Antigenically, there are seven immunologically distinct serotypes: A, O, C, Asia 1, SAT (Southern African Territories) 1, SAT 2, and SAT 3 distributed globally with considerable antigenic and genetic diversities in each serotype resulting into numerous subtypes (Carrillo *et al*., 2005). This characteristic diversity observed at genetic level is used to trace viral origin (Knowles and Samuel, 2003) and was associated with susceptibility of infection by another serotype in animals recovered from infection with one serotype but resistant to challenge by the same serotype (Belsham, 1993). These serotypes have different geographical distributions and epidemiologies though clinically indistinguishable as Asia1 (Ansell *et al*., 1994) has been restricted to the Middle East and Asia with no report of spread into Africa or Europe. SAT3 Serotype rarely occurs in species other than the Cape buffalo in southern Africa **(**Thomson, 1994) while serotype O has widespread distribution across the world (Knowles *et al*., 2001). Numerous strains (quasispecies) have arisen within each serotype from error-prone RNA replication (Domingo *et al*., 2002).

This variability in virus types, sub-types and strains can explain the variations seen in disease occurence due to different strains behaving in peculiar way (specific level of excretion, specific pathologic potency for each domestic species or even breed) (Donaldson and Alexandersen, 2001) thus confounding diagnosis and control.

Most FMDV studies are conducted in countries where control measures are being implemented with sporadic submissions to the World Reference Laboratory (WRL), Pirbright, United Kingdom from sub-Saharan Africa where FMDV is endemic (Ferris and Donaldson, 1992) usually from very limited geographical areas and time scales and as a result may give a very biased impression of the disease situation and epidemiology. However, regions where the disease is endemic are where new strains are most likely to evolve. FMDV typing was originally done by serology cross-protection and on this basis, it was suggested that serotypes O, A, and C had probably not penetrated south of the equator before 1955 (Brooksby, 1982). The SAT viruses were not described until late 1940s in southern Africa (Thomson, 1994) and had not yet moved north of the equator until the late 1950s (Libeau, 1960).

Reviews of literature on submissions to WRL for Africa between 1958 and 1992 (Ferris and Donaldson, 1992) and from 1948 to 2002 (Vosloo *et al*., 2002) suggested occurrence of four serotypes (SAT1, SAT2, A, and O) in western and central Africa, two serotypes O and A have been circulating in Cameroon in the last 70 years (Ekue *et al*., 1990) while serotypes SAT1, SAT2, A and O has been in circulation within Nigeria in the last 54years (1955-2009) (Olabode *et al*., 2012).

2.2.1 Structure of FMD virus

FMD virions appear as round smooth surface particle with diameter of about 25 nm by electron microscopy (Bachrach, 1968). The fine structure viral capsid and structural features of type O1BFS has been determined for a number of serotypes by using X-ray crystallographic techniques (Jackson *et al.,* 2003). The structural proteins, VP1 to -3, fold into an eight-stranded wedge-shaped beta-barrel which fit together to form the majority of the capsid structure (Bachrach, 1968). The VP4 protein is buried within the capsid and has a myristyl group covalently attached to its N terminus (Belsham *et al.,* 1991). The beta-barrel strands of VP1 to -3 are connected by loops which form the outer surface of the virion (Jackson *et al.,* 2003). FMDV has been distinguished from other picornaviruses by the lack of a surface canyon, or pit, which known to be the receptor binding site for the entero- and cardioviruses (Xiao *et al.,* 2001).

The virion also posses a channel at the fivefold axis which permit the entry of small molecules, such as CsCl, into the capsid, this gives FMDV the highest buoyant density amongst picornaviruses (Acharya *et al.,* 1989). Unlike other picornaviruses, FMDV capsid dissociates at pHs of below 6.5 into 12S pentameric subunits (Brown and Cartwright, 1961). The reason for this instability was associated with a cluster of His residues at the interface between VP2 and VP3 which became protonated at low pH, weakening the capsid through electrostatic repulsion (Ellard *et al.,* 1999). This low-pH-induced instability of FMDV brings about differences in the uncoating mechanism upon infection of cells when compared with that of other picornaviruses. It also may play a role in specific tissue and organ viral targeting in susceptible hosts.

2.2.2 Genome organization of FMD virus

The 140S virion particle consist of a single-stranded RNA genome with 60 copies each of four structural proteins (VP1 [1D], VP2 [1B], VP3 [1C], and VP4 [1A]). The FMDV genome has a basic organization similar to other *Picornaviridae* members and their viral protein nomenclature was established (Rueckert and Wimmer (1984). 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 [designation used for the protein]), covalently bound to 5' terminus of the RNA (Grubman, 1980).

The RNA is translated as a single long open reading frame (ORF) into a polyprotein, followed by a series of post-translational proteolytic cleavages to generate both the intermediate and mature structural and NS viral proteins (Robertson *et al.*, 1985). Based on the initial cleavage products, the genome ORF is divided into four regions. The 5' end (L region) which encodes N-terminal component of the polyprotein, contains two in-frame AUG initiation codons that result in the generation of two L proteins, Lab and Lb (Sangar *et al.,* 1987). While both forms of L are synthesized during in vitro translation of viral RNA (Beck *et al.,* 1983) and in infected cells (Clarke *et al.,* 1985). Site-directed mutagenesis showed that deletion of second AUG from an FMDV infectious clone abolished viral replication upon transfection of the transcribed RNA into cells, while deletion of the first AUG had no effect on viral replication (Cao *et al.,* 1995).

In addition, generated synthetic viral genomes lacking L gene and showed that only the genome that initiated polyprotein synthesis at the second AUG codon produced live virus

(Piccone *et al*., 1995). These results strongly suggest that Lb may be the biologically functional protein in vivo. The L protein, a papain-like protease (Lpro) (Roberts and Belsham, 1995), is autocatalytically cleaved from the polyprotein C terminus (Strebel and Beck. 1986). A crystallographic structural analysis of Lpro further suggested that self-cleavage reaction might occur intermolecularly in *trans*, based on C terminus protein position within the active site of an adjacent Lpro molecule (Guarne *et al*., 1998). However, other structural features within the same crystals suggested that an intramolecular *cis* self-cleavage reaction is also possible (Guarne *et al.,* 1998). In addition, Lpro has unique cation concentration and pH range requirements, due to differences within the molecule active site, which distinguish it from other papain-like enzymes (Guarne *et al*., 2000).

The Lpro also plays a role in inhibition of host protein synthesis and has been identified as a viral virulence factor. Directly downstream of the L region is the genome P1 region, encoding the four viral structural proteins VP4, VP2, VP3, and VP1. Following the P1 region is the P2 region, encoding three viral NS proteins, 2A, 2B, and 2C, and the P3 region, encoding NS proteins 3A, three copies of VPg, 3Cpro, and 3Dpol. Historically the 2A region was considered part of P2 region, however, genetic and biochemical evidence showed that FMDV 2A peptide was cleaved as a P1-2A precursor (Vakharia *et al*., 1987) while 3Cpro was identified as a viral protease (Klump *et al.,* 1984) associated with viral polyprotein processing and 3Dpol the viral RNA dependent RNA polymerase (Lowe and Brown, 1981).

The FMDV genome also contained untranslated RNA found upstream (5’ untranslated region [5’UTR]) and downstream (3’ UTR) of the ORF. The FMDV 5’ UTR contain about

1,300 bases (Robertson *et al*., 1985) and can be divided into five functional elements which play roles in virus translation and RNA replication. The most 5' segment, the S fragment, encompasses about 360 bases and folds into a long stem-loop (Bunch *et al.,* 1994). The function of the S fragment is unknown, but analogies with other picornaviral genomes suggested that it may play a role in maintaining genome stability in infected cells (Barton *et al.,* 2001) and may also be involved in genome replication protein binding (Andino *et al.,* 1993).

There have been some suggestions that the S fragment may affect viral pathogenicity, but currently no available direct evidence for this (Cao *et al.,* 1991). Following the S fragment, there exist poly(C) tract comprising over 90% C residues with a small number of U and A residues. This segment contain over 100 bases in length, however, poly(C) tract length can be extremely variable (Costa Giomi *et al.,* 1984) as tissue culture-adapted virus have been shown to have a poly(C) length of over 400 bases (Escarmis *et al.,* 1992). Although an early study suggested poly(C) tract length was associated with virulence (Harris and Brown, 1977), other studies have been unable to correlate poly(C) length with this viral property (Costa Giomi *et al*, 1984). It has also been shown that poly(C) lengths of natural viral isolates can be increased by repeated passage in cell cultures (Escarmis *et al.,* 1992) as seen in genetically engineered viruses (Rieder *et al*., 1993).

Virus without poly (C), virulently replicates in suckling mice with much higher particle/PFU ratio than viruses containing longer poly(C) tracts (Rieder *et al*., 1993). The viral virulence in susceptible animals has not been determined. While the exact role of poly(C) tract in FMDV replication is unknown, studies with poliovirus have shown an

association of the host factor, poly (rC) binding protein (PCBP), with the 5'end of the poliovirus genome (Gamarnik and Andino, 1997), which could regulate the switch from translation to genome replication (Herold and Andino. 2001). After the 3' end of poly(C) tract there exist series of RNA pseudoknot structures of unknown function (Escarmis *et al.,* 1995). Downstream of the pseudoknots there is a short hairpin loop structure called *cis*-acting replicative element (*cre*) (Mason *et al.,* 2002) which has a stem-loop with a conserved AAACA sequence in the loop region. However, the *cre* is located within different regions of ORF in other picornaviruses the *cre* of FMDV is located within the 5' UTR (Mason *et al.,* 2002).

The *cre* is essential for RNA genome replication, the region between the *cre* and ORF contain series of highly conserved stem-loop structures which together constitute the internal ribosome entry site (IRES). Identified IRES element in FMDV genome (Kuhn *et al.,* 1990) contained a high degree of secondary and tertiary structure which was divided into three groups, based on conserved structure as opposed to primary sequence (Pilipenko *et al.,* 1992). The IRES element for aphthoviruses, which is similar in structure to the cardiovirus IRES (group 2 IRES), contained about 450 nucleotides in length (Belsham and Brangwyn, 1990) and has been modeled into five-domain structure (Pilipenko *et al.,* 1992). IRES elements contain a pyrimidine-rich region at their 3' ends immediately preceding the AUG translation initiation codon, and FMDV contains pyrimidine-rich regions directly upstream of each alternative AUG initiation codons (Pilipenko *et al.,* 1992). The 3' UTR which followed ORF termination codon contain a short stretch of RNA which folds into a specific stem-loop structure (Lamphear *et al.,* 1995) then the poly (A) tract of variable

length carried on the genome (Dorsch-Hasler *et al.*, 1975). The 3' UTR also appeared important for genome replication (Melchers *et al.*, 1997).

This was supported by studies that showed the binding ability of 3' UTR to a number of picornaviral proteins involved in RNA replication (Cui and Porter. 1995). However, hybridization of antisense RNA to the 3' UTR of FMDV did not effect in vitro translation of viral RNA but did inhibit RNA replication in infected cells (Gutierrez *et al.*, 1994). Subsequently, studies demonstrated deletion of FMDV 3' UTR reduced in vitro translation efficiency and blocked the ability to recover viable virus from transfected cells (Saiz *et al.*, 2001). FMDV 3' UTR replacement with that of the enterovirus, SVD virus, resulted in a non-viable genome (Saiz *et al.*, 2001) suggesting 3' UTR is specificity for each picornavirus. The poly (A) tract probably functions in FMDV translation (Lopez de Quinto, 2002) and may also play a role in picornavirus RNA replication (Herold and Andino, 2001).

2.2.3 Infectious cycle

FMDV, like other members of the *Picornaviridae* has a relatively short infectious cycle in culture cells. Depending on the multiplicity of infection, newly formed infectious virions begin to appear at between 4 and 6 h after infection. 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). Early interactions involve adsorption, penetration, and uncoating. The interactions of FMDV with cells have been extensively studied for many

years and it was accepted that FMDV receptors, as well as other picornavirus receptors, play a role in tissue and organ tropisms which lead to disease pathogenesis (Verlinden *et al.,* 2000). FMDV bind rapidly to cells in culture at both 4 and 37°C by using a limited number of receptor sites measured between 10^3 and 10^4 per cell (Baxt and Bachrach, 1980). These studies also suggested that while six of the seven serotypes bound to a single class of receptor site, some of the serotypes bound to a second class of receptors which were present at a high copy number (Sekiguchi *et al.,* 1982). Early studies showed limited trypsin digestion of virus resulted in viral particles which were non infectious due to inability to bind to cells in culture (Barteling *et al.,* 1979a). Analysis of trypsin-treated virus revealed a single cleavage of the VP1 protein at Arg144 (Robertson, 1983), which was later shown to be located within a surface loop connecting the beta-G and beta-H strands of (G-H loop) protein (Logan *et al.,* 2002). This suggested that VP1 protein region interacted with the cell surface receptor as other alternative 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).

In addition, an artificial receptor which consist 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 with RGD-deleted virus (Rieder *et al.,* 1996). While penetration and uncoating of FMDV have not been studied in great detail, there have been some observations which suggest possible mechanisms of how they may occur. It has been shown that after adsorption to cell surface 140S virion break down into 12S pentameric subunits, releasing the RNA (Baxt, 1987). This breakdown does not occur at cell surface, since particles which are eluted from the cell after adsorption are fully

infectious and still sediment at 140S (Baxt and Bachrach, 1980) as the virus probably breaks down upon entering an acidic endosome (Carrillo *et al.,* 1985). However, non infectious genetically engineered FMDV was unable to perform maturation cleavage of VP0 to VP2 and VP4 which was adsorbed into cells in culture and were acid sensitive (Knipe *et al.,* 1997).

Thus, 140S virus breakdown to pentameric subunits does not lead to productive infection, but there must be other events after the breakdown. This indicated viral receptor was responsible only for virus docking to the membrane of susceptible cell and played no role in viral uncoating, which was consistent with the ability of FMDV to utilize multiple receptors for infection in cell culture.

2.2.4 Viral transcription and genome replication

Picornavirus RNA replication has a number of unique challenges. Although, very few studies on FMDV system transcription and replication have been conducted but extensive studies on these activities have been performed with enteroviruses (Grubman and Baxt, 2004). The first step in picornavirus RNA replication is the synthesis of a minus-strand RNA molecule as this system has not been studied in FMDV. However, RNA models of 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 Andino, 1998).

The mechanism behind this translation shutdown of the plus strand is unclear, but it has been proposed in poliovirus-infected cells, that when polymerase precursor (3CD) accumulates in the cell, it binds to 5'cloverleaf structure and modify the affinity of PCBP

for IRES, an interaction which is essential for translation. Whether 3CD play similar role in FMDV RNA replication is not known but it has been shown that in FMDV-infected cells, this protein rapidly cleaved to 3Cpro and 3Dpol (Grubman *et al.,* 1984).

2.2.5 Viral translation

The FMDV RNA release into the cytoplasm after uncoating by an unknown mechanism and begins a series of viral translation. The genome-linked protein VPg cleaves by a cellular enzyme prior to translation of the incoming RNA (Ambros and Baltimore, 1980). However, protein synthesis initiation complexes can be formed with mRNA containing VPg (Golini *et al.,* 1980). Unlike most host mRNAs, actively translated viral mRNA does not contain a 7-methyl-G cap structure at its 5'end (Grubman and Bachrach. 1979) and initiates protein synthesis internally at the IRES by a cap-independent mechanism (Kuhn *et al.,* 1990).

Cap-dependent mRNA translation inhibition in infected cells is followed by cleavage of protein synthesis initiation factor eIF4G by Lpro (Kirchweger *et al.,* 1994). Intact eIF4G acts as a bridge connecting the mRNA cap to 40S ribosomal subunit. This is accomplished by binding of the cap binding protein, eIF4E to the N-terminal domain of eIF4G, while the C-terminal domain binds eIF4A and the 40S ribosomal subunit via eIF3 (Lamphear *et al.,* 1995). In contrast, initiation of FMDV RNA translation required only Lpro-generated C-terminal eIF4G cleavage product, which bond to FMDV IRES and interacted with 40S ribosomal subunit-bound eIF4A and eIF3 (Saleh *et al.,* 2001).

In addition, eIF4B bound to FMDV IRES and has been identified in both 48S pre-initiation complexes and 80S ribosomes (Ochs *et al.,* 1999). The FMDV IRES interacted with a

number of cellular proteins, including initiation factors important for normal cellular mRNA translation. A host factor of 57 kDa, subsequently identified as the nuclear polypyrimidine tract binding protein (PTBP) (Niepmann *et al.,* 1997) was shown to interact with at least two regions of the IRES (Pilipenko *et al.,* 2000). Deletion of these two sites inhibited both the binding of protein and in vitro translation (Luz and Beck, 1991). A second host factor, which required for FMDV IRES-driven translation but not for translation of cardiovirus mRNA (Pilipenko *et al.,* 2000), was identified. This 45-kDa protein, IRES-specific *trans*-acting factor (ITAF45), along with PTBP, was required for the formation of 48S translation-initiation complex (Martinez-Salas *et al.,* 2001). A third host factor, PCBP, which was required for translation of poliovirus RNA (Blyn *et al.,* 1997) has not been shown to be involved in FMDV translation.

However, the presence of poly(C) tract upstream of IRES suggested that it may also play a role in FMDV translation, genome replication, or both. It has also been postulated that PCBP facilitated circularization of poliovirus genome to modulate balance between translation and RNA replication (Barton *et al.,* 2001). Initiation and translation resulted in production of a single polypeptide which was subjected to series of cleavages leading to production of both structural and NS proteins. The primary cleavage reactions performed by three different proteases: Lpro autocatalytically cleaved itself from polyprotein. 2A, (18-amino-acid peptide), autocatalytically removed itself from the P2 polyprotein, and remained associated with the P1 precursor (Grubman *et al.,* 1995).

There has been suggestion that this cleavage was not a proteolytic event but rather is a modification of translational machinery by 2A peptide which allowsed the release of P1-2A from the ribosome while permitting synthesis of the downstream proteins to proceed

(Donnelly *et al.,* 2001). This hypothesis, however, has not been confirmed by other laboratories. Nevertheless, the 2A peptide has been used in non-viral systems to cleave foreign genes from polyproteins (Groot Bramel-Verheije *et al.,* 2000). All other cleavages of the polyprotein, (with the exception of maturation cleavage), was performed by 3Cpro (Bablanian and Grubman, 1993). This protein was related to trypsin family of serine proteases (Gorbalenya *et al.,* 1989), and the active site of FMDV 3Cpro to Cys163, His46, and Asp84 have been mapped (Grubman *et al.,* 1995). The protease cleaved at a number of different dipeptide sequences, including Gln- Gly, Glu-Gly, Gln-Leu, and Glu-Ser (Palmenberg, 1990).

2.2.6 Encapsidation and maturation

The final steps in replication cycle are the encapsidation of plus-strand viral RNA and maturation cleavage of VP0 to VP2 and VP4 to form mature virion. The mechanisms of encapsidation and maturation are still unresolved and are probably the least studied of all of the steps in replication cycle as most of the studies that have been done with enteroviruses and analogies drawn for FMDV (Grubman and Baxt, 2004).

In broad terms, 3Cpro cleavage products of P1 region are assembled into a protomer structure containing one copy each of the proteins VP0, VP1, and VP3 as five protomers assemble into a pentamer and 12 pentamers assemble into the final capsid structure. Following RNA encapsidation, maturation cleavage reaction (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 with an uncleaved VP0 lacking RNA (empty capsid) (Grubman *et al*., 1985).

Two unresolved issues in picornaviral maturation are what signals are necessary for encapsidation of RNA and what are the roles of empty capsid and provirion. Picornaviruses encapsidate only plus-strand RNA, linked to VPg, to the exclusion of all other viral and cellular RNAs (Novak and Kirkegaard, 1991). In addition, only newly synthesized plus-strand RNAs are encapsidated, indicating that there is a link between active RNA replication and encapsidation (Nugent *et al.,* 1999). Thus, there may be *cis*-acting packaging signals present within the plus strand RNA to facilitate encapsidation. The putative packaging signal does not appear to reside within the genome P1 region, since defective-interfering particles have been demonstrated in poliovirus-infected cells (Hagino-Yamagishi and Nomoto, 1989), all of which have deletions within the P1 region. Interestingly, no defective-interfering particles have been detected in FMDV infected cells (Polacino *et al.*, 1985).

The final maturation step in virion assembly is the cleavage of VP0 into VP4 and VP2 in the presence of viral RNA (Hogle *et al.,* 1985). An aberrant cleavage of VP0 in FMDV empty capsids has been demonstrated, which suggested viral RNA presence for proper cleavage event to take place (Curry *et al.,* 1997). Cleavage is thought to be autocatalytic and occur from a conserved His residue in VP2 which activates local water molecules, leading to a nucleophilic attack on scissile bond and cleavage (Hindiyeh *et al.,* 1999) as this maturation cleavage is required for generation of infectious virus (Harber *et al.,* 1991). In contrast, FMDV site-directed mutations within VP0 led to the formation of non-infectious

provirions that exhibited receptor binding and acid sensitivity, similar to the case for infectious virus (Knipe *et al.,* 1997).

However, upon acid dissociation, the generated pentamers were more hydrophobic than those from mature virions, suggesting that VP0 cleavage may be necessary for RNA release into the cytoplasm (Knipe *et al.,* 1997).

2.2.7 Antigenic variation in FMD virus

The presence of seven serotypes, multiple subtypes and variants has compounded the difficulty in FMD laboratory diagnosis and control due to new variants generated by continued circulation of virus in the field and the RNA genome quasispecies nature (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 the lack of error correction mechanisms during RNA replication (Drake and Holland, 1999). This high error rate leads to differences of FMDV replicated genomes from the original parental genome of 0.1 to 10 base positions (Haydon *et al*., 2001), and quasispecies concept was developed to explain the effects of errors in replication on the evolution of replicating RNA molecules (Eigen, 1971).

The concept envisaged that within 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). Hence no "wild type" as such but rather an observed "average" phenotype which has adapted to and replicates "best" within any given environment. The environment can be in either tissue culture or a particular host species, and in situation, immunologic pressure or physical conditions such as temperature or pH are influential as

change in environment can lead to emergence of a new "average" phenotypic trait. The quasispecies nature of FMDV genome was described (Sobrino *et al*., 1983), and while the concept was studied at nucleotide level, the variability of FMDV populations manifested when mutations lead to codon changes with a resultant change in viral phenotype. Most of this variation occurs within the genome capsid-coding region (the P1 region) and leads to antigenic variation. While mutations also occur within the genome NS protein-coding regions, they are probably less tolerated, since proteins encoded by these regions are necessary for viral replication and changes are more likely to be lethal. In addition to variation caused by mutation, FMDV has been shown to undergo RNA recombination in tissue culture (Wilson *et al.*, 1988).

These studies indicated that recombination was more likely to occur within the genome coding regions for NS proteins especially RNA recombination within the genome capsid-coding P1 region may contribute to genetic diversity in field isolated FMDVs (Tosh *et al.*, 2002). Antigenic variation in the field increases with time and most probably resulted from immunologic pressure placed on virus by either infected or vaccinated host species (Haydon *et al.*, 2001a). However, antigenic variation in FMDV was reported in tissue culture in the absence of immunologic pressure (Fares *et al*., 2001), indicating that antigenic sites on the virion may also be involved in other virus functions. Regardless of the mechanism, analysis of both genome sequence and antigenic variation has been invaluable in epidemiological studies of outbreaks and analysis of virus within countries where the disease is enzootic, and in the case of a possible deliberate introduction of virus, which will also have forensic value in tracking the source (Knowles and Samuel, 2003).

Antigenic sites (epitopes) on the surface of the FMD virion have been identified for five of the seven serotypes of FMD virus with exception of South African Territories (SAT) 1 and 3 (Marquardt *et al.,* 2000). At least four antigenic sites have been identified, involving one or more of the capsid proteins, VP1, VP2, and VP3 but each serotype may not contain all four sites. Three of the sites have elements located within the flexible loops which connect the viral proteins beta-sheets 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).

This site also contains the RGD receptor-binding recognition sequence. While this site is clearly the major antigenic site, FMDV antigenic variation is associated with mutations leading to amino acid replacements within all of the known antigenic sites (Mateu *et al.,* 1994). Although antigenic variation may be extensive within FMDV, the changes are limited to very specific regions of the viral surface. This may result in changes within other regions of the capsid that could affect either viral structural integrity or virus identity (Domingo *et al.,* 2003). The antigenic variation within FMDV makes control extremely difficult as the best vaccine may induce immunologic pressure within the population that results in emergence of new variant. Furthermore, the observed antigenic variation in tissue culture 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 may eventually be utilized as vaccine antigen may not provide the needed antigenic coverage (Grubman and Baxt, 2004).

2.2.8 Physical properties and preservation of FMDV

The virus is inactivated by pH outside the range of 6 to 9 as well as sodium hydroxide (2%NaOH), potassium hydroxide (KOH), sodium carbonate (4% Na_2Co_3) and citric acid but resistant to iodophores, quaternary ammonium compounds, phenols (Blancou, 2002) and alcohol (Sahle, 2004).

The virus can remain viable on a variety of materials for variable lengths of time, depending on environmental conditions (survival decreases with increasing heat and decreasing humidity), in fecal material: Up to 100 days (liquid slurry), hay: Up to 105 day, bran: Up to 140 days, wool: Less than 21 days, average 18 days, snow-covered soil: More than 185 days, meat and meat by-products in which pH remains above 6 can serve as a source of viable virus (Bartley *et al*., 2002).

The non-enveloped protein shell capsid of Picornaviruses consists of polypeptides, which are devoid of lipo-protein, and hence stable to lipid solvents like ether and chloroform (Cooper *et al.*, 1978). However, in milk and milk products, the virion is protected, and can survive at 70 ^{o}C for 15 seconds and pH 4.6. In meat, the virus can survive longer period in chilled or frozen bone marrow and lymph nodes (Mckercher and Callis, 1983).

The sizes of droplet aerosol also play an important role in the survival or viral drying out as aerosol droplet size of 0.5 - 0.7 μm is optimal for longer survival in the air, while smaller aerosols dry out. In dry conditions the virus also survives longer in proteins especially in epithelial fragments (Donaldson, 1987b).

2.3 Epidemiology of Foot and Mouth Disease

2.3.1 Suceptible host of FMD

The mammalian species susceptible to FMD virus belong to the order of Artiodactyla and family suidae (wild boars and domestic pigs), cervidae (deer), and bovidae (cattle, sheep, goats and antelopes). Other cloven-hoof mammals include buffalo, yaks, and wildlife (Fenner 1993; Lubroth, 2002). Other species include hedgehogs (Insectivora), tapirs (Perissodactyla), elephants (Proboscidea), bears (Carnivora), camelidae (camels and llamas) and alpacas although described as having been infected, have not been proved to be of importance in disease epidemiology (Hedger, 1981). Rats, mice, hedgehogs, and armadillos have been demonstrated to be susceptible under experimental conditions while African Cape buffalo remains major maintenance host for the SAT serotypes (Lubroth, 2002).

2.3.2 Occurrence and distribution of FMDV

FMD is enzootic in parts of Asia, South America, the Middle East, and Africa, (Aggarwal *et al*., 2000). The distribution of different FMD serotypes and subtypes varies according to countries and continents (Patil *et al.*, 2002). In some areas, several serotypes and subtypes can occur at the same time, however, the serotypes and subtypes pattern around the world is not static and may be influenced by disease control type practiced, or level of challenge by emergence of new strains (King, 2007). Outbreaks have occurred in every livestock-containing region globally with exceptions of New Zealand, and the disease is currently enzootic in all continents except Australia and North America (Gurbman and Baxt, 2004).

The widespread nature of the disease has resulted in only 59 countries declared free by the Office International des Epizooties (OIE, 2007c). Trade restrictions imposed on non-FMD-free regions have helped most European Union (EU) member countries to maintain their FMD-free status since the first disease eradication effort from that continent in 1992. However, the risk for FMD virus (FMDV) introduction into the EU is still high, as demonstrated by epidemics in Italy (1993), Greece (1996, 2000), United Kingdom (2001), Ireland (2001), France (2001), and the Netherlands (2001) (Maragon *et al.*, 1994; Chmitelin and Moutou, 2002; Griffin and O'Reilly, 2003). These European outbreaks in the last decade originated from United Kingdom in February 2001 and spread to France and the Netherlands. The outbreak was associated with swine swill contaminated by virus that originated in India (Pan Asian O strain), but the exact method of entry was not known. This outbreak in the United Kingdom which lasted for approximately 7 months highlighted the devastating impact of FMD in a country previously free from the disease (King, 2006) with the last recorded FMD case in the United Kingdom was in September 2002 (Davies, 2002).

The epidemic was controlled by slaughter of affected and in-contact animals ("stamping out" method). Social and economic consequences in some FMD epidemics have been devastating for EU member countries. The epidemic cost was estimated to about 2.7 billion British pounds (excluding lost tourism and associated revenue) and maximum of £8 billion to the UK economy as a result of slaughter of about 4 - 6.5 million animals for its control on 2,000 farms (King *et al*., 2006; Martı´nez-Lo´pez *et al*., 2008) while the natural outbreak in Taiwan in 1997 cost an estimated $378.6 million (Tharratt, 2002).

Foot-and-Mouth Disease was last seen in the United States in 1929. The U.S. Government placed an extremely high priority on keeping the disease out of the country (Anon, 2010). FMD is considered endemic with the serotypes O, A and Asia 1 in both Pakistan (Zahur *et al.,* 2006) and neighboring countries of India, Afghanistan, Iran and China (Tosh *et al.,* 2002; Perez *et al.,* 2005) and those serotypes are a continued problem in Pakistan. The regional Pan Asia II lineage of serotype O and A/Iran/2005 lineage of serotype A was responsible for the outbreaks FMD in suburbs of Karachi in Pakistan and causing huge economic losses amongst the world's largest Buffalo colony with more than 300,000 in contact animals with FMDV infection prevalence at aggregate level showing endemic occurrence of FMDV in the colony, with peaks in August 2006, December 2006 and February 2007 to March 2007. Disease prevalence peaks were significantly associated with the rainy seasons which concided with the coldest time of the year as well as the Muslim Eid-festival (Klein *et al.,* 2008).

FMD serotype A virus responsible for disease outbreaks in Egypt was phylogenetic demonstrated to have a close relationship with recent FMD virus isolates from East Africa, rather than to viruses currently circulating in the Middle East (Knowles *et al.,* 2007)**.** Outbreaks of FMD have occurred repeatedly in Mali and neighboring countries during the last decade (1980 to 1996). The outbreaks in 1980 and 1982 were confined to regions of Kayes and Gao, respectively. Between 1991 and 1992, outbreaks occurred in Segou, Sikasso and Bamako. In 1996, FMD outbreaks were reported in cattle populations throughout Mali, except in Kidal in the Sahara desert, where temperatures reach 45°C. High mortality was reported in young animals, while morbidity approached 100% in adult cattle (Sangare *et al.,* 2004a). Mali been one of the largest cattle and small ruminant population

countries in West Africa (approximately six million cattle) (DNAMR, 2001) with cattle exports to Senegal, Côte d'Ivoire, Guinea, Ghana and Nigeria (Jarrett, 1980) as the first FMD virus molecular epidemiological studies in West Africa demonstrated evidence of FMD transmission across common national boundaries in this region (Sangare *et al.*, 2004a).

2.3.2.1 Epidemiological patterns of FMD in Africa

The FMD epidemiology in Africa has been reviewed (Vosloo *et al.*, 2002; 2004). Emergency FMD audit on Southern Africa was conducted (Batho, 2003). Africa has the greatest diversity of FMD serotypes based on prevalence data, serotype and topotype distribution, expert evaluation of factors such as animal movement patterns, impact of wildlife and farming systems, which lead to proposition of epidemiological clusters for Africa. The epidemiological clusters have the following characteristics: Indian Ocean Island Countries Madagascar, Mauritius and Seychelles are free of FMD, with a recognized FMD status freedom without vaccination. The commercial (large and small) livestock sectors of South SADC countries, i.e. Swaziland, Lesotho, South Africa, Botswana and Namibia are free from FMD and met the conditions of OIE for zonal or country freedom from FMD without vaccination. In some of these countries, however, there are segregated wildlife areas that harbour African/Cape buffaloes known to be infected, asymptomatically, with FMD virus serotypes SAT-1, SAT-2 and SAT-3. These wildlife parks are segregated from livestock through a system of game-proof fencing and vigorous surveillance.

In these countries, game ranching or other wildlife conservation activities with FMD-infected African buffaloes are not allowed within FMD-free zones. The north SADC cluster

comprises Zimbabwe, Zambia, Mozambique, Malawi and southern Tanzania. In these countries, FMD was controlled in livestock during the 1970s and 1980s through intensive vaccination and livestock movement control. In Zimbabwe, livestock remained largely unaffected and the country was able to export beef to the international market including the EU, until 2000 when the maintenance of game fencing deteriorated allowing cattle and buffalo to mix and set up outbreaks at a time when the veterinary services were too inadequately resourced to contain such outbreaks. Consequently, FMD spread within the country and to neighbouring countries. SAT-1 and/or SAT-2 outbreaks in Mozambique, Malawi and Zambia between 2002 and 2004 were either because of outbreaks spreading from neighbouring countries or to internal buffalo-cattle contact. Northern Malawi and Northern Zambia were under constant threat of FMD spread from southern Tanzania.

For this epidemiological cluster, the primary source of FMD seemed to be risk posed by wildlife buffalo herds (Sutmoller *et al.,* 2000; Hargreaves *et al.,* 2004). Several studies in Southern Africa have shown Cape/Africa buffalo's ability to maintain silent serotypes SAT-1, SAT-2 and SAT-3 infection for a long time, independent of circulation in livestock (Vosloo and Thomson, 2004). The carrier buffalo can transmit infection to other buffalo or to impala or cattle by an unknown mechanism, although South African workers have shown experimental viral transmission from buffalo to contact cattle as overt disease in buffalo was rare event. There have been only two documented outbreaks in South Africa, one involving type SAT-2 in 1970 (Young *et al.,* 1972) and SAT-1 in 2001 (Vosloo *et al.,* 2007).

The Angola (and probably western Democratic Republic of Congo) cluster subregion was CBPP and possibly FMD endemic, which threatened to spread into northern Namibia and south-western Zambia. However, the true incidence of FMD in this cluster was unknown. Official data indicated FMD has not been recorded in Angola since 1974 (F. Vissesse, personal communication). The Great Lakes cluster comprised the countries of East African Community (EAC) (Tanzania, Uganda, Kenya, Rwanda and Burundi) plus the eastern part of the Democratic Republic of Congo (DRC) which had both large livestock population and the highest global wildlife concentration. Farming dominated this agro-pastoral and pastoral communities characterized by communal grazing and migrations. Eastern DRC was heavily dependent on trade livestock from Uganda, Tanzania, Rwanda and Burundi.

This Great Lakes cluster probably contained the most complicated FMD situation in the world. The cluster probably contained several FMD primary endemic foci. Five serotypes (O, A, C, SAT-1 and SAT-2) were endemic in this cluster and a sixth serotype (SAT-3) was isolated in wildlife (African buffalo) in Uganda in 1970 (Hedger *et al.*, 1973). However, the role of African buffalo in maintenance and transmission of FMD serotypes that occurred in this cluster has not been systematically studied.

The Horn of Africa/IGAD Cluster (Sudan, Eritrea, Ethiopia, Djibouti, Somalia, Northern Kenya and Northern Uganda), which probably harboured major FMD primary endemic foci as Ethiopia and Sudan have the highest cattle populations in Africa with major ambition for cattle and small ruminants export especially to the Middle East.

The WRL for FMD identified type O topotype in Sudan that was peculiar for this cluster. The Soudan/Sahel Cluster comprised Western Sudan, Niger, Chad, Burkina Faso, Mali, Northern Nigeria, Senegal and Mauritania. The farming system in this ecosystem was predominantly pastoral, characterized by long distance movement of livestock due to either transhumance or trade. This cluster probably also contained important FMD primary endemic areas. Furthermore, it may be an important disease corridor cluster, linking the Inter-governmenttal Authority on Development (IGAD) cluster with West Africa and probably West Africa with North Africa. The 1999 FMD strain in Algeria was found to be related to the West African type O topotype as transhumance and trade were important vehicles in the past for spreading rinderpest in this region (Roeder *et al.,* 2005).

Although the FMD epidemiology in the Coastal belt countries of West and Central Africa has not been extensively studied, it maybe assumed that this cluster probably aquired infection from Soudan/Sahel clusters and described as secondary endemic. North Africa/Maghreb Cluster Morocco, Algeria (last infected by type O) and Tunisia have not reported FMD since 1999, because of routine preventive vaccination and other biosecurity measures.

Libya and Egypt recorded sporadic FMD outbreaks bacuase of routine preventive vaccination enforcement as Libya reported SAT-2 infection in 2003, probably as a result of live animal introductions from neighbouring countries to the south, breaching Sahara barrier. Egypt experienced a type A outbreak in 2006 after many years of freedom from this serotype as this virus was shown to be genetically related to type A strains previously isolated from Eastern Africa (Wadsworth *et al.,* 2006).

2.3.3 Transmission of FMD

FMD virus in unvaccinated herds usually spread rapidly affecting over 90% population within a group while in vaccinated herd virus aerosol production from a single infected animal can infect the entire herd with resultant appearance of clinical disease (Alexandersen *et al.,* 2002).

The virus is transmitted through a variety of mechanisms such as inhalation or ingestion (from infected source to susceptible animals), direct contact with infected animals, mechanical fomites (eg, vehicles, instruments, and feed), Airborne (up to 60 km overland and 300 km by sea) in temperate zones under proper conditions (these include a high viral load [eg, swine herd infection], stable atmospheric conditions, and susceptible population downwind) (Lubroth, 2002).

Foot and mouth disease usually spread by infected animal movement when susceptible cattle come in contact with infected animal whether sheep, goat, pig or wild life species and may be infected by respiratory route, or through skin or mucous membrane abrasion (Kitching 2002a). Calves drinking infected milk can be infected by inhalation of milk droplets during suclking. Of the domesticated susceptible species, cattle are the most susceptible to viral aerosol generated by other infected animals, particularly pigs, because of their larger respiratory volume when compared with small ruminants and their higher susceptibility by this route of infection compared to pigs.

Cattle susceptibility by this respiratory route requires as little as 20 $TCID_{50}$ (tissues culture infective dose) of virus to establish infection but may require 10,000 times more to become infected by oral routes (Donaldson, 1987a). More than 50% of cattle that have recovered

from infection with FMD virus and vaccinated cattle that have had contact with live virus become carriers (Salt *et al.,* 1996). These cattle are capable of transmitting the virus to other susceptible in-contact animals in spite of their subclinical infection nature (Kitching, 2002b).

Milk and semen from infected cattle may contain virus up to four days before the onset of visible signs, when clinical signs appear the virus titre would have peaked at $\log_{10}$6.7 $TCID_{50}$ per ml of milk and $\log_{10}$ 6.2 $TCID_{50}$ per ml of semen (Donaldson, 1987a). Urine may contain $\log_{10}$ 4.9 $TCID_{50}$ and faeces $\log_{10}$ 5.0 $TCID_{50}$ per gram (Kitching, 1992). The virus persists particularly in basal epithelial cells of the pharynx (Zhang and Kitching, 2001) and recovered animals (Salt *et al.,* 1996).

Slaughtered animals may spread airborne FMDV via infected skin cells in livestock infected with foot and mouth disease simply by skin exposure to wind and/or mechanical abrasion (e.g. moving animal carcasses, spraying hides with water) as these skin cells shed off (Dillon, 2011).

The Cape buffalo was identified as natural hosts for SAT serotypes of FMD, although they may be infected by all serotypes (Hedger *et al.,* 1973; Hedger 1976) and frequently become infected, subsequently circulating virus (es) amongst the herd(s) and acting as reservoir. This discovery along with clear spatial associations with outbreaks in cattle gave credence to FMD control strategy in buffalo as a means of disease control in cattle (Thomson *et al.,* 2003).

2.3.4 Communicability of FMD

In general, sheep act as maintenance hosts, pigs act as amplifiers, and cattle act as indicators (Barnett *et al*., 2002). Sheep are often infected and spread virus to susceptible animals and environment without showing obvious clinical signs (Barnett and Cox, 1999). Mild clinical signs complicated by concomitant occurrence of other common diseases such as contagious ecthyma and foot rot can mask lesions which may aid diffused FMD dissemination (Hughes *et al.*, 2002).

Disease spread amonsgt pigs infects the entire herd very rapidly as pigs produces 1,000 times more virus particles in their respiratory secretions than cattle post infection (Donaldson and Alexandersen, 2001). The high level of virus shedding increases aerosol opportunity for spread and environmental contamination. Often the disease is first recognized in cattle because lesions develop sooner in cattle disease course and manifest with more severe clinical signs. Virus is shed in saliva, feces, urine, milk, semen, and breath for up to 4 days before clinical signs appear in acutely infected animals.

Animals that have recovered or have been vaccinated can become carriers with virus persistence in pharyngeal region for more than 28 days post infection and have been considered as carriers. Different animals can be carriers for different duration of time as observed in Cattle: Up to 3.5 years, Sheep: Up to 9 months, African Cape buffalo: Lifelong (herd setting), Pigs: Do not become carriers (Barnett *et al*., 2002).

2.4 Pathogenesis of Foot and Mouth Disease

FMD affects a wide variety of cloven-hoofed animals but the pathogenesis was extensively studied in cattle and pigs (Gurbman and Baxt, 2004). Infection of cattle generally occurs via the respiratory route by aerosolized virus and less efficiently through skin or mucous membrane abrasions which require almost 10,000 times more of virus (Donaldson, 1987b). FMD virus is usually excreted into cattle dairy milk (Ray *et al.,* 1989) as well as in semen, urine, and feces (Kitching, 1992) and calves can become infected by inhaling milk droplets. Infected cattle also aerosolize large viral quantities, which can infect susceptible cattle in addition to other species (Sorensen *et al.,* 2000). Studies have suggested that lung or pharyngeal areas are sites of initial virus replication (Burrows *et al.*, 1981), with rapid virus dissemination to the oral and pedal epithelial areas possibly mediated by cells of monocyte/macrophage origin (Brown *et al.,* 1992).

In situ hybridization (ISH) of cattle experimentally infected via aerosol, showed evidence of virus in the respiratory bronchiolar epithelium, subepithelium, and interstitial lung areas within first 24 hrs and by 72 hrs vesicular lesion was detected in the epithelial cells of tongue, soft palate, feet, tonsils, and tracheobronchial lymph nodes (Brown *et al.,* 1996). Other studies, however, suggested pharynx and not the lungs as initial viral replication site in infected cattle (Zhang and Kitching, 2001).

This observation about the respiratory tract region initially infected in cattle exposed to aerosols may be associated with a number of variables such as aerosol particle size and virus strain (Alexandersen *et al.,* 2003). Vesicles develop later at multiple sites on the feet and tongue, and are usually preceded by fever. Severe lesions often occur in areas subjected

to trauma or physical stress and some animals develop viremia. The incubation period can be between 2 and 14 days, depending on infection dose and route of infection (Gailiunas and Cottral, 1966).

2.4.1 Virulence factors of FMDV

In theory, viral structural and NS proteins, viral RNA element, host proteins and membranes that participate in viral replication cycle can be considered as virulence factor, since defects in the factor or its absence in the cell may lead to virus' inability to replicate and cause disease in host species. Viral and host factors that might be involved in virulence include, capsid proteins (VP1 to 3), Lpro, IRES elements, S region, poly(C), [*cre*, 2B, 2C, 3A, 3B, 3Cpro, 3Dpol], Capsid proteins (VP0, VP1, and VP3), 3Cpro, 2A, viral RNA and host factor such as Integrins, other receptors, entry factors, eIFs, PTBP, ITAF45, PCBP, and Cellular membranes. Some of these listed factors have been shown to play a role in virulence either in FMDV system or that of other picornaviruses (Mason *et al.*, 2003). These viral receptors play role in tissue tropism and disease pathogenesis (Schneider-Schaulies, 2000).

Viral integrin receptors have been identified to aid FMD virus ability to utilize alternative receptors. Virus with RGD sequence of the VP1 G-H loop either mutated or deleted cannot replicate in tissue culture or cause disease in animals (Leippert *et al.*, 1997). A type O virus variant adapted to utilize the HS receptor in-vitro showed the importance of virus-integrin interaction in-vivo. This virus was shown to be relatively avirulent in cattle, while the wildtype virus, which required only integrin receptors to initiate infection in vitro (Ochs *et al.*, 2003) was virulent in bovine (Sa-Carvalho *et al.*, 1997).

FMDV need three integrin receptors to cause disease in susceptible hosts. However, tissue culture demonstrated different virus serotypes exhibited altered efficiencies of integrin utilization (Duque and Baxt. 2003). It is possible that the virus used different receptors during various diseases stages as it appeared disease process in susceptible animals was mediated by virus-integrin interaction, a type C virus containing an RGD sequence has been isolated from a bovine which was not protected from virus challenge following immunization with an experimental peptide vaccine (Tami *et al.,* 2003).

In addition, a tissue culture-adapted type C virus with a genetically engineered RGD sequence, which was unable to bind to heparin, was able to infect cells expressing both HS and integrin receptors and cells which do not express FMDV integrin receptors and HS (Baranowski *et al.,* 2001). The ability of these two viruses to cause disease in susceptible animals has not been demonstrated. Tissue culture-adapted derivative of a type O1 virus, isolated in China, was able to replicate in tissue culture in both an integrin- and HS-independent manner and caused mild disease in pigs (Zhao *et al.,* 2003b). Thus, there is a possibility that non-integrin receptors may be involved in disease pathogenesis.

However, with the exception of the virus isolated after challenge from the peptide-vaccinated bovine (Taboga *et al.,* 1997), no natural isolate of FMDV which does not contain the RGD sequence within the VP1 G-H loop has been identified. Lpro was shown to be a virulence determinant based on experiments with animals with genetically engineered type A12 virus with Lpro deleted (leaderless) (Chinsangaram *et al.,* 1998a). It was thought that this virus would be less virulent than the wild-type virus, since the lack of Lpro would lead to viral inability to cleave eIF4G and shut off cellular protein synthesis. This virus replicated at only a slightly lower rate than wild-type virus in BHK-21 cells

(Piccone *et al.*, 1995), and was markedly avirulent when injected into cattle and pigs and was unable to spread to cohoused animals (Mason *et al.*, 1997b).

The mechanism of attenuation of leaderless type A12 virus was examined by aerosol exposure of cattle. Wild-type-infected cattle had histologically altered respiratory bronchioles and virus-specific ISH signals in bronchioles by 24 hrs, and by 72 hrs they developed clinical disease, including fever and vesicles on the feet and positive ISH signals in epidermal sites corresponding to visible lesion development. In contrast, cattle infected with leaderless virus showed no clinical disease at 72 hrs and no pulmonary changes at either 24 or 72 hrs. These animals had only limited positive virus-specific ISH signals in respiratory bronchioles by 24 hrs and had no evidence of lesions or ISH signals in epithelial tissue by 72 hrs (Brown *et al.*, 1996). Thus, leaderless virus did not appear to replicate well at the site of primary infection and was not able to spread to other sites within the host. It was subsequently shown that infection with wild-type or leaderless virus induced synthesis of alpha/beta interferon (IFN alpha/beta) mRNA both in tissue culture (Chinsangaram *et al.*, 2001) and in lung mononuclear cells from aerosol exposed cattle (Brown *et al.*, 2000), but in tissue culture, IFN activity was detected only in leaderless-virus-infected cells (Chinsangaram *et al.*, 1999).

The latter observation can be attributed to the inability of leaderless virus to inhibit host translation, including IFN synthesis, production of IFN within infected animal probably inhibited initial amplification and spread of the virus. In contrast, wild-type virus infection blocked capped IFN mRNA translation, which allowed rapid virus spread to neighboring cells systemically prior to the induction of adaptive immune response. Molecular characterization and role of 3A protein in viral virulence was demonstrated during studies

with FMDV isolate responsible from outbreak in Taiwan in 1997 (designated O/Taw/97) as the virus revealed 10-codon deletion in the C-terminal half of 3A protein (Beard and Mason, 2000). The location of this deletion was similar with that 19- to 20-codon deletion found in FMDV passaged in chicken embryos with reduced virulence in bovines (Giraudo *et al.,* 1990).

The role of this deletion in the bovine-attenuated phenotype was confirmed by using reverse genetic analysis (Beard and Mason, 2000), and analysis of viruses suggested that in addition to the deletion, mutations in 3A protein around the deletion may also be responsible for the observed phenotype (Knowles *et al.,* 2001).

The molecular basis for porcinophilic phenotype appeared to be related to a reduction in viral RNA synthesis, which is manifested to a greater degree in bovine cells than in swine cells (O'Donnell *et al.,* 2001). The 3A proteins from either porcinophilic or a bovine-virulent isolate co-localized to RNA replication complexes in either bovine or porcine cells or also caused disruption of the Golgi apparatus (O'Donnell *et al.,* 2001). However, no reason was associated with 3A deletion effect on FMDV replication in bovine cells more than in swine cells. It was also shown that a single amino acid change in the 3A protein was responsible for FMDV adaptation to guinea pigs, but the mutation was located in different region other than the deletion associated with porcinophilic phenotype (Nunez *et al.,* 2001).

It has been suggested that IRES elements, and possibly the host factors that bind to them, affect the pathogenicity and virulence of other picornaviruses (Malnou *et al.,* 2002) but in FMDV, rescued from persistently infected BHK-21 cells showed two mutations within the IRES, which suggested increased virulence of the virus in tissue culture (Martinez-Salas *et*

al., 1999). Later, preliminary results suggested that the FMDV-specific IRES-binding protein ITAF45 may play a role in virus virulence by controlling virus tropism within tissues of susceptible species (O'Donnell, 2001).

2.4.2 Host response to FMD

The virus generally elicits rapid humoral response in either infected or vaccinated animals, as virus-specific antibodies protect animals in a serotype-specific manner against reinfection, or against infection in the case of vaccination, and protection is correlated with high levels of neutralizing antibodies (Salt, 1993). The 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 (Capozzo *et al.,* 1997), and IgA, can also be detected in upper respiratory secretions in early infection (Salt *et al.,* 1996).

The neutralization of virus within the host may occur by mechanisms similar to those occurring in in-vitro neutralization but there exist a possibility that macrophages may play a role in clearing the virus from infected animal by phagocytosis of opsonized virus (Rigden *et al.,* 2003) as a means of cellular immunity in protection of FMD infected animals. Specific T-cell antiviral responses, involving $CD4^+$ and $CD8^+$ cells, have been observed in cattle and swine following either infection or vaccination (Bautista *et al.,* 2003), and cell-mediated immunity has been involved in clearance of virus from persistently infected animals (Childerstone *et al.,* 1999). The induction of anti-FMDV antibody called Blast transformation correlated with a lymphoproliferative response in cattle and swine (Collen and Doel. 1990) and T-cell dependent in mice (Collen *et al.,* 1989).

Studies of early acute phase of FMDV infection in swine, prior to antibody detection, demonstrated transient lymphopenia by 2 days after infection involving $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ T cells, which does not appear to be related to either infection of 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 was either reduced or eliminated (Bautista *et al.*, 2003) as both the number of lymphocytes and the altered T-cell function returned to normal levels by 4 days after infection. These results suggested T cells played a role in virus protection and the reduction of both T-cell numbers and function enhancee viral pathogenesis due to virus spread within the host, with resultant increased in viral shedding into the environment. Also immunization of both cattle and swine with a replication-competent human adenovirus 5 (Ad5) vector expressed in the P1 capsid precursor did not yeild virus-specific neutralizing antibody in the serum but partially protected animals from FMDV challenge (Sanz-Parra *et al.*, 1999a) which may further indicate a role for cellular immunity in protection from FMDV infection but it is also possible that innate immune responses may be responsible for the observed protection.

There has been increasing interest concerning the role of the innate immune response of the host to both FMDV infection and vaccination. A number of studies have shown that IFN-alpha, -beta, and –gamma may be involved in host defense against FMDV infection (Alexandersen *et al.*, 2002). In addition to the IFNs, other cytokines may also play a role in the host response. In studies of swine which were immunized with a conventional FMD vaccine, it was shown 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, which suggested monocyte/ macrophage activation (Barnett *et al.,* 2002). Although the levels of IL-6 and IL-8 did not appeared to be related with swine protection upon challenge, IL-12 levels were higher in vaccinated pigs, which were protected from contact challenge, suggested a role for cytokine-induced monocytic cell activity in protection from acute-phase disease (Barnett *et al.,* 2002).

2.4.3 Carrier state

Following FMDV infection acute phase in ruminants, some animals may experience a long asymptomatic persistent infection. Also, animals which have been successfully vaccinated may also become persistently infected if exposed to infectious virus. These animals are referred to as carrier animals and the carrier state is a complication which can occur during outbreak situations (Alexandersen *et al.,* 2002b).

Live FMDV was first demonstrated and recovered from esophageal-pharyngeal fluids of cattle during FMD convalescent phase (van Bekkum *et al.,* 1959). Carrier animals are defined as those from which live viruses can be isolated 28 days post infection (Sutmoller *et al.,* 1968). In domestic cattle, the carrier state can last as long as 3.5 years, and it has also been identified in sheep and goats but not in pigs (Alexandersen *et al.,* 2002b). African buffalo have been reported to carry live virus for up to 5 years (Condy *et al.,* 1985) and other cloven-hoofed wildlife may become carriers (Alexandersen *et al.,* 2002). The number of carrier animals in a population depends on the species, incidence of infection, and the herd immune state (vaccinated or not vaccinated). In the African buffalo, carrier rate can be

as high as 50 to 70% in the field (Condy *et al.,* 1985) and rates in cattle and sheep can vary widely, from 15 to 50% (Alexandersen *et al.,* 2002b).

In general, virus titer in the esophageal-pharyngeal fluids of carrier animals is usually low as virus is not consistently recovered from individual animals but the recovered virus originates in the pharynx, which appeared to be the target region for persistent infection in cattle (Zhang and Kitching. 2001). Virus isolation from esophageal-pharyngeal fluids is the most sensitive method to detect carrier animals, but reverse transcription-PCR (RT-PCR) assays have been developed to increase sensitivity. The role of carrier animals in field virus spread is still controversial. The only direct evidence was that of transmission from African buffalo to cattle during outbreaks in Zimbabwe in the late 1980s and early 1990s (Bengis *et al.,* 1986; Dawe *et al.,* 1994a).

In addition, transmission from buffalo to cattle has been obtained experimentally (Dawe *et al.,* 1994b) alongside proposed transmission might occur through sexual contact (Bastos *et al.,* 1999). However, no experimental evidence to date of transmission of virus from carrier cattle or sheep to uninfected animals. The mechanisms for the establishment and maintenance of carrier state are not well understood, since persistence can occur in animals exposed to virus after either acute disease or vaccination as this long persistence and replication of virus in the host animals can lead to genetic variation in the field, possibly generation of new viral variants (Toja *et al.,* 1999).

Two mechanisms were proposed for the developed persistence in the pharynx (Alexandersen *et al.,* 2002b). One suggested FMDV infected immune system cells, such as macrophages, or other immunologically privileged sites, leading to evasion of the immune

response. Viral replication in porcine peripheral blood macrophages showed virus infection of such cells only when presented as an immune complex, presumably by Fc receptor mediated adsorption (Baxt and Mason, 1995) as the infection was abortive and did not lead to production of new infectious virus. Porcine alveolar macrophages were also not able to support viral replication but the virus bound to macrophages in the absence of specific antibody which elicted promising results (Rigden *et al.*, 2002).

In addition, virus appeared to be internalized by phagocytosis but remained infectious for 12 hrs. The second mechanism proposed that the virus exploited the host response to provide favorable intracellular conditions for long-term persistence, possibly by utilizing cytokine signaling. Studies on the innate immune response mechanisms involved in this phenomenon and possibly help in the development of methods to eliminate persistence was suggested (Gurbman and Baxt, 2004).

2.5 Clinical Signs of Foot and Mouth Disease

Foot and mouth disease (FMD) in cattle is usually obvious in unvaccinated herds in non endemic countries. However, in vaccinated herds and in some breeds indigenous to areas in which FMD is endemic, the disease may circulate undetected (Kitching, 2002). The classical clinical features of FMD in cloven hoofed mammals occur following 2-14 days Incubation period (IP) as described (Remond *et al*., 2002). This IP is dependent on the infection dose, virus strain and individual host susceptibility (Kitching, 2002).

Initial sign include pyrexia [up to 106°F (40^{o}C) lasting 1-2 days before other clinical signs], pyrexia is followed by anorexia, agalactia in milking animals and appearance of vesicles, vesicles in oral cavity are on mucosal surfaces, including tongue, dental pad, gums, and

lips, feet vesicles are on bulbs of heel, interdigital cleft and coronary bands (Clavijo *et al.*, 2004a).

Painful lesions cause profuse drooling saliavation, bruxism, foot stamping and lip smacking with ruptured vesicles within hours to 2 days, leaving erosions and recovery generally occurs within 8-15 days. Associated complications include, tongue erosions, further increasing anorexia, lesion super infection, particularly on feet, hoof deformation or sloughing of hoof horn, mastitis with permanent production milk impairment, myocarditis, abortion, permanent weight loss, loss of heat control ("panters") due to pituitary effects. Death and case-fatality rate is usually greater than 60% in young animals of all susceptible species (Aggarwal *et al.*, 2000) primarily due to myocardial effects (OIE, 2000, Kitching, 2002a).

FMDV serotype O infection has been associated with sub-functional ovarian activity and disturbed oxidative status in buffalo-cows due to FMD virus influence on ovarian function and graafian follicles, oocyte growth and maturation (Kawther and Ahmed, 2008).

2.5.1 Pathology

Vesicles or sore lesions are found on the tongue, dental pad, gums, cheek, hard and soft palate, lips, nostrils, muzzle, coronary bands, teats, udder, snout of pigs, corium of dewclaws and interdigital spaces. Post-mortem lesions are noticed on the rumen pillars, in the myocardium, particularly of young animals (tiger heart) (Patil *et al.*, 2002) usually seen as grey areas especially in the walls of the left ventricle which appear striped (Kitching, 1992). The cells of the stratum spinosum undergo ballooning degeneration and as the cells rupture and oedema fluid accumulates, developed vesicles coalesce to form aphthae and

bullae that characterize FMD, in addition, cells of skeletal muscle may also undergo hyaline degeneration (Kitching, 1992).

2.6 Diagnosis of Foot and Mouth Disease

Initial diagnosis is based on clinical signs, with or without a history of contact between infected herd and suceptible animal, or report of FMD in any vicinity. In a fully susceptible herd, clinical signs are frequently severe and pathognomonic. However, in endemic regions in cattle that have partial natural or vaccinal immunity, clinical signs may be mild and or undetected (Huang *et al.,* 2000). Lesions and/or effects of inflammation that are readily detectable during acute FMDV infection are usually on the feet, either on the coronary band, interdigital cleft and mouth as sore(s) on the lips, muzzles or snouts of sheep, cattle or pigs. The first signs of inflammation maybe noticed as increase surface temperature of inflamed area as remote thermal imaging of feet and heads of FMD infected animals may provide a non invasive method of assessing infection (Nikkhah *et al.,* 2005; Bashiruddin *et al.,* 2007).

Further disease confirmation can be done using various conventional diagnostic tests, however, the threat of deliberate release of FMDV in the era of terrorisim after 11 September 2001 has caused governments, including those of the United Kingdom and the United States to support the development of new diagnostic and disease control approaches. The considerable amount of information that has become available about FMDV at the molecular level and limited understanding of virus-host interactions ushered in development of new rapid diagnostic assays, novel vaccine candidates, and antiviral control approaches (Gurbman and Baxt, 2004).

A pen-side Loop-mediated amplification (LAMP) method for FMDV genomes detection that is rapid, sensitive, and cost-effective for field diagnosis in developing countries was developed (Dukes *et al.,* 2006) which do not require denatured template as DNA is generated in abundance so that positive LAMP reactions can be visualized with the naked eye (Iwasaki *et al.,* 2003). RT-rapid detection of foot-and-mouth disease virus by Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) method was found simple, rapid, cost-effective, efficient and it can be performed in a water bath, which would facilitate its use particularly in developing countries for rapid detection of FMDV in the laboratory or under field conditions (Shao *et al.*, 2010). However, this disposable equipment must not be contaminated during sample preparation and appropriate controls must be included to detect the presence of any cross-contamination during the entire experimental process.

2.6.1 Specimen for laboratory diagnosis

The success of laboratory confirmation of FMD presumptive diagnosis depends on the submission of adequate material sent under suitable conditions. A minimum of $2cm^2$ of epithelium from ruptured vesicles in 50/50 mixture of glycerine and 0.04 molar buffered phosphate (pH 7.4-7.6) should be sent to designated laboratory for handling live FMD virus and equipped with the necessary reagents for typing a positive sample. Whole blood and clotted blood samples as well as probe bang samples could be sent for diagnosis (Kitching, 2002a; Kitching and Hughes, 2002).

Oropharyngeal fliud could be collected by probing cup (speculum) or swabed in transport media containing 0.08 M phosphate buffer with 0.01% bovine albumin, 0.0002% phenol red, and antibiotics (1,000 IU penicillin) with a final 7.2 pH. Sample should be immediately frozen to -40^{o}C and transported on solid carbon dioxide or liquid nitrogen until arrival at laboratory (OIE, 2000; Remond *et al.,* 2002).

2.6.2 Laboratory diagnosis

The OIE Terrestrial Manual (OIE, 2004) described four virological identification methods namely Virus Isolation (VI), Antigen (Ag) ELISA, Complement Fixation Test (CFT) and Nucleic acid recognition methods as reviewed (De Clercq *et al.,* 2008).

2.6.2.1 Virus isolation

Virus isolation remains the ultimate proof of the presence of live FMDV. Primary cells like bovine thyroid cells (Snowdon, 1966) or lamb kidney cells (House and House, 1989) are very sensitive, but laborious to maintain. In addition, the quality of this cell-based detection can differ with each cell batch and therefore require a well-established QA/QC system to guarantee a 24/24 hr availability of cells with the same sensitivity (Sn) (Ferris *et al.,* 2002). Generally, pig cell lines are used such as IB-RS-2, PK15 or SK6, or a baby hamster kidney (BHK) cell line. The cell line must be sensitive enough to isolate FMDV from samples originating from different species as pig cell lines have not always been suitable for isolation of FMDV obtained from goats or sheep with very low amount of virus harvest (Bouma *et al.,* 2001).

The BHK-21T cell line on the other hand, seemed to be less species dependant (Ahl, 2000). Well established low-positive controls are essential within a QA/QC system to prove that each cell batch is highly sensitive. These low-positive controls must be calibrated to internationally accepted standards, which are unfortunately not available. An international ring test is urgently needed for virus detection and this would increase the confidence in the cell system used to give mutual recognition of results by different laboratories and fill a big gap in the framework of accreditation. The first ring test with a limited number of laboratories was organized to prepare a much wider exercise (Ferris *et al.*, 2006).

2.6.2.2 Complement fixation test

The CFT has been of great value in the past in many FMD laboratories but it is still in use in some Transcaucasian countries and regional laboratories in Iran (Mahmoudan *et al.*, 2003) unlike in most parts of Europe because CFT is serotype-dependent and require a good practical knowledge of anticomplement reactions. CFT have been replaced by the more sensitive Ag-ELISA (Roeder and Le Blanc Smith, 1987) but the availability of an ELISA reader, ELISA plates and reagents are essential factors. This is even more of challenge for RT-PCR and real-time RT-PCR. The necessity of using a more sensitive test depends on the FMD control campaign phase.

2.6.2.3 Antigen detection

The Ag-ELISA as described by Ferris and Dawson (1988) can identify FMDV serotypes. In some European laboratories, however, the test is restricted to a subset of serotypes (serotypes for Europe A/O/C and sometimes Asia1 because these strains were considered as

the highest risk for Europe) and therefore, include a risk of false negatives. In this regard, a pan-serotype antibody would be very useful (Grazioli *et al.*, 2004).

Although the Sn of the Ag ELISA is lower than VI (Reid *et al.*, 1998), the success rate is high for samples from cattle and pigs but samples positive for ELISA result in sheep is lower (Alexandersen *et al.*, 2003) as a sample negative ELISA result requires further examination in highly susceptible cell cultures and/or RT-PCR assays. But, as for all tests, the quality of result depends primarily on sample quality. Each ELISA plate should contain the necessary low-positive controls as it is difficult to find figures for test performance. As for many tests, the validation process stopped with determining the analytical Sn (until which dilution the virus is still scored positive) and analytical Sp (cross reactivity).

Although most laboratories claim to work within an accreditation system, hardly anything is known about the rate of false positives and false negatives based on a reliable number of samples. It must, however, be stressed that the required numbers of representative positive samples needed for validation are very difficult to collect for diagnostics dealing with exotic diseases. To be statistically meaningful, the number of samples must be relatively high for checking the presence of FMDV in sheep flocks or in vaccinated cattle with low excretion of virus as probang sampling is highly unpractical on large scale and is only sensitive enough if consecutive samples are taken (Kitching, 2002b). Mouth or nose swabs are alternatives (Callens *et al.*, 1998) that could provide a large number of samples in which low viral antigens can be detected by an improved Sn ELISA.

Pen-side test based on the same reaction between antibody-Ag was demonstrated by chromatography technology. Several companies in collaboration with World Reference Laboratory (WRL) (Ferris *et al.,* 2001; Reid *et al.,* 2001a) have worked on development of these kinds of tests. The assay developed by the latter was based on a pan-serotype reactive monoclonal antibody for Ag capture. Penside tests are extremely fast, easily repeatable and do not require well-organized and safe sample transport. However, they must be very robust and therefore, a full validation is absolutely essential. Pen-side tests brought in the problem of the user and result interpretation. Potential users need to be aware of the nature and methods of samples to be taken as well as interpretation of negative and positive results with corresponding action to be instituted (Crauwels *et al.,* 2001). Pen-side tests should be used by accredited people in the field, while continuous training and validation is advocated even in absence of an FMD outbreak.

The protein or peptide micro-arrays (Biosensors; Ab Microarray) were also designed for used as screening tools (Yuk *et al.,* 2004) and they constitute a clear high-throughput method. A wide range of different monoclonal antibodies (mAbs) are immobilized on a glass surface and a fluorescent image reader is used to read the result. The most difficult element in micro-array platform is the data analysis as expensive software is needed for result interpretation. Ab micro-array using panels of mAbs developed for Ag profiling covering a very wide range of FMDVs have been developed for screening (Grazioli *et al.,* 2004). Some preliminary reports have been published exploring such 'biosensor' applications for veterinary purposes (Feliu *et al.,* 2002; Gomes and Andreu, 2002), although not all applications involved the use of multiplexed arrays, such as the piezo

electric crystal based detection of FMDV (Gajendragad *et al.,* 2001) and the bio-engineered enzyme techniques (Blanco *et al.,* 2002).

2.6.2.4 Nucleic acid recognition methods

Discussion is ongoing whether a positive RT-PCR result really means that live virus is present for FMD in the field. Various PCR procedures have been developed such as the conventional RT-PCR (Pattnaik *et al.,* 1997), RT-PCR ELISA (Callens *et al.,* 1998), nested RT-PCR (Rodriguez *et al.,* 1992), RT-PCR combined with restriction enzymes (Moonen *et al.,* 2003), real-time RT-PCR (Zhang and Alexandersen, 2003; Oem *et al.,* 2005; King, 2006). Some of these methods could distinguish the different FMD serotypes (Callens and De Clercq, 1997; Reid *et al.,* 2001b).

These nucleic acid-based tests are faster than and as sensitive as VI. To use RT-PCR as high throughput tests, expensive automates are necessary (Reid *et al.,* 2003) and even then 'only' 100–140 samples a day could be managed, which is too few for statistically validated screening for the presence of virus in the aftermath of an FMD outbreak. Using tests with high Sn also means that thorough validation, extremely high laboratory standards (to prevent cross-contamination) and QC are needed to allow their routine application. Inherent to nucleic acid amplification techniques is their need for a quality DNA/RNA sample. The importance of optimization of this nucleic acid purification step must be stressed (Scheibner *et al.,* 2000), and considerable efforts have been made to standardize and upscale it (Lemieux, 2000).

The incorporation of non-specific fluorescent dyes or specific fluorescent hybridization probes into a PCR reaction enables detection of DNA fragment copies build-up during PCR cycling using specialized equipment. Real time PCR offers the advantage of performing amplification and detection in a closed tube format, limiting the chance for contamination. The absence of an additional electrophoresis step also makes real-time PCR suitable for automation (Mackay *et al.,* 2002) which led to development of a portable real-time RT-PCR as a kind of pen-side test (Hearps *et al.,* 2002).

The term multiplex real-time PCR was used to describe the use of distinct fluorogenic oligoprobes for the discrimination of multiple amplicons (Mackay *et al.,* 2002). There was only a few multiplexed real-time PCR assays described in literatures, and few of these have been applied to diagnosis of infectious diseases (Scahaw, 2003). This was partly because of the limitations set by the number of fluorophores that can be combined and clearly distinguished in one assay and the number of controls that have to be included. The need to include valuable controls was very relevant. Some teams used plasmids, others synthetic cDNA or RNA. The latter, however, was the only one that covered the whole chain from RNA isolation to PCR product which required standardization.

DNA microarrays are microscopic glass slides on which cDNA fragments are spotted. The cDNA fragments can be hybridized with previously labelled viral genome when present in the sample. In the veterinary world, it has been described for differential diagnosis of pestiviruses (Deregt *et al.,* 2006) and for typing of FMDV (Baxi *et al.,* 2006). The latter results opened interesting perspectives in the generalization of the use of this high throughput micro-array technology for FMDV epidemiological study. However, all

currently described applications required time-consuming DNA or RNA amplification by PCR, followed by fluorescent labeling of amplified nucleic acids prior to micro-array hybridization. This technique is very expensive and making full validation studies is quite difficult.

2.6.2.5 Other Serological tests

The virus infection associated Ag assay (VIAA) precursor of the NSP tests, was applied in Europe for an FMD serological survey (McVicar and Sutmoller, 1970). The VIAA test detected mainly antibodies to 3D protein, which was present in inactivated virus used in vaccines. The level of 3D can be quite high depending on the Ag purity, but maybe present even in purified virion and therefore, VIAA did not reliably distinguished infection from vaccination (Kitching, 2002b). Later, the VIAA was replaced in South-America by a combined system of an indirect ELISA-3ABC with an enzyme-linked immunoelectrotransfer blot assay as confirmatory test (Bergmann *et al.,* 2000).

FMD laboratories worldwide now use well validated serological tests based on protocols described in the OIE Terrestrial Manual (2004) that are continuously monitored (Goris and De Clercq, 2005b). ELISA is been used for mass serology, especially the Liquid Phase Blocking ELISA (LPB ELISA) to detect antibodies against FMDV (Mackay *et al.,* 2001) as well as the more sensitive and specific solid phase competition ELISA (SPC ELISA) (Paiba *et al.,* 2004).

Positive results in ELISA must be confirmed by VNT in an NRL with a P3 biosecurity level as VNT is more strain-specific (Paton *et al.,* 2002) with regard to the different subtypes. Therefore, the test must use an antigenically FMDV closely related to field virus,

otherwise, false-negative results would be obtained. Antibodies detection to FMDV-NSP 3ABC has been shown to be sensitive and specific differentiation method between infection and vaccination (Sørensen *et al.,* 1998; Clavijo *et al.,* 2004a, b).

Other NSPs have also been used in ELISAs as recombinant proteins (Mezencio *et al.,* 1998; Chung *et al.,* 2002) or as peptides (Liu *et al.,* 2002). The antibody response against NSPs has been shown to persist for a year or so following infection (Mezencio *et al.,* 1998), but the long term duration has not yet been established. Where conventional vaccines, which contain traces of NSP are used repeatedly, some animals can develop specific NSP antibody (Mackay *et al.,* 1998b). Vaccine manufacturing methods have been reported where NSP component was reduced to a level that will not cause detectable seroconversion following vaccination (Doel, 2001) with minimal difficulty but not all FMD vaccines are purified to this degree.

Commercial ELISAs currently available using kits developed in production units of European or American Companies such as: Ceditest^{R} FMDV-NS [Cedi Diagnostics B.V., Lelystad, the Netherlands (Jacobs and Moonen, 2004; Sorensen *et al.,* 2005)]; SVANOVIR TM FMDV 3ABC-Ab ELISA [Svanova, Uppsala, Sweden (Persson *et al.,* 2004)]; CHEKIT-FMD-3ABC [Bommeli Diagnostics, Bern, Switzerland (Schalch *et al.,* 2002; Bruderer *et al.,* 2004)]; UBI^{R} FMDV NS ELISA [United Biomedical Inc., New York, USA (Shen *et al.,* 1999; Liu *et al.,* 2002)]. NSP validated and licensed commercial test kits must be used as serological reference standards. These internationally recognized standards are essential for the production of secondary and working standards used as internal QC (Goris and De Clercq, 2005a) and for batch release (Luyten *et al.,* 2004). This would make the

comparison and mutual recognition of results between NRL easier. The FAO WRL developed bovine serum panel for evaluation of newly developed (confirmatory) FMDV NSP antibody tests (Parida *et al.,* 2006).

2.7 Differential diagnosis of Foot and Mouth Disease

The following three diseases are clinically indistinguishable so the require laboratory diagnosis for clarification in the FMD outbreaks. Vesicular stomatitis caused by Vesicular stomatitis virus, (family Rhabdoviridae) Swine vesicular disease caused by Swine vesicular disease virus (family Picornaviridae) and Vesicular exanthema of swine caused by Vesicular exanthema of swine virus (family Caliciviridae). Other diseases considered as differential diagnosis include, Rinderpest caused by Rinderpest virus (family Paramyxoviridae), Infectious bovine rhinotracheitis caused by Bovine herpesvirus 1 (family Herpesviridae), Bluetongue caused by Bluetongue virus 1-25 (family Reoviridae), Bovine herpes mammillitis caused by Bovine herpesvirus 2 (family Herpesviridae), Bovine papular stomatitis caused by Bovine papular stomatitis virus (family Poxviridae), Malignant catarrhal fever caused by Alcelaphine herpes virus 1 (family Herpesviridae), Peste des petits ruminants caused by Peste des petits ruminants virus (family Paramyxoviridae), Bovine viral diarrhea caused by Bovine viral diarrhea virus (family Flaviviridae), Bovine mucosal disease in cattle persistently infected with bovine viral diarrhea virus and Foreign bodies or trauma (Lubroth, 2002).

2.8 Treatment, Prevention and Outbreak control

2.8.1 Treatment

There is no specific treatment for animals affected with FMD (Kitching 1998).

2.8.2 Prevention

Protection of FMD-free areas by animal movement control and surveillance is usually the first recommended protocol, followed by infected animal quarantine and import restrictions on feed, animal products, and livestock from areas without FMD-free status. Finally, emergency and routine vaccinations in enzootic (non-FMD-free) regions using customized vaccines to reduce disease production impact (Kitching 1998).

2.8.3 Outbreak control

Gobal progressive FMD control was regarded as a major contribution needed for international public good (Rweyemamu *et al.,* 2008b). The recent FMD outbreaks in many parts of the world, particularly in developed countries, exacted profound effect on the range of disease control procedures that are currently being considered in event of additional incursions. Thus, emergency vaccination, vaccination-to-live policies, and antiviral approaches are actively encouraged measures approved by the OIE and reports from government-sponsored boards of inquiry (Gurbman and Baxt, 2004).

Procedures for FMD control and eradication are well documented and would depend on existing status of affected country or zone prior to outbreak. In FMD disease free countries, a policy of slaughter for all infected and in contact susceptible animals is usually employed,

where as vaccination would be used to control an outbreak in an endemic area. Vaccination may also be used to surround a focal disease outbreak to virus prevent spread and the vaccinated animals may be subsequently slaughtered to reduce delay in re-establishing trading status (OIE, 2001) The current method of FMD control in Europe still involves culling of infected and contact animals ("stamping out policy"), animal movement restriction and zoosanitary measures as well as ring emergency vaccination (Anon, 2001).

Application of modern FMD vaccines as alternative disease control method has been widely discussed (Niedbalski *et al.*, 2003). A vaccinated animal buffer zone may be used to separate an area within a country where FMD is endemic from an FMD-free area, from which exports of cattle and cattle products are sourced (Kitching, 2002b). However, difficulties usually encountered in regularly vaccinated herds have being the interference in response to vaccination by young animals with high levels of maternally derived antibody (Kitching and Salt, 1995).

The new OIE code as regard FMD freedom without vaccination indicated that a country may use emergency vaccination, but has to either cull all the vaccinated animals or use serological tests that detect antibodies against foot and mouth disease virus (FMDV) non-structural proteins (NSPs) for the differentiation of infected from vaccinated animals (Niedbalski, 2004) during export/import serological examination. Outbreaks can be controlled by one or a combination of two methods: Stamping out (slaughter of all animals) and routine animal vaccination (Domingo *et al*., 2002).

The advocated progressive control strategy for FMD by Rweyemamu *et al.,* (2008b) in endemic settings was based on a seven stage process namely: (1) Assessment and definition

of national FMD status; (2) Vaccination and movement control institution; (3) Viral transmission suppression to achieve absence of clinical disease; (4) Achievement of FMD freedom by vaccination in accordance with OIE standards; (5) Achievement of FMD freedom without vaccination in accordance with OIE standards; (6) Extension of FMD free zones; and (7) Maintaince of FMD Freedom. Concomitant with progressive FMD control, there is the need for risk reduction measures advocacy such as in-country commodity processing in order to encourage livestock commodity regulated trade without unduly increasing the risk of disease spread. This progressive FMD control should also be seen as part of reducing global threat of infectious diseases to human health and economic development which requires integrated roles of different stakeholders.

2.8.3.1 Role of governments and their veterinary services

Foot-and-mouth disease virus control measures enhances prohibition of entry or spread of infection, as the mangnitute of control measures required are synonmus to the level of infectiousness. Therefore it is essential to maximize compliance with disease control measures, which usually require both effective Government Services and significant private sector drivers from livestock producer associations. Government services must shoulder far more of the total responsibility, else failure may occur if these associations are weak (De clerq *et al.*, 2008).

The necessary structures for effective state veterinary services for epizootic control are described in the Office International des Epizooties Terrestrial Code (http:// www.oie.int) and by the UN FAO Good Emergency Management Practices (http://www.fao.org/ag/AGA/AGAH/ EMPRES/GEMP/index.html). Effective service is

rarely provided in countries with very limited public sector funding, with the exception of few countries where there are major private sector push to organize control campaigns. For countries where the rule of law is very weak, movement control cannot be rigorously applied and vaccination is almost the only available tool. Under these circumstances, stability and longer term national economic growth is usually required before Government Services are in a sufficient state to mount progressive control campaigns. Developing the infrastructure required for the control of one disease, or a limited subset of diseases, creates an enabling environment which facilitates progress in more general disease control activities.

The corollary of this experience is that attempting to generate all the paraphernalia of a modern official veterinary service at one time in resource-poor environment rarely achieves anything sustainable, partly because of the short time horizons used. A longer term perspective than the 5 years or so normally adopted is clearly needed for TAD control programmes. However, there are fundamental policy constraints relating to official veterinary service structure and functioning which must be overcome. Veterinary services in many developing countries are in a parlous state and recent years have seen a tendency for structural adjustment programmes to dismantle traditional line-managed veterinary services. While measures which differentiate clearly between functions relating to private good (such as clinical service delivery) and public good (epizootic and zoonotic disease control) are laudable, it must be clearly understood that excessive decentralization/regionalization and down-sizing of official veterinary services does not provide an environment conducive for effective disease control, such as FMD (Holden, 1999; Sidibe´, 2003; De Haan, 2004; Riviere-Cinnamond, 2004a, b).

One of the major problems that official veterinary services in developing countries face is the lack of appreciation and reward for needed specialist expertise. Consequently, it is extremely difficult to recruit and retain high quality staff even if they are trained at national expense. Policy makers must come to terms with the fact that veterinarians offer a spectrum of skills and expertise and that the role of official, state veterinarians is neither the treatment of sick animals nor even the vaccination of animals. Their role, in this respect, is to understand disease behaviour and manage and direct resources to effect an interruption of disease transmission to give effective control. This requires specialist expertise and specialist training. Employing official veterinarians to provide clinical services to farmers, or actually forcing them to provide such services to supplement inadequate salaries, creates a conflict of interests which militates against effective control.

2.8.3.2 Role of vaccination

Vaccination in FMD control has been extensively reviewed (Barnett and Mackay, 2008; Doel, 2003). For the purpose of disease control the main points to be considered are that; Coverage must be sufficient to stop spread; International standards of vaccine quality are essential, with the strain incorporation and potency must be appropriate as well as the vaccine must be effectively inactivated otherwise to prevent it as source of infection and lastly, effective FMD control is not merely a matter of organizing mass vaccination campaigns in the hope that this will subdue infection. Mass vaccination is just one tool to be used (Barnett *et al*., 2002); others include movement control, quarantine, rapid detection and reporting of outbreaks, focused vaccination, disinfection, sero-monitoring, sero-surveillance and public awareness (Davies, 2002; Domingo *et al.*, 2002; Toma *et al*., 2002).

2.8.3.3 Role of epidemiology and surveillance

Progressive control of disease would only achieve success when it is built on sound epidemiological understanding, which discloses determinants of disease occurrence together with mechanisms of virus maintenance and transmission (EFSA, 2006). It is therefore, essential to conceive FMD control as related to a number of independent diseases caused by different serotypes or even topotypes of FMD virus together with a clear understanding of the epidemiology of each of these serotypes. The dilemma exists of whether to promote efficiency of sample collection and analysis for the benefit of disease-free countries; or whether to engage regions where FMD is still endemic to be partners in a common good goal of FMD detection, identification and monitoring. The latter would be intended to lead to a defined strategy for progressive control of FMD (i.e. giving benefit for current endemic regions) and for progressive reduction of FMD risk in free areas [i.e. giving benefit to Europe and other Organization for Economic Cooperation and Development (OECD) countries].

The second option was clearly the more sustainable of the two. Some pertinent concepts for epidemiological analyses include laboratory testing of clinical samples in FMD endemic settings as outlined (Perry and Sones, 2007). In addition, mutually beneficial strategy to stimulate the concept of Research Partnerships as networks on a themed basis more than to organize support for a single institution and/or reference centre was conceived. This could build on the successful practice of inter-institutional and multi-country funding for research within the European Union (EU) and the experience of Research Group of the FAO European Commission for the Control of FMD (EUFMD).

A new development has been the international coalition of FMD and Epidemiological institutions from both FMD-free and FMD-endemic countries, which promoted the concept of a global roadmap for FMD control tool development in endemic settings (Perry and Sones, 2007) as the need to address how to pool data from global surveillance network into an 'early warning system' by all parties (free and infected countries) was eminent. As part of Global Framework for the progressive control of TADs (GF-TADs), the three international organizations involved with animal health [OIE or World Animal Health Organization; UN FAO and The World Health Organization (WHO)] proposed a Joint FAO–OIE–WHO Global Early Warning System for infectious animal diseases and zoonoses to be known as GLEWS (Ben Jebara, 2004; FAO-OIE, 2004) as a global 'honest broker' needed to underpin the global surveillance partnership and network.

2.8.3.4 Role of trade

The control of FMD and other major TADs in both Asia and Africa would need to be linked to promotion of livestock commodity trade through formal channels at all stages (local, regional and intercontinental trade). This way, the livestock dependent communities in Asia and Africa would also increasingly have a stake in effective disease control. Furthermore, globalization of trade in livestock commodities is an inevitable consequence of the rapidly increasing demand for foods of animal origin. It is most desirable, therefore, that such trade be through formal channels, safe and inclusive of as many livestock-dependent communities as possible (FAO, 2005).

The strategy outlined in the global framework may need to be linked to the following elements: local livestock commodity marketing infrastructures, support for low disease risk, health-assured, livestock commodity export zones; commodity processing in the countries

of production in such a way as to minimize the risk of such commodities transmitting infection and lastly, the promotion of commodity-specific standards for all major livestock commodities, including processed ones (Thomson *et al.,* 2004, 2006), which would require further applied research on risk mitigation.

2.9 Historical background of FMD vaccine production

Foot and mouth disease (FMD) occurred in many countries of Asia, Africa, the Middle East and South America (Röhrer and Olechnowitz, 1980). In the past, the United Kingdom (UK), Ireland, countries of Scandinavia, Japan, Canada and the United States of America were able to control the disease by 'stamping-out'. The UK applied this policy by law as early as 1892 (Fogedby, 1963).

In most other countries of Europe, control only became possible after the Second World War, when sufficient vaccine became available, firstly through the introduction of Frenkel culture technique and later through virus production in baby hamster kidney (BHK) line cells. The quality of vaccines incorporated with alhydrogel and saponin as adjuvant gradually increased up to 1991, when vaccination was discontinued in all countries of the European Union. In South America, compulsory cattle vaccination served for many years at best to maintain a status quo of limited outbreaks (Casas Olascoaga *et al.*, 1999).

This was due to poor quality of vaccines used and the consequent negative attitude of farmers. However, from the 1980s, all vaccine batches were controlled by independent, government controlled laboratories, and oil-based vaccines that offer a longer lasting immunity, were gradually introduced. Farming communities regained their trust in vaccination and cooperated. FMD outbreaks became sporadic events in extensive areas of South America. In the 1990s, many countries achieved freedom from FMD and obtained

the status of ‘FMD free without vaccination’ from the Office International des Epizooties (OIE: World organisation for animal health) (Sutmoller *et al.*, 2002).

The history of FMD in South America demonstrated that for the control of this widespread disease, the quality of vaccines employed was crucial for the success of an eradication programme. Bearing in mind the good record of modern vaccines in the control of outbreaks and the possibility of screening vaccinated herds for carriers, was suggested that the OIE reconsider the differences between the requirements for regaining export status following the use of stamping-out as opposed to vaccination in outbreak situations which pre-informed the need for the historical review of FMD vaccine production (Barteling, 2002).

After Sabin developed an attenuated poliomyelitis vaccine, much research was carried out along the same lines for FMD in the 1950s and 1960s. The basic approach was to passage FMD virus in *in vitro* culture systems to select a virus population that would induce an immune response to the virus without causing disease. Many of such vaccines have been developed in the medical and veterinary fields. Production of attenuated (modified-live) virus vaccines in cell cultures is relatively simple and cheap because only a small quantity of infectivity per dose of vaccine was required. However, difficulties were encountered in effecting viral attenuation for all animal types (species, breeds, sexes and ages) in which the vaccines were to be used. The problem of re-version to virulence was a further complicating issue (Röhrer and Olechnowitz 1980; Casas Olascoaga *et al.*, 1999).

Thus it was difficult to ensure safe attenuation of FMD virus for all varieties of animals that needed to be vaccinated in all circumstances. Outside the risks of back-mutation to

virulence, there was the risk of contamination with other viruses, e.g. contamination by calf serum used in the culture medium. These difficulties resulted in the use of attenuated vaccines being abandoned in the late 1960s and early 1970s in most parts of the world. The alternative was to use viruses that had been chemically inactivated ('killed') as the basic immunogen in FMD vaccine. Upon injection into animals, antibodies against the virus surface proteins were induced that protect against future infection. There are three crucial requirements for production of inactivated virus vaccines. First, the viral antigen must be produced in large quantities because each vaccine dose should contain high levels of inactivated virus to be effective. Secondly, the virus preparation must be inactivated in such a way that no residual infectivity remains but at the same time the immunogenicity of the virus surface proteins remain intact and preserved. Thirdly, an adjuvant must be added to the vaccine to potentiate antibody response to viral proteins.

The first inactivated vaccine against FMD employed was the Waldmann formulation developed in Germany. This essentially involved artificially infecting cattle and then killing the animals at the height of clinical disease and harvesting the tongue epithelium to obtain infective virus. Al(OH)3-gel was added to the virus preparation which was later inactivated with formaldehyde, the virus preparation clarified by chloroform treatment and filtered through porcelain candle filters (Waldmann *et al.,* 1937; Barteling and Vreeswijk 1991). In the late 1940s, Frenkel developed a large-scale *in vitro* culture system using freshly cut slices of normal bovine tongue epithelium obtained from an abattoir (Frenkel, 1947).

The harvested epithelium was subsequently infected with FMD virus to produce large quantities of newly replicated virus harvest which was mixed with chloroform, clarified by centrifugation and filtered through Seitz filters. Thereafter, the method which was similar to

that developed by Waldmann (Frenkel, 1947; Barteling and Vreeswijk 1991) was reviewed. The Frenkel-type vaccine worked very well (Bekkum *et al.*, 1963) as this method was adopted by a number of laboratories outside the Netherlands (Fogedby, 1963; Fontaine, 1985).

The main disadvantage of Frenkel system was bacterial and yeast contamination during culture. In addition, uncertainty in substrate source made this method unfit good manufacturing practice (GMP). Nevertheless, the Frenkel method was continuously used in several European laboratories until the discontinuation of vaccination in 1991. FMD virus was reported to multiply efficiently in a BHK cell line (Stoker and Macpherson 1964). These cells were subsequently adapted for growth in suspension culture (Capstick *et al.*, 1965; Telling and Elsworth, 1965) which enabled relatively easy 'scaling-up' to an industrial level (Radlett *et al.*, 1985) as this production system provided basis for almost all FMD vaccine manufactured in the world (Barteling, 2002).

Some FMD outbreaks in Europe have been clearly linked to vaccination and/or vaccine failures (Barteling and Woortmeijer, 1984; King *et al.*, 1981) which lead to the replacement of formaldehyde by aziridin compounds, in particular binary ethylene-imine (BEI) (Brown, 2001). This resulted to the use of double-oil emulsion vaccines for vaccination of pigs and in general outbreak situations in all species. Although the traditional inactivated vaccine has been proved effective, it may lead to a new outbreak of FMD because of either incomplete inactivation of FMDV or live virus escape from vaccine production workshop, which thrust the need to develop a novel, safer, more effective and economical FMDV

vaccines than the traditional vaccine (Doel, 2003) and subsequently the development of a new promising subunit FMDV vaccine (Li *et al.,* 2008).

2.9.1 Current vaccines

The current FMD vaccines contain inactivated whole-virus preparation that is formulated with adjuvant prior to use in the field. A number of countries have established vaccine banks which contain concentrated antigen stored in the gaseous phase of liquid nitrogen (Doel, 2003). Banks contain antigen against a number of virus serotypes and provide member countries with an almost immediate source of vaccine as antigen stored under these conditions is stable for a longer period of time than formulated vaccine (Doel and Pullen. 1990).

The report which provides an in-depth review of the history, production, and utilization of inactivated FMD vaccines and some of the limitations of their use, especially in emergency control programs were highlighted (Doel, 2003).

These limitations with its use in emergency control programs include the following. (i) Requirement of high-containment facilities for vaccine production (ii) Most virus preparations used for vaccines are concentrated cell culture supernatants from FMDV-infected cells and depending on the manufacturer, vaccines may contain various amounts of contaminating viral NS proteins. Vaccinated animals may develop antibody responses against the contaminating proteins, in addition to the viral structural proteins, making it difficult to 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 a window of susceptibility

of vaccinated animals prior to the induction of adaptive immune response. Fourthly, vaccinated animals were prone to been long-term carriers following contact with FMDV.

Some of these concerns are being addressed by development of new marker vaccines that do not require infectious virus and diagnostic tests that are based on NS proteins that are absent or are only minor contaminants of the current vaccine (De Diego *et al.*, 1997). Furthermore, efforts to understand the role of innate immunity and utilize various cytokines to both rapidly induce protection and immune response boost are under way (Gurbman and Baxt, 2004).

2.9.2 Alternative vaccine strategies

Researchers over the past 20 to 25 years have attempted to develop alternative FMD vaccines that do not require infectious virus following the concerns and short comings described above. These include Proteins and peptides using immunologically important VP1 G-H surface loop (Kleid *et al.*, 1981) or chemically synthesized VP1 peptides (Nargi *et al.*, 1999) and inoculation with DNA expressing VP1 epitopes alone (Wong *et al.*, 2000). Live attenuated FMD vaccines developments by classical procedures of serial passage in non-permissive animals or in cell culture have been reviewed (Brooksby, 1982). Attenuated strains were produced by passage in non-susceptible species such as mice, rabbits, and embryonated eggs, until their virulence for cattle was weakened (McKenna *et al.*, 1995; Zhao *et al.*, 2003).

Other experimental FMD vaccines have targeted immunogens that contain the entire repertoire of immunogenic sites present on intact virus but lack infectious nucleic acid

(Grubman *et al.,* 1993) refered as empty viral capsids. The strategy involve molecular cloning of viral genome regions necessary for the synthesis, processing, and assembly of viral structural proteins into empty viral capsids (P1-2A- and 3Cprocoding regions) which have been preliminary demonstrated to elicit significant FMDV-specific neutralizing antibody response to completely protect from clinical or serological evidence of FMD after direct inoculation challenge (Grubman and Mason, 2002).

Antiviral approach have been demonstrated in cells pre-treated with IFN- alpha/beta, with inhibition of productive replication of FMDV by IFN-induced gene products, double-stranded RNA-dependent protein kinase and 2'- 5'A synthetase/RNase L (Chinsangaram *et al.,* 1999) which rapidly induced non-specific protective response against all tested FMDV serotypes (Chinsangaram *et al.,* 2001). Finally, utilization of antisense technology to specifically inhibit FMDV replication in cell culture was executed (Rosas *et al.,* 2003). However, this technology has not yet been extended to susceptible animals.

2.10. Public Health Significance

FMD has no public health threat, although humans can contract the disease. FMD virus can cause pyrexia, anorexia, and vesicular lesions in humans (David and Brown, 2001). Most reported cases in humans have been in laboratory workers and in people having close contact with affected animals (Lubroth, 2002). FMD should not be confused with hand, foot, and mouth disease which affect humans.

2.11 Livestock Population and Distribution in Nigeria

Livestock account for one third of Nigeria's agricultural GDP, providing income, employment, food, farm energy, manure, fuel and transport (Maisamari, 2002) and a major source of government revenue. Traditional livestock production in Nigeria is varied and complex with livestock, especially ruminants, been the most efficient users of uncultivated land and contributing substantially to Animal production (Nuru, 1983). In the past, official livestock population figures were derived indirectly and extrapolated from various administrative sourcessuch as *jangali* cattle tax receipts, vaccination returns, slaughter records, trade movements and exports. The information was frequently out of date, inaccurate and/or incomplete and yielded population estimates of considerable uncertainty that related only to a few of the many livestock species kept. Such figures should obviously be treated with caution (Colville and Shaw, 1950; Fricke, 1979), as this is rarely reflected in official publications. Further doubt has been cast on the validity of livestock statistics by the pervasive impact of human population growth and agricultural expansion on the natural environment and the associated redistribution of livestock within it (Bourn, 1983).

Under the prevailing circumstances of doubt and speculation, the Government of Nigeria commissioned Resource Inventory and Management Limited (RIM) to conduct an independent national livestock resources survey. This study was undertaken in collaboration with the Federal Department of Livestock and Pest Control Services and academic institutions as a component of the World Bank-assisted Second Livestock Development Project, and it included the assessment of both traditional and commercial sectors of livestock production. These livestock which play very important role in the socio-economic and spiritual livelihood of Nigerians consist of Cattle (13,885,813), Goat

(43, 453,003), Sheep (22, 092,602), Donkey (936,832), Horses (206,212), Camels (87,839), Pigs (3,406,381), Cats (3, 265,554) Dogs (4,543,003), Rabbits (1,719,846), Giant rats (64,433), Guinea pigs (474,105), Chicken (72,400,856), Ducks (11,793,968), Guinea Fowl (4,679,907), Pigeons (14,139,866), Turkeys (223,363), Tortoises (96,176), Fish Ponds (1,539,205), Fish Wells (93,047), Snails Farms (5,811) and Beehives (691,443) (RIM, 1992).

Nigeria is a large country of considerable diversity with a wide range of agro-climatic conditions and corresponding varieties of vegetation. The general purpose of the survey was to obtain more reliable, up-to-date information about Nigerian livestock resources and production systems in order to establish a sound foundation for livestock development planning and more effective for targeted initiatives in the future. Specific objectives of the study were to assess the seasonal distribution and abundance of traditionally managed livestock; determine the characteristics and distribution of the major species and breeds; collect baseline herd productivity parameters; describe the features of locally important livestock production systems; identify the major constraints on production; and assess the size and composition of commercially managed livestock holdings (Bourn *et al.,* 1994).

The methods adopted was a two-tiered sampling strategy, combining low-level aerial surveys and complementary ground studies, based on techniques developed over the past decade and extensively employed in many parts of Africa, including Kenya, Mali, the Niger, Nigeria, Senegal, the Sudan and Chad (GEMS/UNEP, 1986). In essence, pastoral livestock were assessed from the air, while those concealed from view in villages were appraised on the ground. The two were linked by the number of rural rooftops counted by both aerial and ground survey teams. "Hidden animal" to rooftop ratios, derived from

ground observations, were applied to aerial rooftop counts to give indirect assessments of "hidden" village livestock. These were added to pastoral livestock numbers, which were determined from direct aerial observation, to give population totals for each species. This integrated approach optimized the use of available resources and avoided the problems inherent to purely ground-based methods of assessment such as double counting of mobile herds and the under-representation of livestock in remote areas.

The Nigerian Livestock Resources Survey presented to the federal government a four-volume, 1 250-page final report, *Nigerian livestock resources* (RIM, 1992), and a computer database accessible to standard software packages provided an objective basis for targeting future development initiatives and a firm foundation for monitoring and evaluation activities. This assessment showed the estimate cattle population in the country was about 13, 900 000 most commonly distributed in the northern two-thirds of Nigeria. Seasonal transhumance was also established with observed contrast cattle distributions during the wet and dry seasons with almost half the total cattle population permanently resident within the sub-humid zone. Humped zebu cattle are by far the most common, but limited numbers of Keteku, Muturu and Kuri cattle occurred in the south-western, southern and north-eastern parts of the country respectively. It is worthy of note that qualitative changes was observed within local systems of agriculture. These include a marked reduction in pastoral nomadism; increasing sedentariness of pastoralists and adoption of crop cultivation in addition to keeping livestock (van Raay, 1975); the taking up of animal husbandry and livestock fattening by arable farmers; the utilization of crop residues by livestock in exchange for dairy products and/or manure; and the use of animal traction for ploughing and carting. Collectively, these incremental changes are indicative of a progressive and

widespread trend towards mixed farming (FAO, 1983; McIntyre *et al.*, 1992). This tendency established in Nigeria and further integration of livestock production within local farming systems was destined to become one of the major strategic goals of livestock development in sub-Saharan Africa (Winrock International, 1992).

2.12 Cattle Breeds in Nigeria

Over 800 cattle breeds are recognized worldwide, some of which are adapted to the local climate and bred by humans for specialized uses. Cattle breeds fall into two main types, regarded as either two closely related species, or two subspecies of one species. *Bos indicus* (or *Bos taurus indicus*) cattle, also called zebu adapted to hot climates. *Bos taurus* (or *Bos taurus taurus*) are the typical cattle of Europe, north-eastern Asia, and parts of Africa – they are referred to as "taurine" cattle and many are adapted to cooler climates. *Taurus/indicus* hybrids are widely bred in many warmer regions, combining characteristics of both ancestral types (such as the Sanga cattle of Africa). In some parts of the world further species of cattle are found (both as wild and domesticated animals) and some of these are closely related to taurine and indicus cattle interspecies hybrids such as include the Dwarf Lulu cattle of the Nepal mountains with yak blood, (Takeda *et al.*, 2004).

The Beefalo of North America with bison genes, the Selembu breed of India and Bhutan with gayal genes. The Madura breed of Indonesia may have banteng in its parentage while the Dzo of Nepal is an infertile cattle-yak crossing bred for agricultural work. Like the infertile mule they have been continually bred from the parent species (Lenstra and Bradley 1999).

In Nigeria, cattle are kept in all parts of the country, but most concentrated on Oyo, Kwara and the northern states. Over 90% cattle owned by the Fulani are kept under a variety of traditional management systems (nomadic pastoralism to intensive backyard management) and less than 20% are kept in towns or rural settlements. The three categories of indigenous cattle kept include: zebu *Bos indicus* [Bunaji (White Fulani), Rahaji (Red Bororo), Sokoto Gudali, less frequently, Azwak and Wadara]. These constitute 99% of national cattle herd, which are beef and dairy cattle (RIM, 1992).

The Red Bororo also known by Abori, Rahadji, Babadi, Brahaza, Diafoun, Fellata, Fogha, Gabassae, Gadehe, Hanagamba, Kreda, Mbororo, Rahaza, Red Fulani, and or Red Longhorn are found in the eastern Niger, northern Nigeria, Western Chad and northern Cameroon. They belong to the West African Zebu type (Long lyre-horned) with mahogany normal coloration (Mason, 1996).

The second group is the West African Dwarf short horn *Bos Taurus* (Muturu), which was the breed once kept throughout the whole Southern Nigeria as they are now replaced by Ketekus and Zebu. At present they are found in Akwa-Ibom, Cross River states and among the Tiv in Benue State. They are found in low density throughout the Igbo land and in residual population in River State, Bendel (now Edo) state and parts of yorubaland. They are primarily beef producers with lower milk yield than the Zebus and often referred to as trypano-tolerant cattle.

The third category include crosses such as Keteku (Muturu X Ndama) in Sokoto and Niger States, and those in the Government Livestock Centres at Ado-Ekiti, Ndama cattle at

Fashola in Oyo and Adalu in Anambra states and the Kuris- the largest breed of African cattle restricted to a semi- aquatic habitat in the Lake Chad region (RIM, 1992).

N'dama otherwise called *Boenca* or *Boyenca* (Guinea-Bissau), *Fouta Jallon*, *Fouta Longhorn*, *Fouta Malinke*, *Futa*, *Malinke*, *Mandingo* (Liberia), and *N'Dama Petite* (Senegal). Other incorrect names that are sometimes used include *Dama* and *Ndama* and been the most representative of "Bos Taurus" breed in West Africa with approximately 7 million head of N'Dama which originated from the Fouta-Djallon highlands of Guinea (Conakry) and the sudanian and Guinean regions. Being, trypanotolerant it has been used for large scale dissemination for grazing savannah in Congo, Central Africa, Gabon, Nigeria and Zaire, especially in tse-tse fly infested regions. The N'Dama cattle breeds are hardy with medium size body (100 cm at shoulder height for cows; 120 cm for bulls), large and strong head with lyre-shaped horns and matured steer average live-weight between 250 to 300 kg. Their skin is characterized by short, thin hair and fawn colored which may vary from sand to black color and sometimes spotted. Cows produce only 2 to 3 liters milk per day during 7 to 8 months. The N'Dama breeds are used for meat and with about 50% carcass/live weight ratio which have good flavor without much fat (Mason, 1988).

2.13 Participatory Epidemiology

Participatory Epidemiology (PE) involves the use of community-based participatory approaches and methods to collect detailed information, in order to improve animal disease and veterinary services understanding for implementation of designed improved livestock disease problem solution (Jost *et al.*, 2007) based on intimate knowledge of local conditions obtained from first hand and at village level assessment (Johnston and Clark, 1982) at a

substantial reasonable cost (Farringnton and Martin, 1988). Although community participation has various meanings, the term in PE usually convey some form of interaction between local people and outsiders in which the former play a role in identifying, implementing or even controlling development activities. Overtime, participatory methods have attracted increasing interest from veterinarians and are now used by a wide range of organizations (Catley and Mariner, 2002) for disease eradication (Narayan, 1996) and epidemiological studies that form the 'web of causation'(Schwabe, 1984).

PE methods are qualitative in nature that evolved in the 1990s (Thrusfield, 2005). Standardization and repetition of PE methods produced quantitative data that estimated disease incidence, mortality or other variables. The process of triangulation in PE improves the validity of findings and involves cross-checking information from different sources or methods, including conventional veterinary investigation methods (Rufael *et al.,* 2008).

The approach was developed in small-scale, community animal health programmes and then applied to major international disease control efforts. The Global Rinderpest Eradication Program adopted participatory epidemiology as a surveillance tool for rinderpest control. This approach was subsequently used in both rural and urban settings in Africa and Asia, for foot and mouth disease (Rufael *et al.,* 2008), peste des petits ruminants and highly pathogenic avian influenza as well as controlling both rare and common diseases (Jost *et al.,* 2007) based on participatory techniques for the harvesting of qualitative epidemiological intelligence contained within community observations, existing veterinary knowledge and traditional oral history dependent on widely accepted techniques of participatory rural appraisal, ethno-veterinary surveys and qualitative epidemiology (Schwabe, 1984). The obtained information was used to design better animal health

projects and delivery systems, more successful surveillance and control strategies or as new perspectives for innovative research hypotheses in ecological epidemiology.

Community knowledge related to animal health has been termed *existing veterinary knowledge* which has made important contributions to Western medicine (FAO**,** 2011) and the best source of traditional livestock knowledge are from the pastoral and agro-pastoral systems with large animal husbandry components based on clinical, gross pathological and epidemiological observations (Pretty, 1994). The reliability of community base diagnosis can be comparable with clinical diagnosis in the absence of laboratory support. However, the two information types are not equivalent as the community often detects disease problems long before and more frequently than clinicians (Catley 2006).

The use of rapid rural appraisal or participatory rural appraisal techniques as project design and monitoring tool have been shown to be timely, accurate and cost-effective means of collecting essential information for project formulation (Moris and Copestake, 1993) at community level (Theis and Grady, 1991). Participatory rural appraisal (PRA) evolved out of RRA and placed more emphasis on community empowerment to process and utilise locally generated information (McCracken, 1988). As the move from RRA to PRA, the role of outsider shifted from being primarily an 'extractor' of information to community development facilitators. These RRA and PRA techniques have been applied to areas of existing veterinary knowledge (Pretty *et al.,* 1993). The development of participatory techniques has been hand-in-hand with the advancement of the concept of community empowerment. Livestock owners are no longer seen as an inert substrate upon which development is being practiced but are active participants in design, implementation, monitoring and review that intellectually contribute towards successful development of

trustworthy projects conducted (Pretty, 1994) for better acceptance of disease control interventions by the animal-owning public (FAO, 2011).

Field epidemiology was defined as epidemiological practice in response to real, time-bound problems of magnitude significant enough to require a rapid or immediate response (Goodman and Buehler, 1996). This aspect of epidemiology is timely, judgmental in process based on quantitative science, common sense and the need to design practical interventions, therefore methods appropriate to situations that make optimal use of time and resources available must be used as provided in participatory epidemiology methodologies of intelligence gathering that further defines the complex process called 'common sense' (Chambers, 1983). In quantitative studies, statistical analysis can measure associations but cannot order causal relationships. Therefore, judgmental process of establishing cause requires insights based on qualitative analysis (Moris and Copestake, 1993). These qualitative factors that need to be considered are temporality, biological gradient, consistency and plausibility (Rothman, 1986). Culture also plays pivotal in PE as culture and community dictate many of the traditions or norms that determine husbandry systems and the nature of human relationships with the physical and animal world.

This comprehensive, multidisciplinary, flexible and holistic methodology of participatory appraisal suited for development of conceptual models of epidemiological causation are used by many countries and international organisations committed to developing effective infectious disease epidemiological surveillance systems for control. These systems include both general disease surveillance systems and purposive surveillance systems for the eradication or control of specific disease agents (FAO, 2011). In Nigeria, the potential use

of PE in addressing issues of animal health and management practices is increasingly been appreciated and explored (Idowu and Babalobi, 2010).

2.13.1 Existing veterinary knowledge

Livestock keeping communities possess rich vocabulary to describe livestock, production systems and animal health information. With regards to animal health, livestock vocabularies are community dependent and include terms to cover clinical syndromes as well as specific signs and symptoms in addition, to major vectors, pest and medicinal or harmful plants nature are named. Not all things must have a name to be understood as local knowledge and language are not equivalent. However, language manifest as proxy indicator for the importance a community places on a particular subject (FAO, 2011).

Existing veterinary knowledge (EVK) focuses on clinical and epidemiological manifestations of disease and EVK terms do not necessarily correspond to specific disease entities in western medicine. As the livestock owners are mainly concerned with the clinical, gross pathological and epidemiological disease observation and atimes the terms correspond to clinical case definitions. However, disease terminology can vary from one community to another or even within clans of the same community. Thus, epidemiologists must define local terms carefully and prepare traditional case definitions or profiles that list the criteria for application of each traditional term.

Traditional disease terminology often, but not always, has a literal meaning that refers to some aspect of the disease. In some cases, the literal meaning has been lost or never existed. These are the exceptions, rather than the rule. For some disease, a distinguishing sign or symptom provides continuity between communities in regard to the literal meaning

of names. Traditional name for liver flukes as ‘leaves’ in selected cultures from Asia, Africa and Central America as the liver fluke shape was associated with leaf shape. Disease can also be named by the organ affected. The Karamojong of Uganda refer to anaplasmosis as *lopiid*, a term that means ‘of the gallbladder’, with the English name as gallsickness. In some cases, specific disease was named after prominent but non-specific symptom. The Somalis of the Gedo Region named classical rinderpest as *shifow*, the term literally mearnt the sap staining of a tree trunk after a limb is cut but by analogy, *shifow* refered to the tear stain commonly seen in rinderpest, while the Maasai named MCF after the wildebeest as traditional names can also have epidemiological significance. The Pathans of Afghanistan use the term *mach* to denote *Trypanosoma evansi* infection of camels. *Mach* means fly and biting flies are vector of *T. evansi*. Southern Ethiopia pastoralists on Borana plateau identified FMD as hoyaale, contagious bovine pleuropneumonia (CBPP) as sombessa, lumpy skin disease (LSD) as sukii, blackleg as haarka and mastitis as nakarsa (Rufael *et al.*, 2008). Conversely, some disease terminologies are not specific. Local communities may refer to bottle jaw or pneumonia as though it were a specific disease, whereas several etiological agents may be responsible. Communities may have specific terms for some types of pneumonia, such as CBPP, and a term for pneumonia in general**.** These terms must be carefully identified and differentiated.

2.13.2 Participatory and rapid appraisal techniques

Both participatory rural appraisal and rapid rural appraisal techniques include systematic and documented methodologies for qualitative intelligence gathering (McCracken, 1988) over a period of two weeks and two months depending on the desired depth of enquiry (McCracken *et al.,* 1988). Thus, RRA and PRA are timely methods for gathering action-

oriented information based on flexible programmes and checklists; systematic, documented methodologies; detailed and specific data recording; triangulation (cross-checking of results); respect for local people and local knowledge; willingness to learn and iterative analysis of results (Pretty *et al.,* 1993).

PRA are epidemiological study directly linked to community development, in contrast with RRA activity because in classical PRA epidemiology, community level interactions occur at the level of data collection (Moris and Copestake, 1993), analysis and final decision-making and out come implementation (Townsley, 1993). Although RRA and PRA are different, they represent two poles of spectrum of approaches as Participatory epidemiology can be utilised primarily as a data collection method closely related to RRA (Welbourn, 1991) and as a component part of local development programme where the participants themselves formulate an action plan to improve animal health and productivity (Theis and Grady, 1991). This latter case show more 'participatory' and closer relatedness to PRA which are often useful as follow-up mechanism in formal study investigation.

2.13.3 Principles of rapid appraisal methods

Rapid and participatory appraisals are based on two important principles which are designed to improve the quality and reliability of gathered information. These include triangulation and flexibility. *Triangulation* regards to information gained from several intentionally different perspectives while *Flexibility* of Rapid Appraisals indicate no rigid pre-planned execution without deviation, as techniques used and questions asked can be changed at any point during the investigation (David-Case, 1990).

2.13.4 Data sources in rapid appraisals

In applying the principles of flexibility and triangulation, a number of data sources must be tapped. These may be classified either as primary or secondary, depending on their closeness to the actual study subject (Conway *et al.,* 1987). Sources within the communities studied would be seen as primary while all other sources would be secondary (Grandstaff and Messerschmidt, 1993). *Primary sources*: - include direct observation, group and individual interviews of farmers, interviews with key informants such as village elders, local religious leaders and government officials familiar with the area. Also included are the outcomes of participatory activities undertaken to study and evaluate local animal health problems while *Secondary sources*: - include a number of possible secondary data sources such as previous studies and reports, government statistics and records, study area maps, research papers and historical texts. Robust consultations with as many secondary data sources as possible before undertaking field work garantee successful project outcome.

2.13.5 Potential sources of bias in participatory appraisals

All epidemiological studies need to be designed so as to minimise bias. Therefore, planning PRA work is of great importance, as the samples are purposively selected. While it is true that appraisals can often yield a depth of information not possible with formal surveys, they are not usually planned on the basis of randomized sampling frame of survey localities. This means that survey planners need to be aware of possible biases and must consciously take steps to avoid them. Classically, there are six sources of bias that affect rapid appraisals the include spatial biases, project bias, person bias, dry season bias, diplomatic biases and professional biases which may require researcher's attension (Chambers, 1983).

2.13.6 Advantages of participatory methods in epidemiological studies

Despite possible pitfalls and the need for experience, there exist a number of distinct advantages associated with participatory method use (David-Case, 1990). They have been mentioned at various points in this text, but may be summarised as follows: Often the only way of gathering data from certain areas (particularly remote or strife-torn areas), usually cheaper and more feasible than full-scale randomised surveys, thus often an attractive option for poorly-resourced veterinary services, results are usually available very rapidly, more flexible and able adaptive new issues are uncovered during appraisal and effective method for design of more conventional studies through better identification of the breadth, depth and priority issues that may merit quantitative study. Participatory methods also build on what local people already know and enable them use their own knowledge and skills in disease surveillance and control. Finally, poor and rural women participation in appraisal acts as a tool for empowerment.

2.14 Satellite Techniques and its Application in Africa

Satellites techniques are new and modern methods that are largely technologically driven. These techniques include: remote sensing, geographical information system and global positioning system. Although, these satellite techniques started as technological tools they have rapidly evolved into a systematic form of science in their own right (Rothman, 1986). A parallel exist between these techniques and epidemiology with their tenets have been established piecemeal with contributions coming from a number of disciplines, particularly the earth sciences (Rothman, 1986).

Satellite techniques use in developing countries health systems was accepted as a needed fashion (Sepulveda *et al.*, 1992). Several tools such as computers, satellites, aerial cameras, radars systems and scanners have made satellite technique possible. Their introduction into the management of disease control programmes was feasible. These techniques used spatial variation, analysis of data obtained from extrapolation of measurements made at local levels to regional scales, ecological variations, automation and analysis of aerial photography and bio-geographical and bio-climate studies to predict the transmission and prevalence patterns in control of diseases (Sepulveda *et al.*, 1992).

In Nigeria, an earth observation satellite called Nigeria sat 1 was successfully launched into space on 26th September 2003 in Siberia, Russia inorder to improve availability of real-time data from satellite for problem solving applications integrated with Geographic Information System (GIS). Nigeria sat 1 satellite capture and records Nigerian earth surface on daily basis at a 32m pixels resolution (NigeriaSat-1, 2003). This low resolution equipment and is suitable for large scale mapping only not for detailed small scale mapping that would show individual buildings, such as homes, streams, roads, and built cities clearly. This may not be useful without adaptation, for precise animal health risk studies but could be regarded as a fore runner of technology awareness (Esuruoso *et al.*, 2005). However, in order to carry out geographical mapping of cattle herds' movement pattern and their problems, across the country on a GIS, one would require a high resolution equipment like the Ikonos satellite capable of producing 0.5 to 1m pixel resolution images (Adewale and Olugasa, 2005).

This would effectively aid in visualization of spatial distribution of cattle and their movement pattern especially in relation to land use, vegetation cover, water resources and

annual seasonal changes within the country (Esuruoso *et al.,* 2005). Spatial data can then be integrated with obtained non-spatial data **(**Longley *et al.,* 2001**).** Satellite data can be made available continuously to permit examination of short-term changes in disease study such as FMD. With the consciousness of the many likely benefits derived from the use of satellite techniques in disease control, several attempts have been made to apply them in the management and control of some parasitic diseases in Africa (Njemanze, 1996; Dozie et al., 2007).

Beside the fact that Africa has the largest disease burden in comparison to other continent of the world, with several environmental factors that contribute significantly to disease prevalence, intensity and distribution (WHO, 1990). This therefore, maked satellite techniques in disease surveillance, control and prevention particularly relevant. A few studies have shown that satellite techniques permit the planning of interventions where necessary and sustainable success can achieved. In spite of a few constraints, satellite techniques hold considerable promise for the control of veterinary diseases especially FMD and other zoonosis as well as management of other health research in Africa including Nigeria. For the purpose of this research work, Geographic information system was considered and highlighted.

2.14.1 Geographic information system

Geographic information system (GIS) a new cutting edge technology was used as a biological risk visualization, management and tracking tool in veterinary epidemiology (Esuruoso *et al.,* 2005). GIS use in veterinary medicine and epidemiology dated back to early 1970s when a Canadian scientist Dr. Rowland R. Tinline applied GIS retrospectively

to FMD epizootic spread pattern during 1967-68. He applied the GIS technology to generate data from which FMD incubation period was obtained. This provided a better understanding of disease pattern and spread between herds which could be applied to control future outbreaks worldwide (Ramirez *et al.,* 2004).

Spatial and non-spatial capture data related to animal population, distribution, and their problem are major component of GIS risk study. Therefore, GIS application in health risk studies consist of visualization, measurement (evaluation), assessment and management aspects as visualization aspect maked the use in epidemiology a precise science (Fite, 2000). The scientific method of animal health risk visualization when incorporated with computer (especially graphics) transforms data into visual models (Dent, 1999). The application of scientific visualization to locational and spatial data study was termed GVIS (Dent, 1999). This animal health risk may then be described as application of Geographic Visualization methods to assessment of health risk in animal groups/population at a specific location and time.

Geographic information system (GIS) provided computerized capture, storage, management, analysis, retrieval and display of spatial and descriptive data that are geographically referenced to common coordinates system. GIS comprised of database (spatial and attribute), data input (digital and image), cartographic display system, database management and geographic analysis system (overlay process and buffer zone creation) (Njemanze *et al.,* 1999). The spatial database contains information held in the form of digital coordinates, which describes spatial features (Loslier, 1995). The use of points for homes, lines for roads, or squares for local government areas was well illustrated (Njemanze *et al.,* 1999).

The digitalization system allows existing paper maps to be converted into digital format. The image processing system allows conversion of remotely sensed imagery into maps. Data from other sources can be interfaced with the GIS database. The cartographic display system produces maps, and gives the user opportunity to select spatial features and attributes for display and printing. The database management system is used for creation, maintenance and accessing of GIS database, while the process of "overlay" provides comparison of different entities based on their common geographical occurrence, as this involves overlaying transparent maps of two entity groups on top of one another (Eastman, 1992) and buffer zone creation is of particular benefit in investigation of disease at or near pollution and other hazardous sites (Openshaw *et al.*, 1987).

GIS software that was found suitable for animal group/population health risk assessment was the community Viz (The Orton Family Foundation, 2003; Ramirez *et al.,* 2004; Adewale and Olugasa, 2005), while scenario constructor (SC) (Ramirez *et al.,* 2004) was used to analyze potential impact and incidence of accidental biological agent release on cattle. SC enabled the epidemiologist to estimate the number of sick cattle that could occur and visualize the necessary decontamination areas. It also provided information needed for cost effective animal health and production practices, which made community Viz software a suitable efficient tool (Olugasa, 2004).

Other GIS hardware and software of benefits in risk studies include personal digital assistant (PDA) (Ashok, 2000; Ron, 2003) which are hardwares in form hand help computers used for field data collection and re-laid back to a central GIS, where analysis could be made up to date for validation of risk predictive models (Ashok, 2000) such as the probability of FMD outbreak and success of ring vaccination strategies against disease in a

particular area. In addition, laser range finder mapping tool which are available with much easier user-friendly software and at much lower costs (Ron, 2003).

This device use provides a system that relies on GPS positioned control points and a laser range finder to remotely position farms, abattoir, veterinary laboratories, veterinary clinics, quarantine stations and wildlife locations (Ramirez *et al.*, 2004) to enable identification of risk areas, active disease areas and convalescent carrier animal group or population or reservoir host animal areas for risk studies and disease control strategy implementation. Two types of mobile hardware that could be used to run GIS and GPS software concurrently are the Dell Axiom PDA (Dell, 2003) and Tremble GeoXT PDA (TGE, 2003). Summarily, these other GIS/GPS hardware and software when used with a central GIS hardware and software like a desktop PC running community Viz on Arc view can provide positive animal health service delivery at community level in developing countries like Nigeria due to the flexible capabilities in health risk studies (Dent, 1999).

GIS was applied for distribution, diffusion and understanding of disease and its relationship to environmental factors and also for health programme monitoring and evaluation (Njemanze *et al.*, 1999) as well as in probability case study of FMDV accidental release and or deliberate use in quantifying spatial spread and disease impact on cattle group especially in FMD vaccination herd status (Olugasa, 2004). Other application of GIS parasitic disease study was also well highlighted (Dozie *et al*., 2007). The African Programme on Onchocerciasis Control (APOC) identified onchocerciasis endemic communites that qualify for mass treatment with ivermectin using GIS (Noma *et al.*, 2002) and was intergrated with data from Rapid Epidemiological mapping of Onchocerciasis (REMO), which then allowed the priority areas for mass distribution of ivermectin to be visualized. On that basis endemic areas were delineated into three operational areas for

ivermectin treatment namely; “definite community-directed treatment with ivermectin (CDTI)” area; “no-CDTI” areas and lastly “possible-CDTI areas (Nwoke and Dozie, 2001). GIS application in schistosomiasis control in Botswana was conducted to monitor prevalence rate reduction trend in relation to changing environmental factors (Nkambwe, 1994). GIS has also been used to generate models for malaria occurrence (WHO, 2002a) seasonality (Transer *et al.,* 2002) and transmission (Thomson *et al.,* 1996) using climate and remotely sensed data. The outputs of such data have been combined with population data to estimate population exposure, mortality and morbidity and analyze project effects of climate on malaria (Hay *et al.,* 2002). The mapping of vectors of malaria and their habitats as well as transmission intensity was possible with GIS (Omumbo *et al.,* 1998).

2.14.2 Other techniques relevant to satellite research in Africa

A number of complimentary satellite techniques relevant for disease research in Africa including human and robotic teams and telemedicine are at various stages of development. Space robots are being developed for integration in human and robotic team (Erickson, 1995; Njemanze, 1996). Supervised intelligent robotics with vision guidance and dexterous grasp of objects and living organisms can be utilized to acquire samples from remote and dangerous areas where infection risk for medical personnel or areas not accessible to humans (e.g wildlife habitats). Human and robotic teams were used to approach remote areas via telemedicine workstations (Simmons and Billicia, 1995) provided their findings to regional, command, and control centers.

Telemedicine technology is dedicated to improving access to medical care from remote areas on the earth and in space. This technology was developed by the US military (Edwards, 1995; Jenkins, 1995) composed of patient, consultant, on site personnel, sample

collection, transport system and communication connection (Njemanze, 1996). To access the data required remote diagnosis, audiovisual capture of patient ‘s physical findings, biotelemetry of vital signs including electrocardiogram waveforms, blood pressure reading and still images of the patients were recorded in comprehensible form (Njemanze, 1996).

2.14.3 Constraint in the use of satellite techniques in disease research in Africa

The absence of adequately trained personnel prevented many satellite techniques especially GIS projects from surviving the donor-involvement phase in Africa (Taylor, 1991). Satellite projects in Africa are currently supported and funded by international aid agencies or initiative and many are pilot or research projects as opposed to fully operational systems. In this case, the operators are foreigners and non African scientist who understand both the technological and socio-economic context in which the system operates (EIS-AFRICA, 2002). The high initial cost of providing the technology for majority of impoverished Africa countries has also limited satellite application use. Remote sensing projects required satellite and radar systems needs to be fully operational. Lack of GIS data set a major impediment to the growth of GIS in Africa. The access to continous spatial data was reported to be difficult and expensive (Briggs and Elliot, 1995). The in ability to convince government, policy makers and other stakeholders of the cost benefit /effectiveness of satellite application in the health sector was also the striking challenge (Clarke *et al.*, 1996). Even though these technologies are in use in the international scientific community, some skepticism still exists surrounding their use in the health sector.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection and Analysis Materials

3.1.1 Field sampling materials

Syringe (10 mls) and 18 ½ G needles; Universal plastic sample bottles; transport medium [glycerol, PBS tablets, Penicillin, Neomycin sulphate, and polymyxin B sulphate]; 5 ml EDTA plastic bottles (maxiCom^{R}); 5 ml serum bottles (ANTEC^{R}); Cryo-vials; ice packs and coolers.

3.1.2 Serological screening and Serotyping kits, Reagents, Biologicals and Chemicals

3.1.2.1 Kits

PrioCHECK^{R} FMDV-NS detection kit [Five test plates (90 wells per plate); conjugate (30X); DilutionBuffer (2X); additives (lyophilized); Demineralized water; washing fluid (200X); positive control (Ready- to- use); weak positive control (ready-to-use); negative control; chromogen (TMB) substrate; and stop solution].

3.1.2.2 Reagents

The reagents were 3,3',5;-Tetramethyl benzidine dihydrochloride (TMB) tablets; 1 mg 0.05 M Phosphate-Citrate Buffer with Urea and Hydrogen Peroxide, pH 5.0; Sodium Chloride, A.C.S., F.W. 58.44 g; Sodium Phosphate Dibasic, Anhydrous, A.C.S., F.W. 141.96g; Sodium Phosphate Monobasic monohydrate, A.C.S., F.W. 137.99g; Polyoxyethylene Sorbitan Monolaurate (Tween-20), pH standards, certified: pH 4.00 ± 0.01, pH 7.00 ± 0.01, pH 10.00 ± 0.02; Super-Freeze Peroxidase Conjugate Stabilizer, (Pierce^{R}); 2 M Sulfuric acid; Sodium Bicarbonate, Anhydrous, A.C.S., F.W. 84.01g; Sodium Carbonate,

Anhydrous, A.C.S., F.W. 105.99g; 1.0M Tris-HCl pH 7.5; Sodium azide, F.W. (65.01g); D-Sorbitol, F.W. (182.2g); 30% Hydrogen Peroxide (Sigma^{R}); and 1% Virkon.

3.1.2.3 Biologicals

The biological were recombinant His-Tagged 3ABC antigen, in 8M Urea; Detector antibody – mAb anti-3B peptide (prepared at NCFAD); Conjugate–polyclonal goat anti-mouse IgG (H&L) conjugated to horseradish peroxidase; Reconstituted lyophilized conjugate in the required suspension volume indicated with SuperFreeze Peroxidase Conjugate Stabilizer (Pierce); Aliquot of 100 µL volumes in cryovials and stored at -20°C; Strong (Q1) and weak (Q2) FMDV-positive bovine serum, (prepared at NCFAD); Reconstituted lyophilized sera in Milli-Q water (as indicated on vial), Prepared appropriate dilutions for each lot of positive bovine serum (Heat-inactivate at 56 ± 2°C for 30 ± 5 minutes); Negative Serum (Q3); Normal Bovine Serum (Sigma^{R}), heat inactivated at 56 ± 2°C for 30 ± 5 minutes and Bovine Serum Albumin, (Fisher^{R}); Captured antibody - rabbit anti-strain specific FMDV; Antigen-Strain specific FMDV antigens; Detector antibody - guinea pig anti-Strain specific FMDV; Conjugated-polyclonal donkey anti-guinea pig IgG (H&L) conjugated to horseradish peroxidase; Normal rabbit serum (filtered 0.22 µm); Normal bovine serum (filtered 0.22 µm); Non-irradiated Fetal Bovine Serum; Normal porcine serum (filtered 0.22 µm); Strong positive serum (Q1); Weak Positive serum (Q2) and Negative Serum (Q3).

3.1.2.4 Chemicals

The chemicals used were OPD (o-phenylenediamine dihydrochloride), (Sigma^{R}) Fast tablets, (SIGMA Chemical Company); Sodium bicarbonate, anhydrous, A.C.S., F.W. 84.01g; Sodium carbonate, anhydrous, A.C.S., F.W. 105.99g; Sodium chloride, A.C.S., F.W. 58.44g; Sodium phosphate dibasic, anhydrous, A.C.S., F.W. 141.96g; Sodium

phosphate monobasic, monohydrate, A.C.S., F.W. 137.99g; Polyethylene sorbitan monolaurate (Tween-20), pH Standards, certified, pH 7.0 + 0.01, pH 10 + 0.02; Glycerol, A.C.S., F.W. 92.09g and 2 M Sulfuric acid.

3.1.3 RNA extraction materials and reagents

3.1.3.1 Equipment

The equipment used were Class III Biological safety cabinet; latex free gloves; single channel pipettor; RNAase/DNase free filter-barrier pipette tips; sterile RNase/ DNase tubes; 2 ml collection tubes; steriled nuclease free plastic pipettes; pipette aid; reagents vortex; auto clave bags and containers; micro centrifuge; QIAvac 24 plus vaccum manifold with pump; Qiagen centrifuge with 96-well plate rotors; model stignma 4-15^{o}C; Dry block heater; ultra low freezer-70^{o} C and Whole Blood RNA ZR MiniPrep^{TM} extraction kit and reveRT™ First Strand cDNA Synthesis Kit.

3.1.3.2 Reagents

RNeasy mini kit (Qiagen^{R}) with Triphasic reagent: QIAzol lysis reagents; disinfectants; RNase Zap^{TM}; Chloroform; Absolute Ethanol; DEPC, (Diethylprocarbonate), and Isopropanol

3.1.4 Real-Time polymerase chain reaction equipment and reagents

3.1.4.1 Equipment

The equipment used were Cepheid smart cycler II^{TM} Real-Time PCR machine with Cepheid smart cycler II reaction tubes; smart cycler II tube centrifuge and specialised smart cycler II reaction tube holder with cooling block; ABI sequence Detection System^{TM} (SDS) 7900HT real time PCR machine with ABI PRISM^{TM} 96-well optical reaction plates; ABI PRISM^{TM} optical Adhesive covers; plastic adhesive seal applicator; ABI PRISM^{TM} optical

cover compression pads; Qiagen centrifuge model sigma 4-15C; Pipettes designed for PCR; sterile aerosol-resistant filters; pipette tips for PCR use; micro-centrifuge vortex; RNase/DNase-free tubes; amber tubes; Nitrite gloves; Nalgeae bench-top coolers; freezers; disinfectants.

3.1.4.2 *Reagents*

Quanti-Test probe one step PCR kit (Qiagen^{R}); TBE buffer; Nuclease free water; Forward 1186F and Reverse 1237R primers; FMDV Taqman 6 'FAM/MGB probe; FMDV synthetic positive control Taqman; Beta-actin internal control 831F forward primer; Beta-actin control 1036R Reverse primer; Beta-actin positive control RNA and Beta actin internal control probe 880

3.1.5 Sequencing materials

Sequencing kit containing reagents and biologicals; primers to A, O and SAT1-3, (IAH-Pribright, UK); designed primers of O and A serotypes to VP1 produced by NCAD, Canada (Alfonso Primers); sequence stop solution and sequencing software.

3.1.6 Geo-reference recording and spatial analysis materials

3.1.6.1 Equipment

The equipment used were Garmin GPS receiver (GPS MAP 76csx) etrex^{R} [LEGEND/GARMIN] (TAIWAN); Digitizing tablet (summer sketch 3^{R}); Computer (laptop/desktop) with Arch view GIS 10.1 Demonstration Edition software license.

3.1.6.2 Data type

Spot Satellite Imagery (Google earth pro); Administrative map (NASRDA); Shuttle Radar Topographic Mission (USGS) and livestock location coordinates. The satellite based data used include spot 5 metre and 2.5 metre resolution with a wide imaging swath which covered 60 x 60km or 60 km x120 km in twin-instrument mode.

3.2 Study Area

Kwara state with capital as Ilorin is located on the longitude N 8° 30' 0" and latitude E 5° 0' 0" 8.5 / 5 (Geo Name Id : 2332785) (Anon, 2010). The state shares common boundaries with Niger and Kebbi States to the North, Oyo, Ondo and Edo States to the South, Benue, Plateau and Federal Capital Territory to the East and maintains an international boundary with Republic of Benin to the West. It is also referred to as 'gateway' state because of the location between Northern and Southern parts of Nigeria with 15 Local Government Areas namely: Asa, Baruten, Edu, Ekiti, Ifelodun, Ilorin East, Ilorin West, Irepodun, Isin, Kaiama, Moro, Offa, Oke-Ero, Oyun, and Pategi.

Ilesha Baruba is a district in Baruten Local Government Area of kwara state (Anon, 2011), which has the Cattle International markert for animal trade from Mali, Togo, Benin, Cote de voire/Burkina Faso and Senegal. Baruten Local Government Area is uniquely located in the North western part of kwara state and shares boundaries in the West with Benin Republic, in the South with Irepo Local Government Area of Oyo State, in the North with Babana district in Borgu LGA of Niger State and in the East by Kaiama LGA of Kwara State (Akande and Monehin, 2004). The road distance from Kosubosu (LGA headquarters) in Yashikira district to Ilorin (administrative division) is about 503km as this facilitates human and livestock traffic. The people mainly engage in agricultural and livestock

keeping and trading activities with Batonu being the major language, but Hausa, Fulani and Yoruba and Bokobaru are also spoken in the area (Anon, 2011). The availability of water and pasture during the dry season, also favors settlement of migrating pastoralist (Bororo Fulani) from extreme northern part of Nigeria, as well as relocation of the international cattle market from Shaki (Oyo state) to Kwara state which shares an international boundary with Benin republic thus characterizing its high cattle population density and traffic, hence premised the choice of this study area.

3.3 Sample Size Determination

The sample size for FMD antibodies assay was calculated using the formula described by Mahajan (1997).

$n=Z^2pq/L^2$

Where: n= sample size

Z=1.96 (Appropriate value for normal desired confidence)

q = 1-p

L= allowable error = 5%

P= calculated prevalence from previous study = 55.17% (Chukwuedo *et al.*, 2008).

Therefore, 1.96^2 x 0.5517 x (1- 0.55)/ $(0.05)^2$ = n samples

3.8416 x 0.5517 x 0.4483/0.0025= 380 samples

Sample size was increased to 450 inorder to increase precision.

Total of Ninety (Six per herd) blood samples were collected for sero-diagnoses and seven epithelial tissues obtained from ruptured vesicular buccal lesions for molecular analysis

3.3.1 Delimitation of study (specific sampling areas)

Blood samples to obtain sera were collected from five cattle markets in the different Local Government Areas of Kwara state; namely Oke ose cattle market Ilorin East LGA, Ilesha Baruba cattle international market Baruten LGA, Bode sadu cattle market in Moro LGA, Ajasse cattle market in Irepodun LGA and the cattle market in offa in Offa LGA**.** Epithelial tissues were collected from buccal and tongue mucosae as well as whole blood of FMD infected cattle during outbreaks investigation at Ilesha Baruba LGA in Kwara State. Outbreaks occurred in three locations namely, Bode I, Bode II and Bukaru- Rontuwa during the study period. The sampling locations were indexed by latitude and longitude with a GPS device and coordinates were used for geo-referencing as described by Maheshwaran and Craglia, (2004).

3.4 Sample Collection

In phase one (outbreak investigation), epithelial tissue samples were collected from ruptured vesicles of buccal, tongue mucosae, and the foot of affected cattle, using a pair of scissor and forcep. The tissues were kept in transport medium composed of equal amounts of glycerol (50% v/v) and 0.04 M phosphate buffer, (pH 7.2–7.6), with added antibiotics (penicillin [1000 International Units (IU)], neomycin sulphate [100 IU], polymyxin B sulphate [50 IU], mycostatin [100 IU]). Samples were kept on ice and transported to the laboratory and stored at 4^{o}C and -20^{o}C until use as recommended by OIE (2008).

Whole blood was also collected using 10 ml Syringe and 18G $1^{1/}_{2}$ inch needle through the Jugular vein of well restrained FMD affected cattle. Half was transfered into 5 ml EDTA containing plastic bottles (maxiCom^{R}), while the other half was transferred into 5ml plain plastic bottles (ANTEC^{R}) for sera collection. All the samples were packed on ice and

transported to the laboratory for storage until use at 4^{o}C and later -70^{o}C for prolonged storage.

In phase two (market survey), blood (5 ml) was aseptically collected through the jugular vein of well restrained cattle with sterile hypodermic 18G $1^{1/}_{2}$ inch needle and 10 ml syringe during market sampling and or directly as animal bled post slaughter. A total of ninety samples were collected per location. The blood was transferred into clean labeled 5 ml-plastic bottles (ANTEC^{R}), without anticoagulant and allowed to clot at an angle of 45^{o} slant at room temperature. The sera was collected in cryovials and packed on ice for transportation to NVRI, Vom for storage at 4^{o}C and -70^{o}C until use.

3.4.1 Sampling techniques and procedures

The first phase was a purposive study that involved twelve (12) cattle herds in 15 outbreaks over a period of 4 months (December, 2010 – March, 2011). The herds were sampled based on owner cooperation and presence of clinical signs during examination. From each herd, six (6) animals were randomly selected; examined and whole blood as well as epitheial samples were collected from those with characteristic lesions of FMD. Participatory appraisal was conducted using semi structured interviews to evaluate the Fulani herdsmen's knowledge on identification and management of FMD. Some information on cattle movements, history of herd vaccinations and FMD vaccination, as well as contact with other herds and wildlife were also gathered. The herdsmen, during outbreak investigation were considered as informants based on their existing ethno-veterinary knowledge as described (Rufael *et al.,* 2008).

The second phase was a sero-survery conducted using random sampling technique. Samples were collected from major cattle markets and slaughter houses in five different

LGAs of Kwara State, while noting their corresponding demographic data such as age, sex and breed as described by Isamah and Otesile (1997). The ages were classified into two groups based on existing local veterinary knowledge of the herdsmen/marketers as described by (Rufael *et al*., 2008). This phase was carried out over a period of 3 months between July and September, 2011. The sample size was limited by lack of cooperation from most of the cattle dealers to allow blood collection from animals which were about to be transported for slaughter or sales.

3.5 Laboratory Examination of Samples

Sera samples were processed following World Reference Laboratories (WRL), Pirbright, UK and Office International des Epizooties (OIE, Paris, France) guidelines (Kitching *et al.*, 2000) at the Foot and Mouth disease laboratory, National Veterinary Research Institute, Vom. Serotyping of representative positive sera was conducted at the FMD laboratory, National Centres for Animal Diseases, Winnipeg, Canada.

3.5.1 Serology

3.5.1.1 Examination of sera using FMDV-NS ELISA

In vitro dectection of antibodies against bovine FMDV in sera collected from outbreak and surveillance samples was conducted as decribed by Sorensen *et al*. (1998) using PrioCHECK^{R} FMDV-NS blocking ELISA. Working solutions were prepared in advance; these include Dilution buffer working solution, additives, ELISA buffer, conjugate dilution and washing solution as prescribed by the manufactures [Prionics Lelystad B.V. : The Netherlands]. On the second day, the test plates were coated with monocolonal antibody (mAb), using 80 µl ELISA buffer for each well in the plates, 20 µl negative control was

dispensed in wells A1 and B1, and 20 µl of weak positive control was dispensed in wells C1 and D1 while 20 µl of positive control was dispensed in wells E1 and F1. The test was performed by dispensing 20 µl of test sample to the remaining wells in test plate. The test plate was then sealed using enclosed plate sealer and shaked gently before overnight incubation at 25^{o}C. After incubation, plates were washed six times with 200-300 µl of washing solution and 100 µl conjugate solution was added to all wells. The test plates were sealed using enclosed sealers and incubated for 60 minutes at 25^{o}C.

After incubation, plates were washed six times with 200-300 µl washing solution (plates were tapped firmly after the last washing) and chromogen (TMB) substrate (100 µl) was dispensed to all wells. After incubation at room temperature (25^{o}C) for 20 minutes, color development was stopped by addition of 100 µl stop solution. The color development was measured optically at 450 nm wavelength to show presence of antibodies directed against FMDV within 15 minutes after addition of stop solution.

The OD_{450} values of all samples including controls obtained from the ELISA reader were expressed as percentage Inhibition (PI) relative to OD_{450} max by first calculating the mean OD_{450} value of wells A1 and B1 (Negative Control= OD_{450} max). Then using the formula below

$PI = 100 – [OD_{450}$ test sample/OD_{450} max] x 100

Result interpretation

A PI of < 50% was considered negative and it was interpreted that the animal has not been exposed to FMD within 40 days prior to screening test. A PI of ≥ 50% was considered as a positive exposure (<40> days) to FMD. More specifically, a PI value of ≥ 50% but < 70% was considered a weak positive result and a PI value of ≥70% was considered a strong positive result (Soresen *et al.,* 1998).

3.5.1.2 Examination of sera using FMDV-cELISA

Competitive ELISA (cELISA) based on a recombinant 3ABC fusion protein with 5-histidine tag was used for FMD antibody assay as described by Clavijo *et al*. (2004a). In this 3ABC cELISA, serum samples compete with an anti-3B monoclonal antibody (mAb) for specific epitopes on recombinant protein and the result was expressed as a percentage of inhibition. Positive test serum contained anti-FMDV antibodies, which inhibited binding of mAb to FMDV at various degrees. The reaction was completed by addition of detector antibody, peroxidase conjugated anti-mouse antibody and lastly chromogenic substrate. The signal emitted was inversely proportional to amount and/or strength of anti-FMDV antibodies in each test sample.

This assay was conducted at the FMD laboratory, National Centres for Animal Diseases, (NCAD) Winnipeg, Canada.

Result interpretation

Results were expressed as percentage inhibition (PI) and calculated based on the mean optical density (OD) values of duplicate samples, compared with a standard negative reference serum corrected for background signal by subtracting the OD of the high positive reference serum.

Test results were derived by the following formula: PI = [(negative reference serum OD - test sample OD) / (negative reference serum OD - positive reference serum OD)] X 100%.

Based on NCAD Canada testing of over 500 bovine, ovine and porcine field sera from an FMDV-free country (Canada) and the frequency distribution of their PI values, samples were considered positive if they inhibited 50% or more of signal strength of standard negative control.

3.5.1.3 Serotyping assay using Solid Phase Competition ELISA

Solid-Phase Competition enzyme-linked immunosorbent assay was conducted for detection of foot and mouth disease virus serotypes antibodies as described by Clavijo *et al*. (2004b). This competitive ELISA for Foot-and-Mouth Disease (standard antigen capture ELISA) detected anti-FMDV antibodies in test sera. FMDV antigen was bound to anti-FMDV antibodies on a solid support, followed by simultaneous incubation of test sera with guinea pig anti-FMDV antibodies. The guinea pig FMDV antibody was inhibited to various degrees in positive test sera that contained anti-FMDV antibodies. The detector antibody, peroxidase conjugated anti-guinea pig antibody and chromogenic substrate were added to complete the test procedure. The signal emitted was inversely proportional to the amount and/or strength of anti-FMDV antibodies in test sample.

Result interpretation

Results for test sera were expressed as percentage inhibition of signal strength for FMDV capture assay. The PI value for each sample was calculated using the formula:

PI = (100 - mean OD of 2 replicate test samples / (mean OD of the 4 replicate target standards) X 100.

Based on NCAD, Canada test for over 900 cattle field sera and 600 porcine field sera from an FMDV-free country (Canada) and the frequency distribution of their PI values, samples were considered positive if they inhibited 50% or more signal strength of the standard vesicular FMDV antigen capture assay.

3.5.2 Molecular studies

3.5.2.1 Sample processing

Epitheial tissues were homogenised post-centrifugation and the liquid supernatant was kept as backup. For such tiny tissues, glass bead preparation procedure was used for tissue break down. For larger tissues mortar and pestle was used to make their suspensions, Then 10% tissue suspensions were made from all the samples and stored for RNA extraction, Real-Time PCR, and sequencing in the Foot-mouth-disease laboratory at National Centre for Animal Diseases in Manitoba-Canada in accordance with World Reference Laboratories (WRL), Pirbright, UK and Office International des Epizooties (OIE, Paris, France) guidelines (Kitching *et al.*, 2000).

3.5.2.2 RNA extraction using Spin-Columns technique

Extraction of RNA from whole blood samples using ZR whole blood RNA Mini prep^{TM} kit was conducted as described by the manufacturers (ZYMO-RESEARCH CORP), which is based on the use of a unique buffer system combined with Fast-Spin Column technique as described by Knowles and Samuel, (1998); Reid *et al*., (2001c) The RNA was eluted in >6 µl of RNase-free water and stored at -20^{o}C until used.

3.5.2.3 RNA extraction using RNeasy Spin-Columns method

Total RNA was extracted using the OIE/WRL Pirbright protocol as described by Knowles and Samuel (1998) and recommended by Qaigen^{R}. Each tissue sample suspension was lysed using equal volume of 460 µl Lysis buffer and 70% alcohol was added and mixture loaded on RNeasy spin Column and washed twice. The RNA was eluted with 50 µl DEPC-dH_2O into clean collection tube and spun in a microfuge for 60 sec at 10, 000 rpm. Extracted RNA was kept on ice for immediate use and or stored at -20^{o}C until use for RT-PCR.

3.5.2.4 Real-Time Polymerase chain reaction

Reverse transcription of FMDV RNA and Polymerase chain reaction amplification of Reverse transcribed RNA was conducted using automated Real-Time PCR (rRT-PCR) as prescribed by OIE, (2008) and described by Moniwa *et al*. (2007) regarded as Smart cycler II and Beta –actin rRT-PCR. Briefly, 2 µl and 5 µl of the RNA was added onto 23 µl FMD master mix and 20 µl Beta-actin master mix respectively in tight tubes and inserted in small cycler machine slots and subjected to thermal cycle programme 50^{o}C (30 mins) 95^{o}C (15 mins) 95^{o}C (10 sec) 60^{o}C (1 mins) in an automated smart cycler machine connected to a computer loaded with "smart cycler "software using 10 pmol/µl concentration of primers.

Primers:

Forward: FMDV 1186 F sequence 5'-ACTGGGTTTTAYAAACCTGTGATG-3'

Reverse: FMDV 1237 R sequence 5'- TCAACTTCTCCKGATGGTCCCA-3'

After the completion of the RUN a summary view of cycling profile was displaced in the FAM (FMDV detection) and TET (SPC or Beta-actin detection) graph and the Ct results was viewed upon click of "Result Table" button.

Amplication in FMDV sample (Ct<35.99), was considered positive. Amplication with Ct value between 35.99 and 40.00 and or low curve to threshold sample was considered suspicious or doubtful. Ct value greater than 40 was considered negative. Ct value must be obtained for beta-actin to ensure RNA extraction procedure was successful. Beta-actin positive must have exhibited amplication of acceptable Ct range between 12 and 35.99.

3.5.2.5 Reverse Transcription of Virus RNA

Oligonucleotide primers for RT-reaction and subsequent PCR amplification were used as previously described (Knowles and Samuel, 1998). Briefly, the mixture (11 µl) of 10mM

dNTPs, 5x RT Buffer (with DDT), MMLV (200 U/ µl) RNasin (3.3 U/ µl), DEPC-H_2O, and primers (25 pmol/ µl) was added to 14 µl of each RNA template to make up a total volume of 25 µl in sterile 0.75 ml eppendorf tubes. The mixture was then spun briefly in a microfuge before heating at 42°C for 60 min and at 94°C for 10 min in a thermocycler. "RT product" tubes was labeled and stored at -20°C.

3.5.2.6 Polymerase Chain Reaction for FMDV detection

PCR amplification of reverse transcribed RNA reaction was optimized as previously described (Knowles and Samuel, 1998) for PCR using degenerative primers for O-A-C (Alfonso primers produced by NCAD, Canada) and SAT 1-3 serotypes primers based on published oligonucleotides (Knowles and Samuel, 1998). Briefly, into a sterile 0.75 ml tubes, 25 mM $MgCl_2$ with concentrations 1.5mM, 10mM dNTPs (200µM), 10x buffer, primers (0.5 pmol/ µl), Taq DNA polymerase (5U/µl) 2.5U, RT product and DEPC-H_2O were added to make a final volume of 50 µl. Then 20 µl mineral oil was added to top the mixture, before it was spun. This mixture was heated in thermocycler at 94^oC, 4 min, 1 cycle: 94^oC, 60 sec, 55^oC, 60 sec, 72^oC, 90 sec in 30 cycles and lastly, 72^oC, 5 min, 1 cycle. The PCR product was stored until used

3.5.2.7 Sequencing of PCR products

The PCR amplicons were directly sequenced for strain characterization of capsid region VP1/1D gene as described by Knowles and Samuel (1998) using Sequencing primers designed by the Pirbright laboratory for SAT1-3 serotypes and Alfonso primers (NCAD, Canada) for serotypes O, A, C and promega f-mol^R kit based on manufacturer's instruction. Briefly, purification of PCR Products was done using Promega Wizard Preps™ and cloned

into pGEM-T easy vector and stored -20^{o}C in 0.75 ml eppendorf tubes. End-Labelling of Oligonucleotide primers was then carried out using T4 Polynucleotide Kinase. Isolates were labeled and 1 µl of the appropriate dd/dNTPs was added, spun briefly in microfuge and stored at 4^{o}C. In a separate tube, template DNA, 5x sequencing buffer, ATP labeled primers, sequencing grade Taq polymerase (5U/ µl) and DEPC-H_2O were added for each reaction set, mixed and spun, then mineral oil was addition to top reaction mixture. The mixture was placed in a pre-heater thermal cycler at 94^{o}C and 4 µl stop solution was added and spin down. The reaction mixture was then heated at 80 - 90^{o}C for 2 – 5 min before gel electrophoresis. Sequenced recombinant plasmids data generated were compared to GenBank data base.

3.5.2.8 Gel Electrophoresis of cycle sequencing products

Gel electrophoresis of cycle sequencing products was conducted as described Knowles and Samuel (1998) using serotype A-O-C (Alfonso NCAD, Canada) and SAT1-3 (Pirbright, UK) sequencing primers. PCR product (5 µl) was electrophoresed in a 1% agarose gel made in TBE and stained with ethidium bromide and visualized under a UV transilluminator. Auto-radiograph developed was labeled with the name of each sample.

Oligonucleotide primers used for RT-PCR and cycle sequencing of foot-and-mouth disease A-O-C viruses serotypes were Alfonso primers designed at NCAD Canada for small size amplicons

Forward (Positive) sequence:

O-A-C: 5'-GCCGGCAAAGACTTTGAGCT-3'

VP1.2: 5'-TCAACGTCTCCGGCCAACTT-3'

Reverse (Negative) Sequence:

Universal FMD-2B58 (NK61): 5'-GACATGTCCTCCTGCATCTG-3'

The primer set used for RT-PCR amplification and sequencing of South African Territories (SAT) serotypes of FMDV was as stated below (Knowles and Samuel, 1998)

Name	Primer sequence (5' to 3')	Gene	Nt.	Use
SAT-1D209F	CCACATACTACTTTTGTGACCTGGA	VP1	209-234	Forward primer
FMD-2B208R	ACAGCGGCCATGCACGACAG	2B	208-227	Reverse primer
NK72	GAAGGGCCCAGGGTTGGACTC	2A/2B	34-48/1-6	Sequence primer

This primer set worked for all three SAT serotypes and resulted in PCR products of approximately 730 bp, 715 bp and 718 bp for SAT 1, SAT 2 and SAT 3, respectively.

3.6 Preparation of Digital FMD outbreak Maps

GPS machine named etrex^{R} [LEGEND/GARMIN] (TAIWAN) was used to map out the outbreaks recorded between December and March, 2011 as described by Esuruoso *et al.* (2005). A paper map (analogue) of Ilesha baruba was collected from NASRDA Abuja and converted to a digital map using digitizing tablet (summer sketch 3^{R}). Geographic Information System (GIS) was used for geospatial analysis and monitoring of Foot and Mouth disease outbreaks spread, proximity and topography of outbreak locations,

distribution of disease and closeness to features that can spin off virus transfer within the study area using Cartographic Operation and spatial statistical methods as described (Maheshwaran and Craglia, 2004).

3.6.1 Data projection and geo-referencing

The study area spot satellite imagery overlay was obtained from Google earth pro by the conversion of data set to .Kml (Keyhole Markup Language file). The data frame for Arc GIS 10.1 was projected into WGS 1984 of Universal transverse Mercator for overlay of features. Features such as roads and river bodies in the study area were digitalized and geo-referenced with coordinate system to give data view mode which was transferred into layout view enabled insertion of map title, north arrow, scale bar and legend.

3.6.2 Geospatial recording

Spatial analysis was conducted using buffering operation method carried out in 500m to 1km range based on proximity analysis to determine closeness of animal herds to water bodies and roads which were potential media of disease spread. Hill shade operation was further performed using shuttle radar topography mission (SRTM) Data of 90 m to illustrate terrain topography, and to determine each cattle herd elevation in relation to water bodies and its flow. This analysis was conducted at GEO-APPs Limited a subsidiary of National Space Research and Development Agency, (NASRDA) Abuja.

3.7 Participatory Appraisal

The Fulani herdsmen's knowledge of identification of clinical signs and FMD management was appraised using semi structured interviews in Ilesha Baruba LGA. The herdsmen

during the outbreaks were chosen as informants based on their existing ethno-veterinary knowledge as described (Rufael *et al*., 2008). Further interview, with marketers and butchers in four LGA (Ilorin East, Irepodun, Moro and Offa) including Ilesha baruba LGA was also conducted. Ageing of cattle was locally conducted and classified in Hausa/Fulani languages during participatory epidemiology with Fulani's herdsmen, cattle marketers and butchers. The ages were designated in Hausa as Maraki (male)/ Maraka (female)/Wege (Fulani) for cattle aged from 6 months-3years. Bijimi (male)/Karsana (female) in Hausa while Nghari (Fulani) for cattle aged from > 3 years and above. Age was further grouped into two categories: 6 months - 3 years and >3 years above. The sex was noted and breeds were identified as described by (Mason 1988; Bourn *et al*., 1994).

3.8 Hematological Examination

The corresponding pair of the whole blood collected during FMD outbreaks in Ilesha Baruba was subjected to hematological analysis as described by Jain (1993) and Kumshe *et al*. (1997). The Packed Cell Volume (PVC) was determined by microhaematrocit technique and total white blood cell counts were conducted using Neubauer haemocytometer. The values were noted for association with FMD.

3.9 Statistical Analysis

Participatory appraisal data, FMD positive reactors and hematological parameters obtained was subjected to simple descriptive statistics (percentage) and chi square analysis, (SPSS, 2002) to establish association between FMD occurrence and interview responses, positive reactors, and hematology respectively.

Geo-referenced data obtained was subjected to the nearest neighborhood index analysis to determine spatial distribution of cattle herds. In order to establish specific origin (location) and disease spread in relation to the study area. This was expressed as either clustered for spread within or dispersed pattern for spread across different axis of the study area. The Nearest Neighbor Index was expressed as ratio of Observed Mean Distance to the Expected Mean Distance. The expected distance was the average distance between neighbors in a hypothetical random distribution. For index less than 1, pattern exhibited was clustering, for index greater than 1, the trend was toward dispersion or competition (Krivoruchko and Krause, 2012).

Sequence analysis was conducted using TREEVIEW software as described (Page, 1996). The dendogram tree-view was produced using BLAST pairwise alignments and Fast Minimum Evolution based on blastn program from NCBI. The Blast computed a pairwise alignment between a query and the database sequences searched. In this sequence tree presentation was an implicit alignment between the databases sequences constructed, based upon the alignment of database sequences to the query.

CHAPTER FOUR

RESULTS

4.1 Enzyme Linked Immuno-Sorbent Assay (ELISA)

4.1.1Occurence of foot and mouth disease antibodies in serum samples of cattle

Out of the 450 samples tested for foot and mouth disease antibodies, 338 (75.11%) were positive and 112 (24.88%) were negative. The results of NSP- blocking ELISA (Priocheck^{R}) test showed that percentage of Bovine Foot and Mouth Disease in Kwara state was 75.11% (338/450) as shown in Table 4.1

Table 4.1: Percentage distribution of antibodies to Bovine Foot and Mouth Disease Virus as detected by NS-ELISA

Serum sample tested	Frequency	Percentage (%)
Positive	**338**	**75.11**
Negative	**112**	**24.88**
Total	**450**	

n=450

4.2 Demographic distribution of Foot and Mouth Disease Antibodies in Kwara state

4.2.1 Sex distribution

Out of the 268 serum samples from female (cows), 210 (78.4%) were positive for NS antibodies. One hundred and twenty eight (70.3%) of the 182 serum samples from male (bulls) were positive for Foot and Mouth disease antibodies. FMD were found to affect cow than bulls ($p< 0.05$) (Table 4.2)

4.2.2 Breed distribution

Cattle breed distribution showed that out of the 306 White Fulani (Bunaji) sampled 261(85.29%) were positive and 45 (14.70%) negative. The Red bororo (Rahaji) had a frequency of 79, with 37 (46.83%) being positive and 42 (53.16%) negative, while the Sokoto Gudali had a frequency of 65, with 40 (61.53%) positive and 25 (38.46%) negative. White Fulani breed showed association with FMD than other breeds ($p< 0.05$) (Table 4.3)

4.2.3 Age distribution

The age group 6 months – 3years designated as young had a frequency 298 out of the 450 cattle sampled with 254 (85.23%) positive and 44 (14.76%) negative. The adult group 3 years and above had frequency occurrence 152 out of 450 cattle sampled with 84 (55.26%) positive and 68 (44.73%) negative. FMD was found to affect younger cattle than older ones ($p< 0.05$) (Table 4.4)

4.2.4 Distribution based on sampling location

The distribution of sero-positive cattle by sampling locations indicated that Ilesha baruba LGA recorded 76 (84.44%) positive. This was closely followed by Ilorin East LGA with 70 (77.77%) positive. Offa had 67 (74.44%) positive, while Bode Sadu had 65 (72.22%) positive and 25 (27.77%) negatives and Ajasse had 60 (66.67%) positive and 30 (33.33%) negatives. Location was found to affect prevalence of FMD ($p< 0.05$) (Table 4.5)

Table 4.2: Distribution of NSP antibodies to Bovine Foot and Mouth Disease Virus as detected by ELISA according to sex

Sex	Positive (%)	Negative (%)
Male	128 (70.3)	54 (29.7)
Female	210 (78.4)	58 (21.6)
Total	**338 (75.11)**	**112 (24.88)**

Sex: X^2=3.737 df=1 p=0.0532 OR=0.6547 CI=0.4254 to 1.007 n=450

Table 4.3: Breed distribution of Foot and Mouth Disease Virus antibodies as determined by NS-ELISA

Breed	**Results of NS-ELISA**	
	Positive (%)	**Negative (%)**
White Fulani (Bunaji)	261 (85.3)	45 (14.7)
Sokoto guduali (Bokoloji)	37 (46.8)	42 (53.2)
Red Bororo (Rahaji)	40 (61.5)	25 (38.5)
Total	**338 (75.11)**	**112 (24.88)**

X^2=57.16 p<0.0001 df=2 n=450

Table 4.4: Age distribution of Foot and Mouth Disease Virus antibodies in cattle as detected by NS-ELISA

Age	**Results of NS-ELISA**	
	Positive (%)	**Negative (%)**
Young (6 mths-3 years)	254 (85.2)	44 (14.8)
Adult (>3 years)	84 (55.3)	68 (44.7)
Total	**338 (75.11)**	**112 (24.88)**

X^2=43.70 df=1 p<0.0001 OR=4.398 CI=2.787 to 6.942 n=450

Table 4.5: Distribution of Bovine Foot and Mouth Disease Virus antibodies as detected by NS-ELISA according to sampling locations

Location	Results of NS-ELISA		Total
	Positive (%)	Negative (%)	
Offa	67 (74.4)	23 (25.5)	90
Bode Sadu	65 (72.2)	25 (27.8)	90
Ilesha Baruba	76 (84.4)	14 (15.5)	90
Ilorin	70 (77.8)	20 (22.2)	90
Ajasse	60 (66.7)	30 (33.3)	90
Total	**338 (75.11)**	**112 (24.88)**	**450**

X^2=17.80 df=4 p<0.0014 n=450

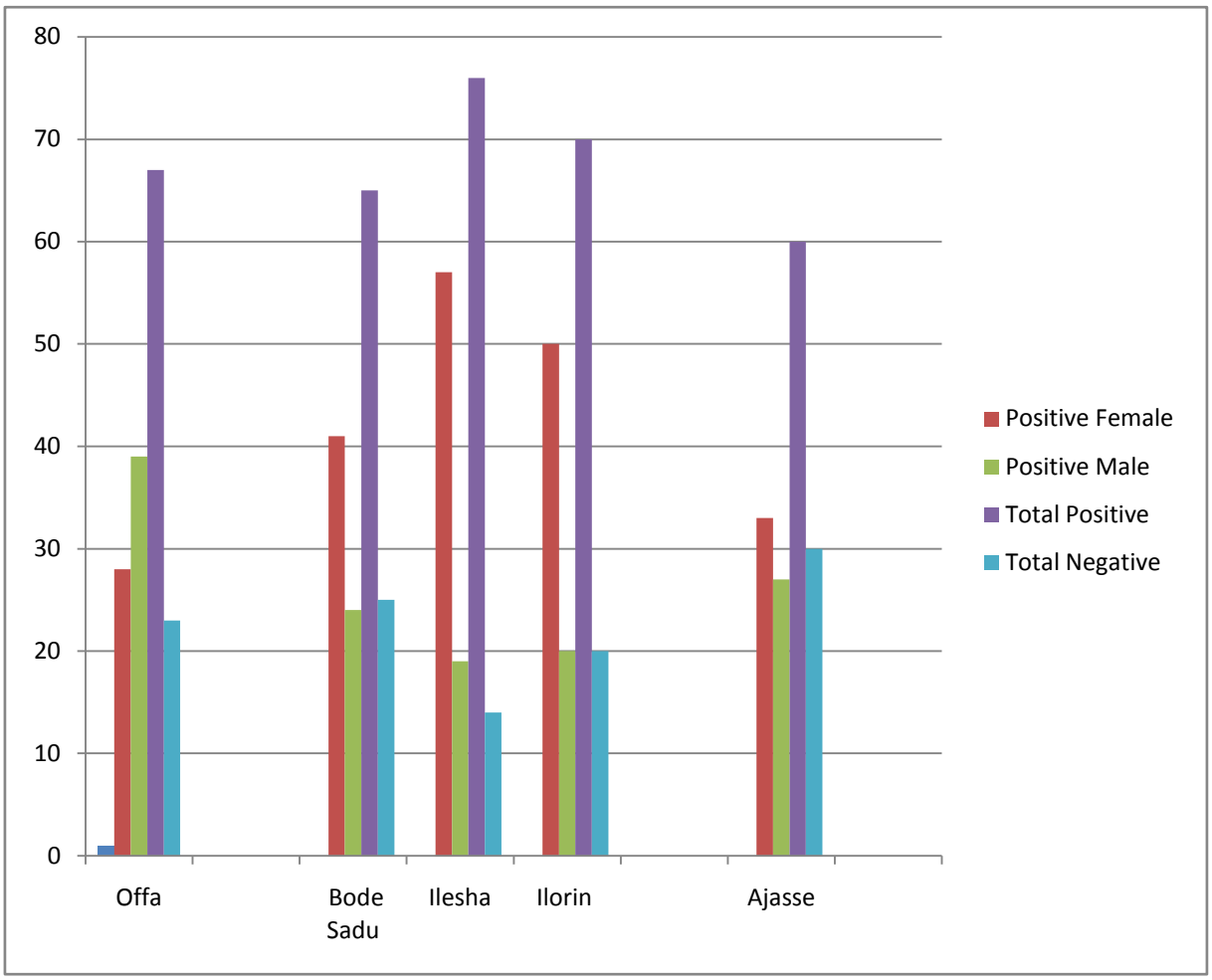


Figure 4.1: Distribution of FMD sero-positive trade cattle according to sex and location in Kwara state

4.3 Solid-Phase Competitive Enzyme-Linked Immuno-Sorbent Assay

4.3.1 Occurrence of foot and mouth disease virus serotype antibodies in trade cattle sera

Out of the 338 positive foot and mouth disease antibody sera as determined by NSP ELISA test, aliquots of 253 subjected to 3ABC ELISA and Solid Phase Competition ELISA test. 188 sera showed evidence of FMD specific serotype antibodies to solid phase competitive ELISA for serotype A (18.6%) 47, serotype O (46.6%) 118, SAT1 (21.3%) and SAT2 (13.4%) 34 sera as shown in Table 4.6. However, 3ABC ELISA test showed NSP negative (absence of non structural proteins) for positive serotype in 13 sera of serotype A, 31 sera of serotype O, 7 sera of SAT1 and 14 sera of SAT2.

In addition, a hundred sera sample showed evidence of multiple serotype combination of O and A, A and SAT2 as well as O and SAT2 serotype antibodies which resulted in a higher frequency of serotype occurrence when compared with the positive sera tested.

Table 4.6: Distribution of Foot and Mouth Disease Virus serotype antibodies in trade cattle sera

TEST		SEROTYPES					TOTAL
ELISA	**NSP**	**A**	**O**	**SAT1**	**SAT2**	**SAT3**	
Blocking	+	47	118	54	34	-	253/338
3ABC	-	13	31	7	14	-	65 (25.7%)
$_c$ELISA		34	87	47	20	-	188 (74.3%)
%		**18.6**	**46.6**	**21.3**	**13.4**	**-**	**253**

4.3.2 Distribution of FMD serotype antibodies in cattle outbreaks in Ilesha baruba district

The distribution of serotypes recorded in the outbreaks was Serotype O 83.0% (53), serotype A 7.8.0% (5), multiple serotypes O and A 4.7% (3), O and SAT2 1.6% (1) Vaccine O 1.6% (1) and Vaccine SAT2 1.6% (1) respectively. In outbreak number 1 serotypes O, multiple serotypes of O and A was identified and serotype O was also associated with the repeated outbreaks, while in outbreak number 7 serotypes O and A was initially detected as serotypes O, SAT2 was later observed in the re-occurred in outbreak number 12. The outbreak in herd 8 showed serotype O with subsequent re-occurrence of serotypes O and combined O, A as well as evidence of vaccine SAT 2 use (Table 4.7).

Table 4.7: Distribution of Foot and Mouth Disease serotype antibodies in cattle outbreak sera in Ilesha baruba district.

Outbreaks	Location (Ward)	Herd size	No. Sick	NSP-ELISA	SPC- ELISA			3ABC ELISA	
					Serotype O	Serotype A	Multiple Serotype	Vaccine O	Vaccine SAT-2
2,3,8,13*,14,15	Bode I	1518	212	30	23	4	O,A	1	1
1,4,5*,6,9,10	Bode II	1680	440	21	20	-	O,A	-	-
7, 11, 12*	Bukaru-Rontuwa	1050	190	13	10	1	O, SAT2; O,A	-	-
15		4,248	842	64	53	5	4	1	1

***Repeated outbreaks**

4.4 Molecular Diagnosis

4.4.1 RNA extraction from blood and epithelial tissue

RNA extraction protocol conducted on sixty (60) samples showed no FMD was found in any of the blood tested (Ct values were all 0). The beta-actin results for blood ranged from Ct=30 to 34.

4.4.2 Real Time polymerase chain reaction

The Real-time RT-PCR assay, which targeted FMDV 3D gene showed six (6) positive with Ct values ranging from 15.28 to 26.42 and only one epithelial sample was negative as shown in (Table 4.8 and Plate I) respectively. The control test for RNA quality, real-time RT-PCR assay for beta-actin, suggested sample sub-optimal quality in 2 samples which showed no amplification and the other 5 were weak with Ct values ranged from 28.56 to 33.08.

4.5 Sequencing

4.5.1 Gel electrophoresis of cycle sequencing products

Agarose gel bands showed FMD serotype A from positive amplicons of sequenced RT-PCR using O-A-C (Alfonso) sequencing primers (Plate II) and SAT2 from positive amplicons using SAT 1-3 sequencing primers (Plate III) at 715bp and 730bp respectively.

4.5.2 Sequence analysis and tree-view dendograms

The observed weak RT-PCR product cloned in pGEM-T easy vector prior to sequencing, confirmed as Serotype A sequence based on NCBI blastn program. This was closely related with the genbank A/NIG/21/2009 sequence with genetic homogeneity >93% genetic

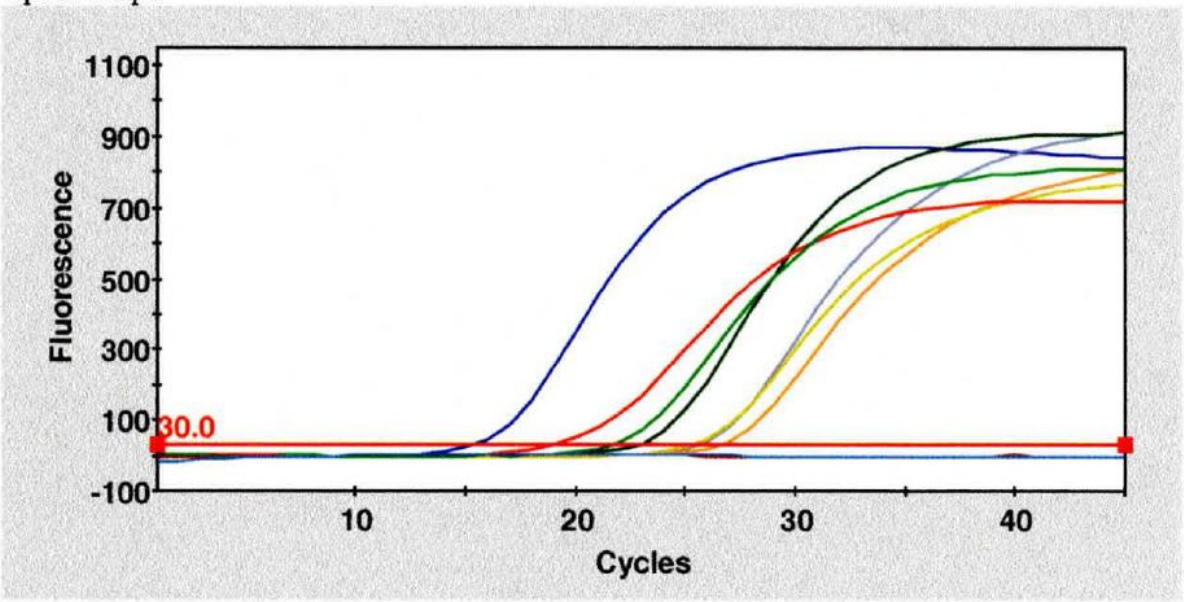
identity in the VP1 region, though multiple NIG/2009 sequences were also identified as closely related as shown in Plate IV.

The strong RT-PCR product sequenced directly using Pirbright sequencing primers to VP1 for SAT serotypes was confirmed to be SAT2 serotype based on NCBI blastn program. This was closely related with genbank sequence SAT2 isolate LIB/7/2003 genetic homogeneity >93% genetic identity in the VP1 region, as shown in Plate V. Hence, observed isolates was designated following the conventional nomenclature for viruses as serotypes A/NIG/1/2011 and SAT2/NIG/3/2011 respectively.

Table 4.8: Real-Time Ct Values for positive FMDV outbreak epithelial samples

Sample ID	Location	Protocol	FAM Ct	FAM Std/Res	TET Std/Res	TET Ct
Herd 2	Bode I	FMD	22.74	+Ve	-Ve	0.00
Herd 5	Bode II	FMD	25.36	+Ve	-Ve	0.00
Herd 7	Bukaru-Rontuwa	FMD	18.79	+Ve	-Ve	0.00
Herd 8	Bode I	FMD	26.42	+Ve	-Ve	0.00
Herd 10	Bode II	FMD	25.53	+Ve	-Ve	0.00
Herd 12	Bukaru-Rontuwa	FMD	15.28	+Ve	-Ve	0.00
Herd 13	Bode I	FMD	0.00	-Ve	-Ve	0.00

Optics Graph



Site Legend

Site ID	Sample ID	FAM Ct	Protocol
A1	1 - FMD	15.28	FMD

Data Type	Line Type
Primary Curve	———

Plate I: rRT-PCR optic curves showing florescence versus cycles for FMD samples

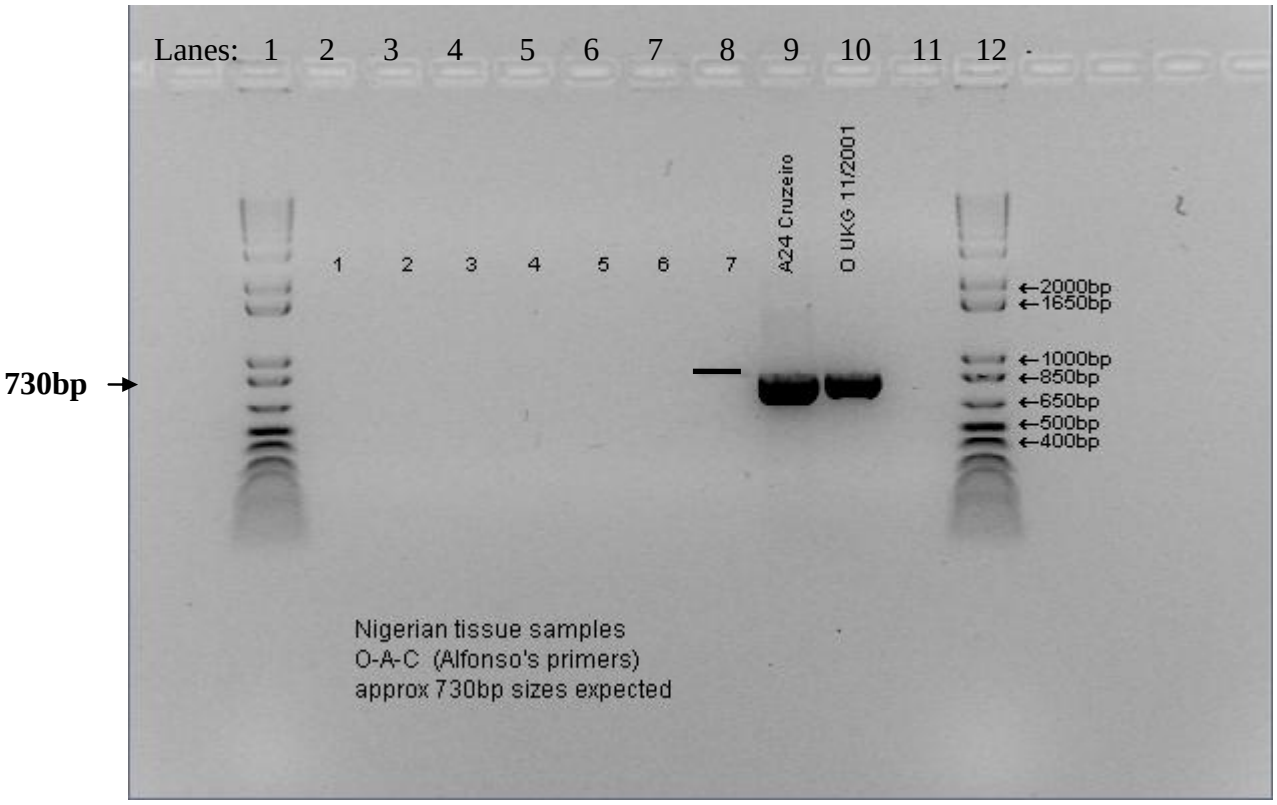


Plate II: Agarose gel band showing positive Serotype A amplicons of sequenced RT-PCR products FMD using O-A-C primers

A 730 bp PCR product resulting from amplification of FMDV RNA extracted from epithelial tissues. Lane1: DNA size marker, ladder 100, Negative sample, Lane 2-7: Positive sample 7, Lane 8: Positive control of Serotype A Lane 9: Positive control of Serotype O, Lane 10: Negative control Lane 11 and Lane 12: DNA size marker, ladder 100.

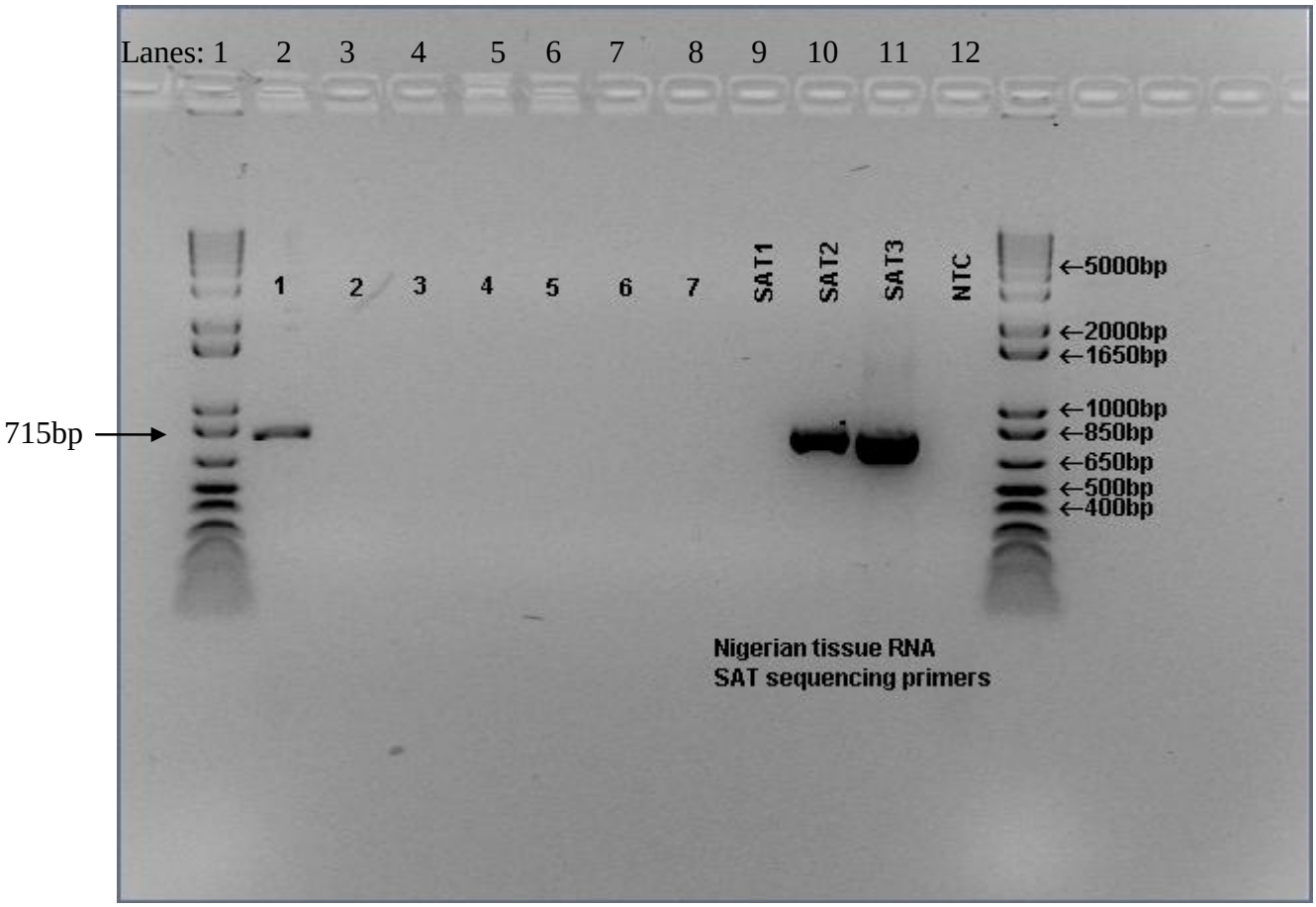
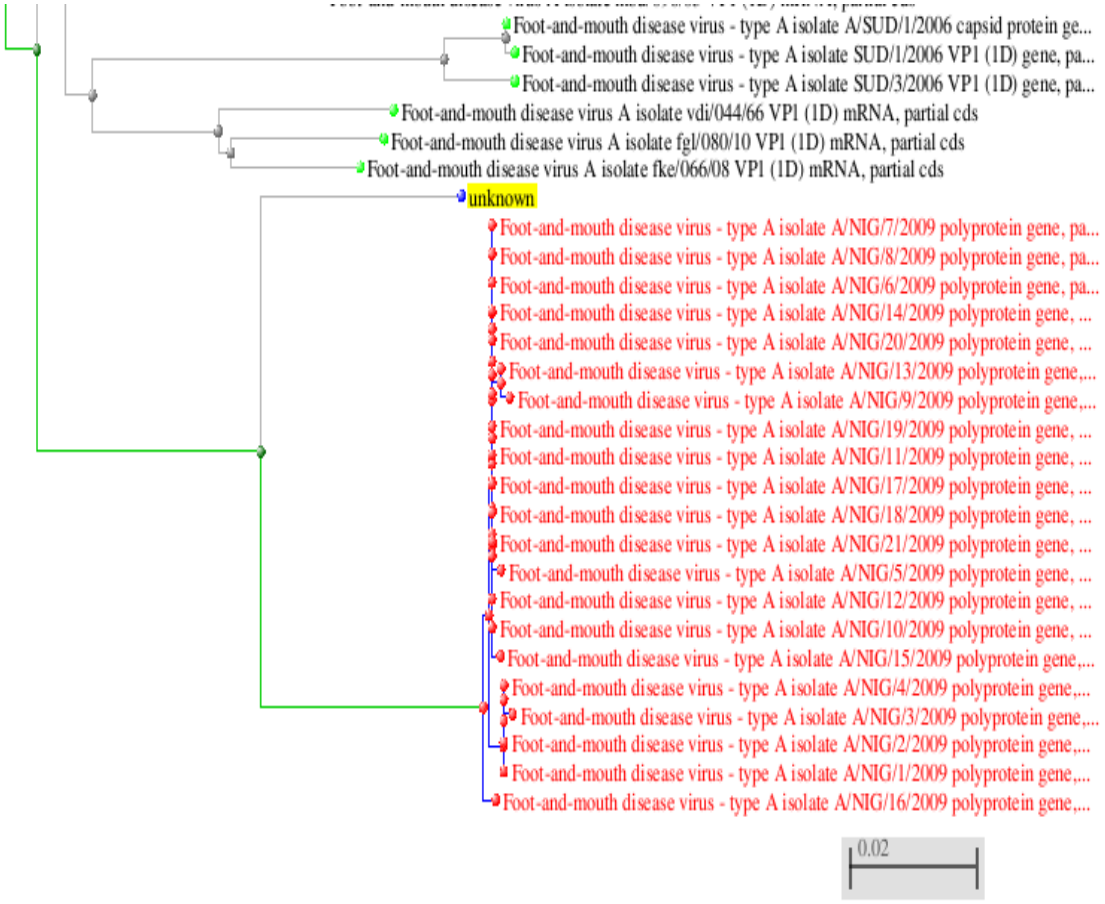


Plate III: Agarose gel band showing positive SAT2 amplicons of sequenced RT-PCR products using FMD -SAT 1-3 primers

A 715 bp PCR product resulting from amplification of FMDV RNA extracted from epithelial tissues. Lane1: DNA size marker, ladder 100, Lane 2: Positive sample 12, Lane 3-8: Negative samples, Lane 9: Positive control of SAT1, Lane 10: Positive control of SAT2, Lane 11: Positive control of SAT3, Lane 12: Negative control and Lane 13: DNA size marker, ladder 100.



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Plate IV: Subset phylogenic tree view of Nigerian Serotype A obtained in this study as compared with NCBI sequence database

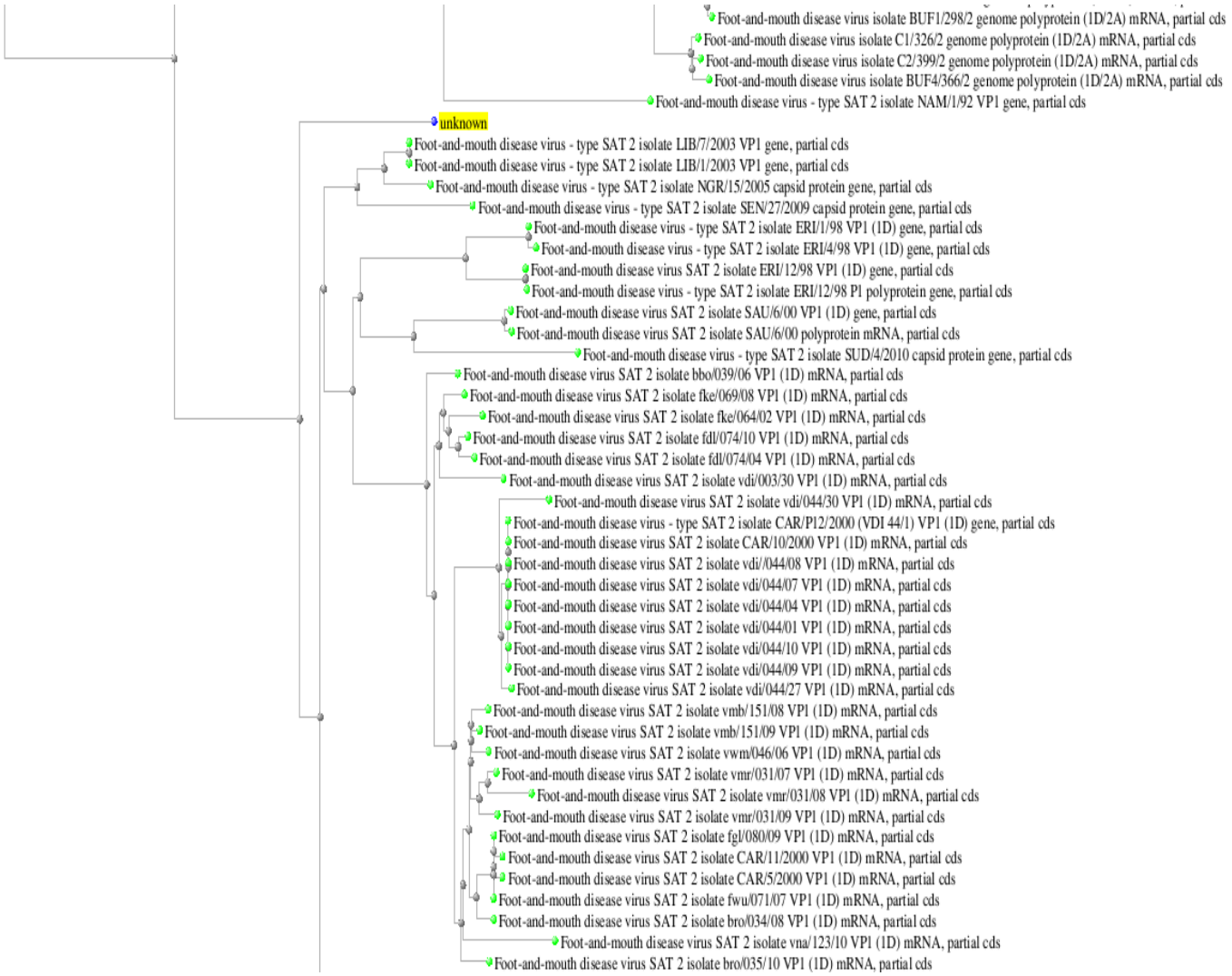


Plate V: Subset phylogenic tree view of Nigerian SAT-2 obtained in this study as compared with NCBI sequence database

4.6 Geo-spatial Distribution of FMD outbreak location and serologically detected Serotypes in Ilesha baruba district

4.6.1 Distribution and occurrence bovine FMD outbreaks in Ilesha baruba district

The investigation showed 15 outbreaks were recorded and three (3) herds experienced multiple outbreaks within the study period. Outbreaks occurred in 12 herds in three locations namely Bode I, Bode II, and Bukaru – rontuwa. The first and last investigated outbreaks in Bode II oocurred within the same herd which was designated as outbreak 1 and 5 respectively. Other herds that experienced repeated occurrence was indicated as outbreaks 8 and 13 (Bode I) as well as outbreaks 7 and 12 (Bukaru- Rontuwa) as shown Table 4.9

4.6.2 Geospatial analysis of FMD virus serotypes in Ilesha baruba district

The geospatial analysis carried out for Image overlay, buffer operations, Hill shade effect and spatial statistics revealed spot imagery overlay (©2010 Google) and layout-view maps of the study area with various scenic features such as towns, rivers, road network, and forest as shown in Plates VI and VII respectively. The number of infected/ sick cattle in each herd was displayed in a graduated proportion which showed more cattle herds was infected in Bode, Gbedeberru wards (Plate VIII), displayed FMD serotype distribution in relation to outbreak zones showed serotype O, A circulated in Bode ward while O, A and SAT2 was detected in Bukaru-rontuwa ward (Plate IX). Cattle location in 500m buffer zones in and around the Ilesha market, slaughter slab as well as Bode I and II in Gbedeberru wards showed close proximity to the access roads coming from Bukaru to Ilesha and that which linked Tabetebere to Gbedeberru but herds in Bukaru-rontuwa ward were over a kilometer to the main road from Kosubosu (LGA headquarters) in Yashikira

district through Gidan Magajiia to Ilesha baruba which ultimately linked to ilorin (Plate X) while 1km buffer zone showed additional association of water source around Ilesha, Bukaru and Bode I (Plate XI).

The Nearest Neighborhood Index (NNI) showed Euclidean distance of cattle herd locations as clustered (NNI Ratio=0.095054) which accepted alternate hypothesis contrary to random as predicted by null hypothesis (Plate XII). The terrain height of cattle herd location in relation to water bodies and kwara National Park using area elevation data from shuttle Radar topographic mission satellite (SRTM) of 90 meters (Plate XIII) showed that these outbreak locations are on higher elevations.

Table 4.9: Distribution of Foot and Mouth Disease Outbreaks according to locations in Ilesha Baruba district

Outbreak ID	Location	Herd size	No. affected	No. of dead animals
2	Bode I	380	60	5
3		78	22	1
8*		200	40	2
13*		200	20	2
14		380	20	3
15		280	50	1
1*	Bode II	300	50	3
4		220	80	3
5*		300	100	3
6		260	60	2
9		250	70	2
10		350	80	4
7*	Bukaru-rontuwa	450	100	4
11		150	30	1
12*		450	60	3
Total		4,248	842 (19.82%)	39 (1.0%)

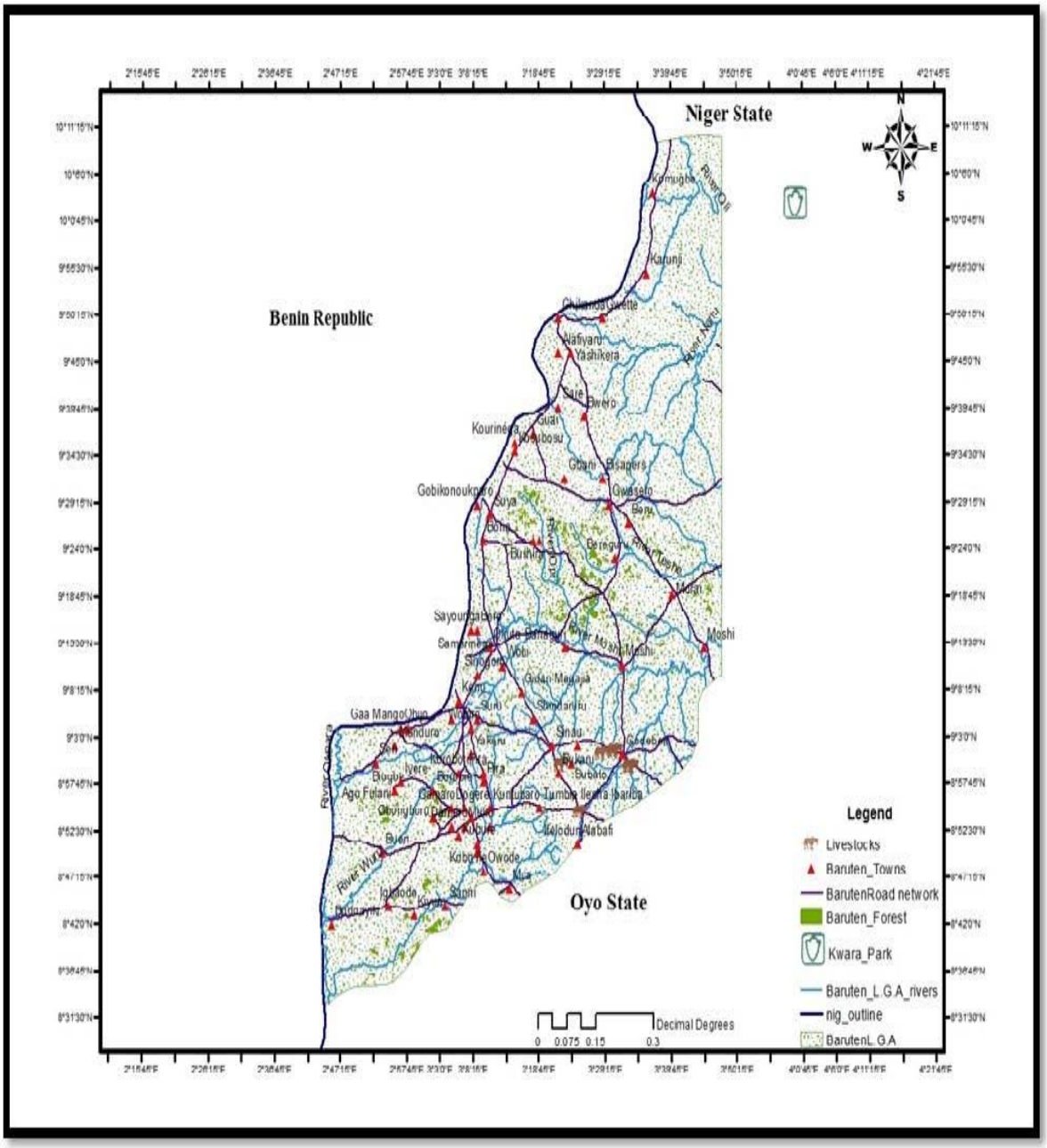


Plate VI: Study area (Ilesha Baruba) overlay map showing interconnectivity of roads, rivers and towns

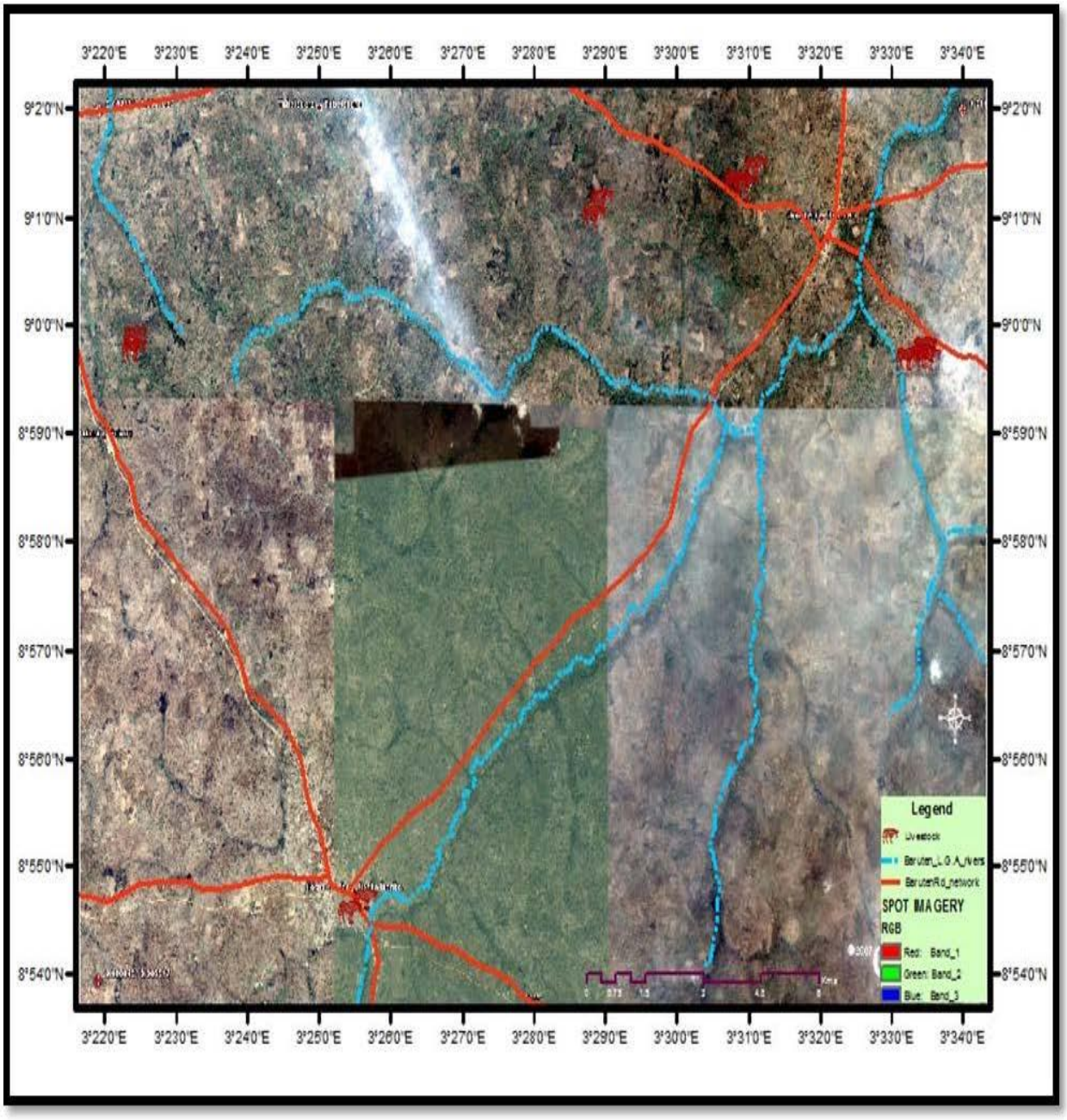


Plate VII: Study area spot imagery map showing scene features and outbreak locations

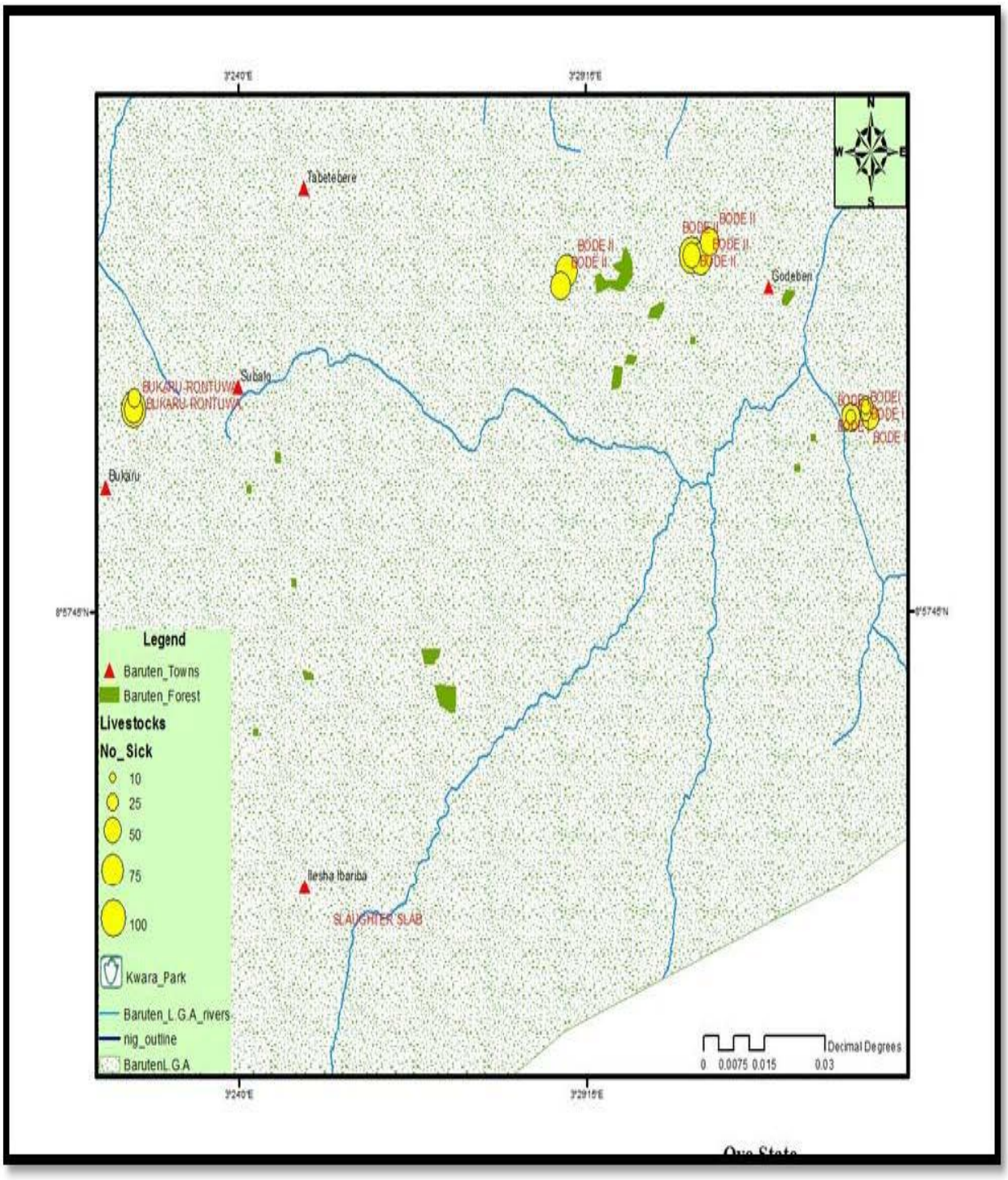


Plate VIII: Study area map showing graded quantities of FMD infected cattle distribution within the herds

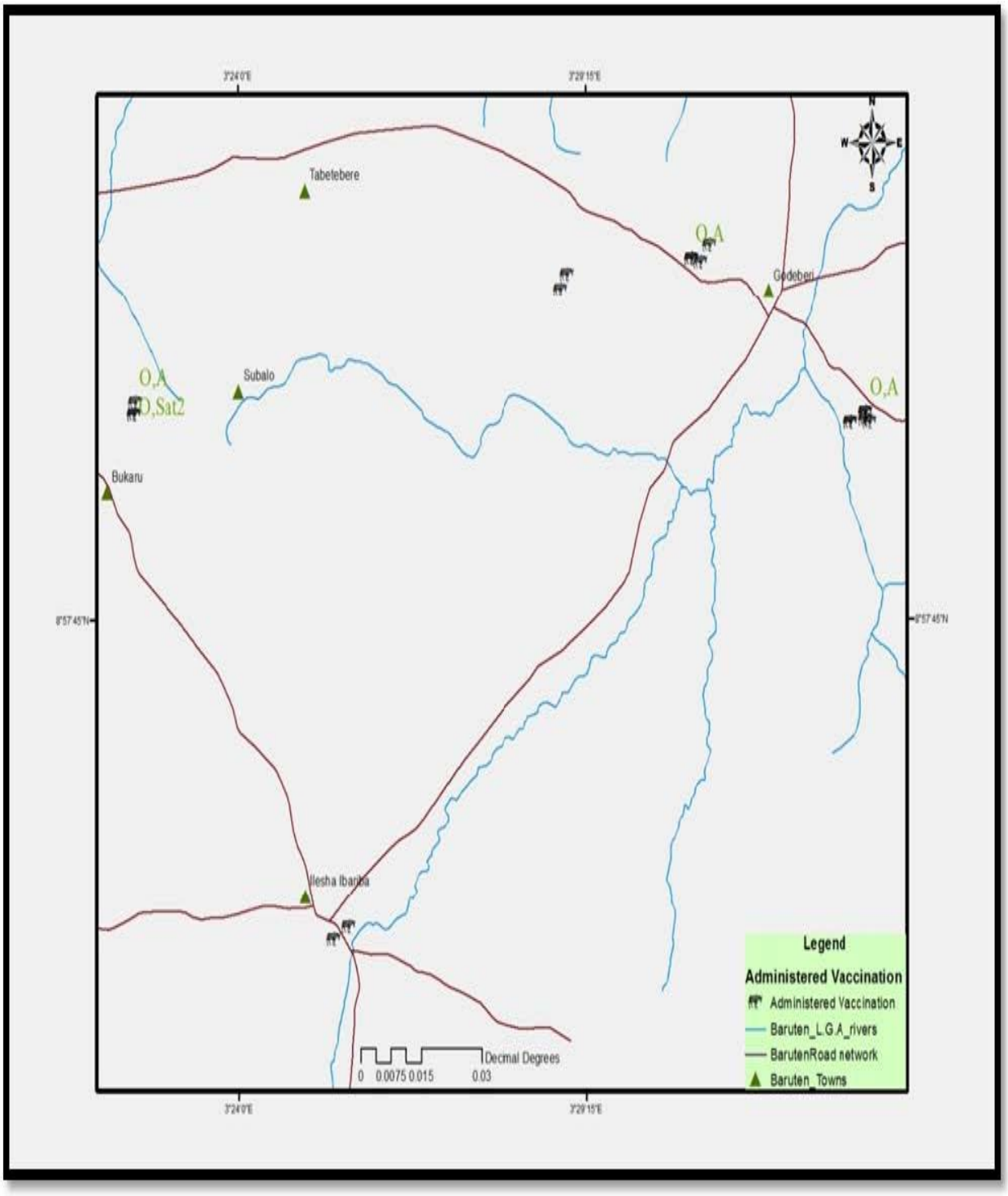


Plate IX: Study area map showing distribution of FMD outbreak serotypes

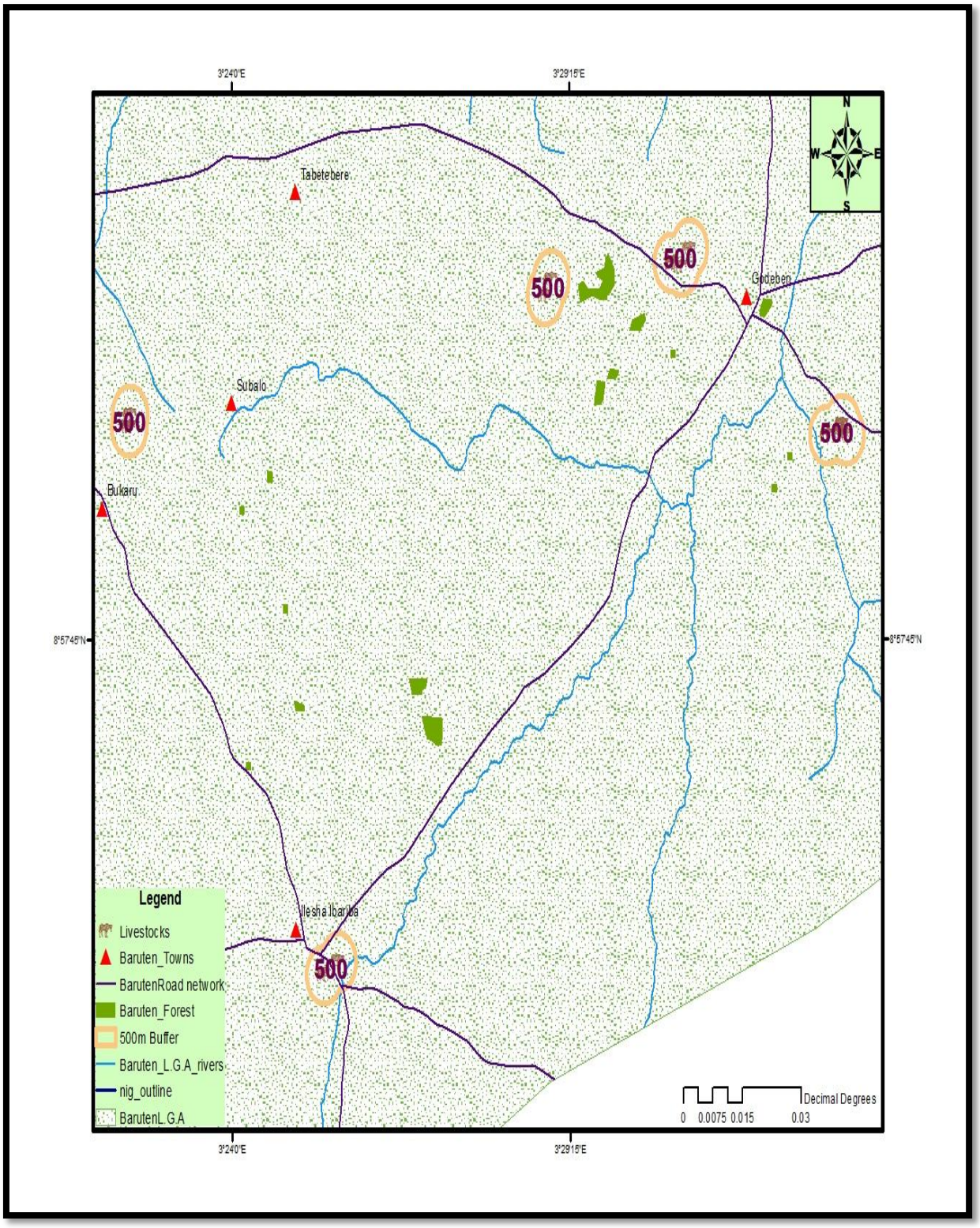


Plate X: Study area showing geographic features within 500m buffer zone around the outbreak herds

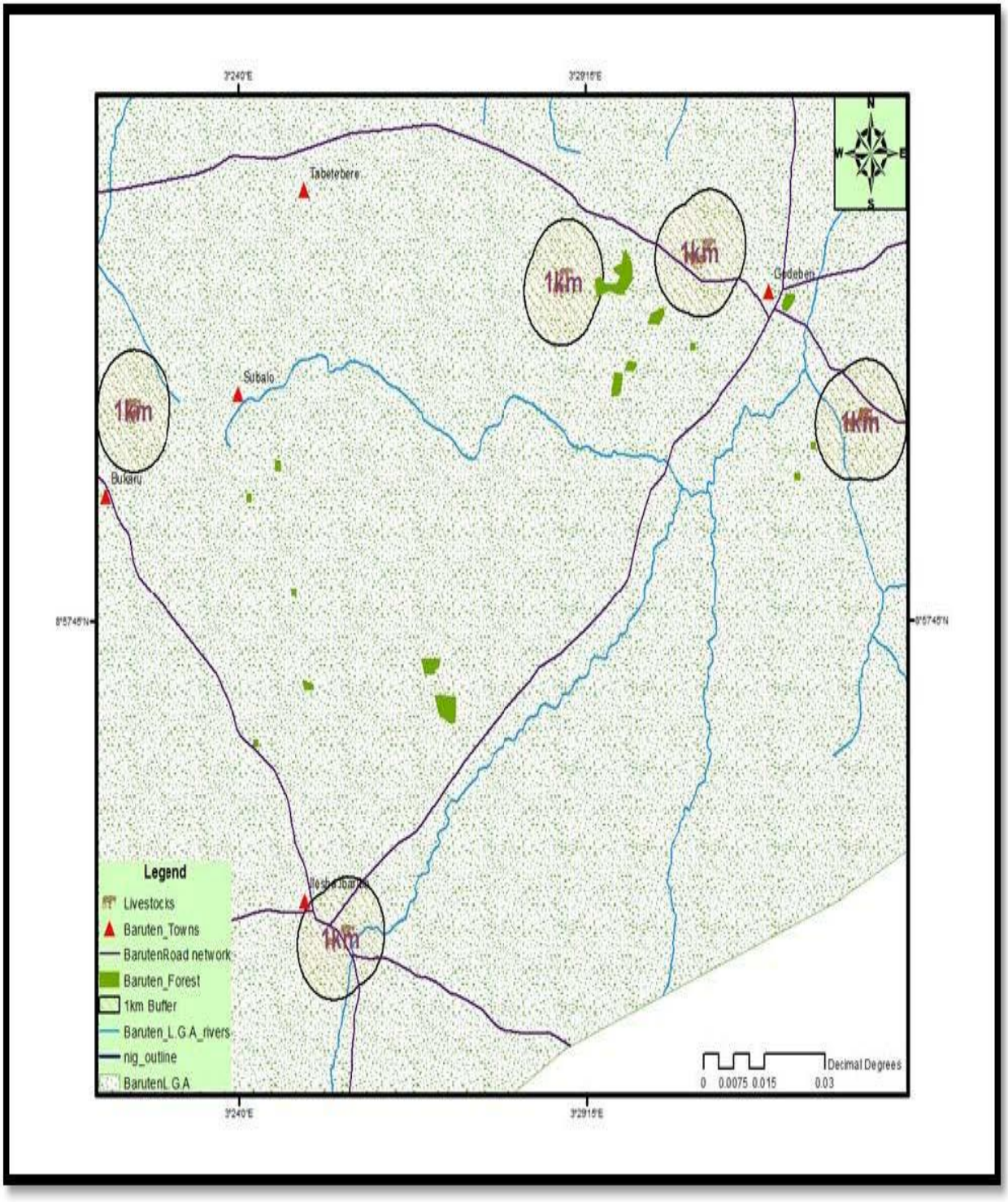


Plate XI: Study area map showing geographic features within 1Km buffer zone around the outbreak herds

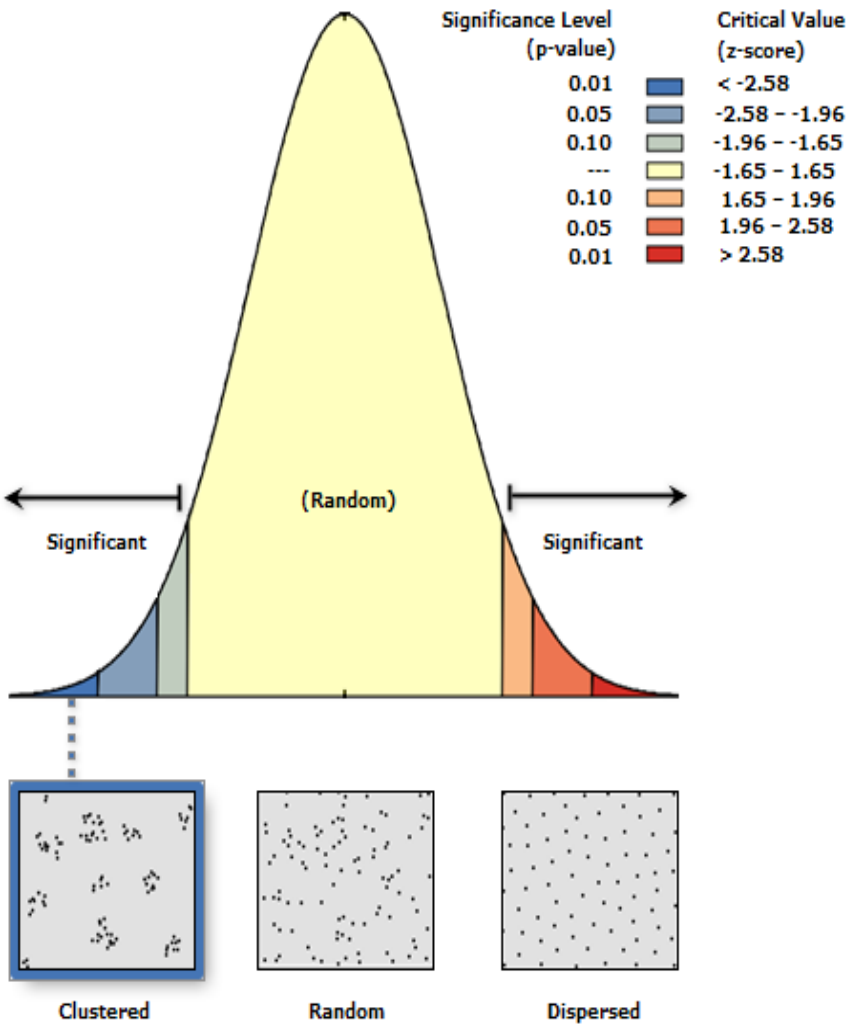


Plate XII: Nearest Neighbor Index illustrating clustered nature of cattle herd locations.

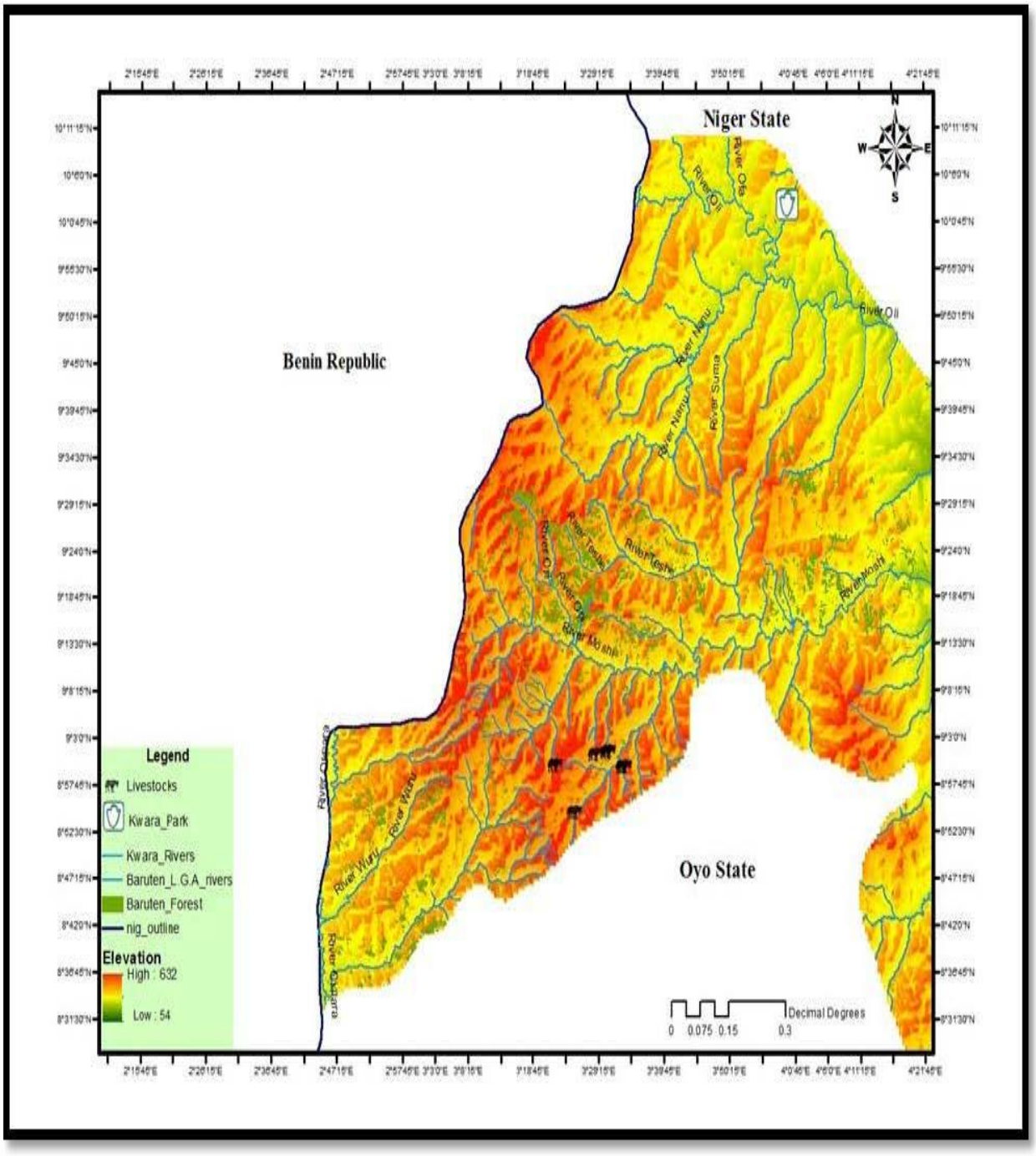


Plate XIII: Study area map showing Hill shade elevation of outbreak locations

4.7 Participatory Appraisal of FMD knowledge, Identification and Management practices

4.7.1 FMD appraisal response amonsgt Fulani herdsmen in Ilesha baruba district

Validation of Fulani herdsmen's knowledge on the identification and management of FMD using semi structured interviews during outbreaks investigations revealed all responses were independent and statistically significant by chi square ($P<0.05$) [SPSS] (Table 4.10). Out of fifteen (15) recorded FMD outbreaks, semi nomadic husbandry management was mostly practiced in herds involving all cattle breeds with no history of FMD. However, some herdsmen vaccinate their cattle against Hemorraghic septicemia (HS), Contagious Bovine Pleuropneumonia (CBPP), Black Quarter (BQ) and at times Brucella. Investigation with regards water source revealed rivers Yepti and Janburku /lassa as well as the surrounding streams and water bodies in Ilesha district as source of water which predisposed cattle contact with other herds but not wildlife. In depth discussion with herdsmen revealed no history of recent animal introduction and or distant migration but with in-contact animals within the herds such as sheep, goats and donkeys. The cattle graze around neighboring settlement (Bode) during the wet season and Mosi Korfar during the dry season. Further discussion showed the herdsmen can recognize FMD and some of them mentioned signs such as hair loss around the horns, upper hoof regional swelling which later resulted into wounds. Others indicated sores on the muzzle, tongue and salivation as common signs. Although, herdsmen reported young female cattle could be affected by FMD with exception of donkeys and no particular season was being associated with disease occurrence. It was also gathered that some herdsmen use local herbs and drug medication (Penicillin). They lamented their inability to access veterinary services which compelled them to engage a common service provider (area livestock superintendent) as well as

limited FMD vaccination due to unavailability and or high cost of limited imported FMD vaccines. Finally, non responsive animals to treatment were sold to butchers for human consumption at reduced market value as death of affected cattle was often avoided, hence uncommon.

4.7.2 FMD appraisal responses amongst cattle marketers and butchers in kwara state

The participatory appraisal with cattle marketers and butchers provided as highest percentage frequency of response as shown in Table 4.11 revealed FMD was commonly regarded as Chabu or Boro. All sex, age and breed were affected by FMD in cattle brought to the market and slabs for sales and slaughter respectively. Affected cattle showed typical signs of FMD in both dry and rainy season in the markets as responded by marketers. Affected cattle were usually treated locally and un-responsive cattle are sold to butchers for human consumption with no case of human occurrence as stated by the marketer and butchers. Further discussion with cattle dealers revealed these cattle were brought from Bauchi, Borno, Taraba (Bali), Oyo (Ibgeti, Kishi), other parts of kwara state (Jebba, Bode Sadu, Ilesha baruba, Kiama, Ilapa, Ajasse) as well as the borders of the Cameroon (Kafanchin). The Ilesha baruba international cattle marketers responded cattle were brought from Mali, Togo, Benin, Cote de voire/Burkina Faso, Senegal as well as those from neighboring Local Government Areas in Kwara and Oyo states.

Table 4.10: FMD appraisal responses amongst Fulani herdsmen in Ilesha Baruba, Kwara state

Variable	Category	No. examined	Response	X^2	P-value	95% CI
Husbandry	Semi nomadic Intensive Extensive	15	12 1 2	14.8	0.001	-10.11 to 20.11
History of FMD vaccination	Yes No	15	1 14	11.2667	0.001	-75.09 to 90.09
History of vaccination against other diseases	Yes No	15	15 Nil	15	0.00	-87.80 to 102.80
History of recent introduction of animals	Yes No	15	2 13	8.06667	0.005	-62.38 to 77.38
Type of in-contact animal	Sheep, goat donkey None	15	15 Nil Nil	30	0.00	-16.51 to 26.51
Contact with wildlife	Yes No	15	Nil 15	15	0.00	-87.80 to 102.80
Source of water	River Yepti Janburku /lasa	15	8 7	0.0667	0.796	1.147 - 13.853
Location of grazing (wet)	Within the neighborhood	15	15			
Location of grazing (dry)	Kofar Morsi Distant migration	15	12 3	5.4	0.02	-49.68 to 64.68
Season of FMD occurrence	Dry Wet	15	8 7	0.0667	0.796	1.147 - 13.853
Recognition of FMD	Yes No	15	15 Nil	15	0.00	-87.80 to 102.80
Some signs mentioned	Mouth Lesions Foot lesions Both	15	2 3 10	7.6	0.022	-5.83 to 15.83
Cattle breed affected by FMD	Yakana Bokoloji All breed	15	Nil Nil 15	30	0.00	-16.51 to 26.51

Age affected by FMD	Young Adult	15	15 Nil 15	30	0.00	-16.51 to 26.51
Sex affected by FMD	Female Male	15	15 Nil	30	0.00	-16.51 to 26.51
Availability of Veterinary service	Yes No	15	4 11	3.267	0.071	-36.97 to 51.97
Medication	Drugs (Penicillin) Herbs	15	9 6	0.6	0.439	-11.56 to 26.56
Management of sick cattle	Sales Death	15	15 Nil	30	0.00	-16.51 to 26.51

Table 4.11: FMD appraisal responses amongst the cattle marketers and butchers in Kwara state

Variable	Category	Respondents	Frequency	(%)
Local	Chabu	50	27	54
names	Boro		23	46
Sex	Male	50	12	24
	Female		16	32
	Both		20	40
Age	Young	50	17	34
	Adult		11	22
	Both		22	44
Breed	W/Fulani	50	10	20
	Red bororo		9	18
	S/Gudali		4	8
	All breeds		27	54
Season	Wet	50	10	20
	Dry		12	24
	Both		28	56
Medication	Local	50	10	20
	Veterinary		8	16
	Self		32	64
Other	Sales	50	45	90
intervention	Death		5	10
Human	Yes	50	50	100
infection	No		-	-

4.8 FMD outbreak incidence and hematological picture in clinically infected cattle herds in Ilesha baruba district

4.8.1 Incidence of Foot and Mouth disease outbreaks in Ilesha baruba district

This study revealed overall Foot and Mouth Disease incidence of 18.9% in fifteen (15) outbreaks amongst twelve (12) herds with a total of 4, 248 cattle. A total of 842 were identified as FMD infected cattle based on clinical signs and history as indicated in Table 4.12.

4.8.1.1 Sex distribution

Out of ninety representative blood samples randomly collected across the herds for sero-diagnosis, female (cows) had frequency of 53 and 37 were male (bulls). Forty 40 (75.5%) out of the 53 females were positive for foot and mouth disease NS-antibodies and 13 (24.5%) was negative. While 24 (64.86%) out of the 37 males were positive for NS-FMD antibodies and (35.14%) was negative. The percentage distribution the 64 positive reactors showed 40 (62.5%) cows and 24 (37.5%) bulls were positive for FMD antibodies. Sex showed association with FMD occurence ($p<0.05$) Table 4.13

4.8.1.2 Breed distribution

White Fulani breed sampled had a frequency of 41, with 30 (73.17%) positive and 11 (26.82%) negative. Sokoto Gudali cattle had frequency of 29 with 22 (75.86%) positive and 7 (24.14%) negatives, while Red Bororo breed had a frequency of 20 with 12 (60.0%) positive and 8 (40.0%) negatives. The percentage distribution of representative sero-positive cattle was 46.9% (30/64) for White Fulani breed, 34.4% (22/64) for Sokoto gudali breed, and 18.6% (12/64) for Red Bororo breed. No association between breed and FMD occurence ($p>0.05$) Table 4.14

4.8.1.3 Age distribution

Age group designated young cattle (6months-3yrs) had a frequency of 47 with 35 (74.46%) positive and 12 (25.54%) negative while Adult (>3 years) group had a frequency of 43 with 29 (67.44%) positive and 14 (32.56%) negatives. The percentage distribution of sero – positive cattle was 54.7% (35/64) for young cattle and 45.3% (29/64) for adult group. Age showed no association with FMD occurence ($p>0.05$) Table 4.15

4.8.2 Clinical hematological picture of FMD sero-positive cattle in outbreak herds

FMD observed hematology as shown in Table 4.16 revealed that 40.9% (27) were anemic, 31.8% (21) showed leucopenia, 9.1% (6) indicated Leucocytosis, 3.0% (2) showed anisocytosis and 4.6% (3) presented poikilocytosis while 10.6% (7) showed both anisocytosis and poikilocytosis. These hematology indicators showed no association with FMD occurence ($P>0.05$).

Table 4.12: Occurrence of Foot and Mouth Disease Outbreaks in Ilesha Baruba district

Outbreak ID	Location (Wards)	Herd size	No. Sick	No. of dead animals	No. Tested +ve	Morbidity (%)	Mortality (%)	Case fatality rate (%)
1*	Bode II	300	50	3	4	16.67	1.00	6.00
2	Bode I	380	60	5	5	15.78	1.31	8.33
3	Bode I	78	22	1	5	28.20	1.28	4.54
4	Bode II	220	80	3	4	36.36	1.36	3.75
5*	Bode II	300	100	3	3	33.33	1.00	3.00
6	Bode II	260	60	2	4	23.07	0.76	3.33
7**	Bukaru-Rontuwa	450	100	4	4	22.22	0.88	4.00
8***	Bode I	200	40	2	5	20.00	1.00	5.00
9	Bode II	250	70	2	3	28.00	0.80	2.85
10	Bode II	350	80	4	3	22.85	1.14	5.00
11	Bukaru-Rontuwa	150	30	1	4	20.00	0.66	3.33
12**	Bukaru-Rontuwa	450	60	3	5	13.33	0.66	5.00
13***	Bode I	200	20	2	4	10.00	1.00	10.00
14	Bode I	280	50	1	6	17.85	0.35	2.00
15	Bode I	380	20	3	5	5.26	0.78	15.00
Total		**4,248**	**842**	**39**	**53**	**19.82**	**1.0**	**4.63**

*Repeated outbreaks ^Overall Incidence 19.82%

Table 4.13: Distribution of Bovine sero-positive reactors observed in FMD outbreaks according to sex as determined by NS-ELISA

Sex	Positive (%)	Negative (%)	Total
Bulls (Male)	24 (64.86%)	13 (35.14%)	37
Cows (Female)	40 (75.5%)	13 (24.5%)	53
Total	**64 (71.11)**	**26 (28.88)**	**90**

X^2= 53.0 df=1 p<0.001 n=90

Table 4.14: Distribution of Bovine sero-positive reactors observed in FMD outbreaks according to breed as determined by NS-ELISA

Breed	Positive (%)	Negative (%)	Total
White Fulani	30 (73.17)	11 (26.82)	41
Sokoto gudali	22 (75.86)	7 (24.14)	29
Red Bororo	12 (60.0)	8 (40.0)	20
Total	**64 (71.11)**	**26 (28.88)**	**90**

(X^2= 1.6, df=2) n=90

Table 4.15: Distribution of Bovine sero-positive reactors observed in FMD outbreaks according to age as determined by NS-ELISA

Age	**Positive (%)**	**Negative (%)**	**Total**
Young (6mths-3 years)	35 (74.46)	12 (25.54)	47
Adult (>3 years)	29 (67.44)	14 (32.56)	43
Total	**64 (71.11)**	**26 (28.88)**	**90**

(X2=0.5, df=1) n=90

Table 4.16: Hematological picture of FMD outbreak sero-positive reactors in Ilesha Baruba district

Blood parameter	FMD sero-positive reactors (%)	FMD sero-negative reactors (%)	Total
Anemia (PCV< 24)	27 (40.9%)	11 (45.8%)	38 (42.2%)
Leucopenia (TWBC < $4.0x10^9L$)	21 (31.8%)	9 (37.5%)	30 (33.3%)
Leucocyctosis (T WBC > $12.0x10^9L$)	6 (9.1%)	1 (4.2%)	7 (7.7%)
Anisocytosis	2 (3.0%)	-	2 (2.2%)
Poikilocytosis	3 (4.6%)	-	3 (3.3%)
Both Anisocytosis Poikilocytosis	7 (10.6%)	3 (12.5%)	10 (11.1%)
	66	24	90

X^2= 5.0, df=5 (p>0.05) n=90

CHAPTER FIVE

DISCUSSION

The sero-positive cattle observed during this survey with PI of ≥ 50% were considered as exposed to Foot and Mouth Disease within minimum of 40 days (Sorensen *et al*., 1998) showing no clinical signs but have evidence of detectable antibodies. The 75.11% bovine FMD prevalence obtained in this survey is higher than seroprevalence from previous studies in Nigeria, which indicated a positive rate of 55% for National surveillance (Abegunde *et al.*, 1988), 64.30% in nomadic herds from Plateau state (Ehizibolo *et al.*, 2010) and 56.3% in Jos south LGA in Plateau state (Ishola *et al.*, 2011).

The increased occurrence in this study may be associated with extensive movement of livestock and high rate of contact between animals at livestock markets, common grazing places as well as at watering point along cattle routes as earlier suggested (Abegunde *et al*., 1988). The high livestock marketing activites within this Nigerian border town (study area) with Benin republic where cattle are often off-loaded at Paraku and trek through cattle routes over a three day journey to the cattle international market for on-ward distribution to other part of Nigeria cannot be under-estimated.

The percentage occurrence obtained in this study is similar to the results of Lazarus *et al*., (2012) which reported a prevalence of 72.62% evidence of FMD for cattle in North West and North Eastern Nigeria associated with high number of livestock markets along the borders and the pulling together of animals from different sources for marketing purposes.

Sex distribution indicates highest sero-positivity amongst females (Table 4.2) which shows association with FMDV antibody reactors. This disagrees with previous report of Jenbere *et al*., (2011) that stated no difference in risk association of FMD transmission between male and female animals. The variation observed maybe attributed to high number of female sampled at various locations as more cows were found in markets following culling probably due to failing reproductive performance.

The distribution of sero-positive FMD cattle according to breeds (Table 4.3) show highest occurrence in white Fulani [Bunaji] in comparison with other cattle breeds. This finding could be associated with predominance of white Fulani breed within cattle markets and ultimately their availability at the time of sampling.

FMD seropositivity according to age (Table 4.4) indicates high occurrence amongst young cattle group. These young cattle were mostly encountered during sampling as their heavy market availability for trade could be associated with affordability and high turnover. This may also have fast tracked migration and contact with other FMD exposed cattle that suffered infection from multiple serotypes thus producing antibodies against all serotypes of FMD. This observed FMD association with age was similar to previous report (Ishola *et al*., 2011) that cattle within the age of 1-2yrs were mostly affected with FMD due to high frequency of exposure to risk factors.

The distribution of sero-positive cattle according to sampling location (Table 4.5) and (Fig.1) indicates highest occurrence in Ilesha Baruba which was closely followed by Ilorin.

Location shows association with occurrence of FMD. The variation in occurrence by location is similar to previous report of Ishola *et al*., (2011).

The observed FMD association in Ilesha and Ilorin, could be attributed with influx of large number of cattle and sheep to meet high demand for meat to satisfy the robust human population in the state capital (Ilorin), and or the Muslim festivals (Eid el Fitri and Eid kabir respectively) celebrations in the months of August and October, 2011 which coincides with the period of this study sampling. Other possible reasons for observed FMD occurrence could be associated with heavy human and livestock traffic and cattle congregations from different sources at the Ilesha cattle market, especially by those from West African trade route thus increasing FMDV contamination in the environment.

Specific serotype antibodies to Foot and mouth disease using Solid Phase ELISA (Table 4.6) indicates occurrence of serotypes A, O, SAT1 and SAT2 as well as multiple serotypes of O and A, A and SAT2 as well as O and SAT2. Previous study by Chuwuedo *et al*., (2008) reported only the occurrence of SAT1 and SAT 2 in trade cattle in some North-Eastern states of Nigeria. The multiple serotype occurrences in this study could be attributed to cross reactivity absence and multiple infections from within various herds where trade cattle originates and or during transit. The long distance cattle travel stress across 2-3 ECOWAS countries before entering Nigeria through Kwara State as well as those trade cattle from far Northern Nigeria being transported to Kwara state where cattle concentration is high could be contributing factor. Also intra and inter market transmission of FMD multiple serotype infection may possibly occur during cattle trade which can not be underrated in the epidemiology of FMD.

The observed multiple high serotype antibodies in some sera samples by this ELISA indicates sera might have being from a vaccinated animal or animal with low levels of FMDV specific antibodies as previously reported by Moonen *et al.,* (2000); Moonen *et al.,* (2004). The negative NSP (3ABC ELISA) sera indicates positive attributes to vaccination or an indication of NSP-specific antibody decay following infection. Although, vaccination against FMD is not common in Nigeria, the latter scenario seems applicable, as these trade cattle may have originated from different parts of African where vaccination against FMD was conducted such as Cameroon and Mali (Ekue, 1990; Sangare *et al*., 2004a) prior to their entry into Nigerian cattle markets and the study area through uncontrolled cattle trade and borders of the North-East and South-Western Nigeria as substantiated by marketers responses during participartory appraisal.

Serotype O predominance in this study is in line with reports of Samuel and Knowles, (2001) as been the mostly widely distributed in occurrence with previous reports in Nigeria cattle trade partners such as Algeria, Cote d' Ivoire Guinea, Morocco, Niger, Ghana, Burkina Faso, Tunisia and Sudan. Other trade partners with Nigeria that have reported serotype A include Mauritania, Mali, Cote d' Ivoire, Ghana, Niger, Cameroon, Chad, Senegal and Gambia (Vosloo *et al.,* 2005). SAT1 has been reported in Sudan and Niger (Sangare *et al.,* 2003) while, SAT 2 has been reported in Senegal, Liberia, Ghana, Mali, and Cote d' Ivoire (Vosloo *et al.,* 2005). However, SAT3 has been limited to East and South Africa Reid *et al.,* (2001b); Bastos *et al.,* (2003a, b) and Asia 1 only restricted to Asia (FAO, 2007) as SAT3 and Asia 1 were not detected in this survey. The serotypes O, A, SAT1and SAT2 detected amongst trade cattle in this study conforms to FMD epidemiological reports of Soudan/Sahel clusters in West and Central Africa.

Foot and Mouth Disease serotypes observed in cattle outbreaks in Ilesha Baruba (Table 4.7) indicates serotypes O, A, and SAT2 were associated with these outbreaks as indicated by ELISA. This is similar to previous report of Durojaiye (1981) which indicated serotype A and SAT2 while Abegunde, (1987) reported serotype A, SAT1 and SAT2 and Knowles *et al*., (2008) reported serotype O and SAT2. However, this study further indicates occurrence of multiple serotype infections of serotype O and A and O and SAT2 as well as evidence of vaccination against O and SAT2 serotypes.

Serotypes O, A, and SAT2 seen in this study have been previously recorded in central African and West African since 1958 (Volsoo *et al*., 2002) especially in Mali by Knowles *et al.,* (1998); Sangare *et al.,* (2004 a, b). Mali, been one of the largest cattle populations holders in West Africa with approximately six million along side with large number of small ruminants (DNAMR Mali, 2001) exports cattle to Senegal, Côte d'Ivoire, Guinea, Ghana and Nigeria (Jarrett, 1980) as Nigeria assumes a principal consumer livestock market in West Africa, therefore it is not unlikely that the serotypes in this study may have their origin from these cattle trade source in Mali.

Foot and Mouth disease SAT2 outbreak in this study also confirm previous reports (Thomson, 1994; Sangare, 2002) as SAT 2 is most frequently associated with FMD outbreaks in southern and West Africa livestock as evident in continuous cattle migration from in and out of representative countries such as Senegal, Liberia, Ghana, Mali and Cote d' Ivoire (Bastos *et al.,* 2003a). In addition, occurrence of Serotype A in this study outbreaks have been previously documented in Mauritania, Mali, Cote d' Ivoire, Ghana,

Niger, Cameroon, Chad and Senegal (Volsoo *et al*., 2002) which are Nigerian cattle trade partners and potential source of these serotypes.

In this study, observations indicates recovered cattle were not often removed from outbreak herds causing subclinical infections which may constitute important reservoir for FMD virus as previously reported by Zhang and Kitching, (2001). This may substantiate reasons for repeated outbreaks within some herds observed in this study as previously reported by Salt *et al*., (1996) where carrier cattle have been associated with new outbreaks.

Sequences obtained for serotype A and SAT2 genes were closely related to A/NIG/21/2009 and SAT2 isolate LIB/7/2003 as both isolates showed marked genetic homogeneity with >93% genetic identity in the VP1 region. The sequence of serotype A in this study with locus in between the phylogenic cluster of serotype A/SUD/3/2006 and that of Nigeria isolates cluster of 2009 as shown in a subset phylogenic tree view (Plate IV). While the sequence of SAT2 align in between the cluster of SAT2/NAM/1/92 and SAT2 isolate /LIB/7/2003 as seen in a subset phylogenic tree view (Plate V), although multiple A/NIG/2009 sequences were also identified as closely related.

This heterogeneity amongst serotypes in this study is similar to reports of Abdulkadir, (1989) which occurs due to quasispecies nature of FMDV within each serotype following error-prone RNA replication as reported by Domingo *et al*., (2002). The variations in disease course and manifestations due to different subtype evolution behaving in peculiar way within animal species and or breed were reported by Donaldson and Alexandersen,

(2001). This evolved related multiple subtypes of serotype A in this study may be associated with the observed repeated outbreak confounding disease control.

Outbreaks of SAT 2 serotypes in this study is similar to recent outbreak reports in both Libya and Egypt (FAO, 2012). Although, previous reports of SAT2 serotypes from Nigerian samples indicates close relationship between viruses isolates in West and North-Eastern Africa, ascribing a possible origin of Nigerian type O and SAT 2 viruses to the Horn of Africa countries (WRLFMD, 2008; WRLFMD, 2010), especially Sudan (FAO, 2012), Cameroon-south of the Sahara (Bronsvoort *et al*., 2004). This continous cycle of re-occurrence could be associated with extensive normadic flock movement across the Sudano-Sahelian region into sub-Saharan Africa as majority of livestock trade enters into Nigeria, following protein demand for a growing population which amount to one-fifth of African total population (Di Nardo *et al*., 2011).

Further evidence for link between livestock movements from West African countries to North Africa and FMD spread is evident by the SAT 2-type outbreaks in Niger, 2005 caused by viruses closely related to the Libyan isolate (Bastos *et al.,* 2003a). The diverse linkage of this serotype is principally associated with pastoralism in sub-Saharan Africa livestock trade especially in West and Central African countries which is characterized by cross-border transhumance, either as departure countries or as reception or transit countries. The main import basin for livestock trade comprises Benin, Cameroon, Chad, Niger and Nigeria. While some trade flow come from Burkina Faso, Mali and even less frequent flow occur from Chad and Niger towards the countries of North Africa (Kamuanga *et al*., 2008).

The successful adaptive strategy of pastoral production systems in sub-Saharan Africa is the high mobility of herds (legal and illegal) and animal products along well-defined migratory routes, dictated by the availability of natural resources, social conflict and animals' dietary needs as reported by FAO, (2001). These factors were known as possible keys for the spread of outbreaks complicated by presence of sheep role in some herds as these small ruminants habitually share grazing areas with cattle, and their living areas were often in close proximity (Sangare *et al*., (2004b), as this co-habitations senerio exist within this study.

Although no wildlife sample was recovered in this study despite proximity of various game reserves in Niger, kwara and Oyo states, occurrence of SAT 2 in this study, therefore, may pose potential danger to Nigerian un-robust wildlife as it appears to be the most efficient SAT virus type in crossing species barriers (Bastos *et al*., 2001) causing wildlife clinical disease (Keet *et al*., 1996; Bastos *et al*., 2000) and maintaining carrier status for SAT serotypes (Vosloo *et al.,* 1996) reported in South Africa (OIE, 2000). However, transmission from buffalo to cattle has never been convincingly reproduced experimentally in-spite of considerable efforts (Dawe *et al.,* 1994b). In view of the above, there exists a risk possibility that FMD outbreaks could be linked to cattle movement in and around the parks during search for water and pasture where potential wildlife subsists. However, Nigerian wildlife role in FMD epidemiology remains unknown in this current endemic situation as wildlife appears not to be of immediate threat probably due to un-robust wildlife population.

In comparison, serological and molecular diagnosis of these outbreaks indicates serotypes O, A and SAT2 were identified from sera using SPC-ELISA while serotype A and SAT2

serotypes were obtained from epithelial tissues using rRT-PCR. This could be due to small sample size and sample status submitted for the latter procedures which is related to delay and under reporting of outbreaks, logistic reasons and or absence of visible epithelial tissue rather than actual disease serotype occurrence.

The comprehensive serotype occurrence of A, O, SAT 1 and SAT 2 in both trade cattle and herd outbreaks in this study in the North-western Nigeria agrees with previous FMD reviews by Nwanta and Ojemiren, (1999) and Olabode *et al*., (2012) that affirms four serotypes as been responsible for previous disease outbreaks in Northern Nigeria. The distribution and occurrence of Bovine FMD outbreaks in Ilesha Baruba district (Table 4.9) further shows repeated outbreaks within some herds in the three sampling locations. This repeated occurrence within the same herd is associated with absence of cross immunity between serotypes as previously reported by Abdulkardir, (1989) as recovery of cattle from one serotype infection usually develops resistance to challenge by the same serotype but susceptible to infection by any other serotype (Belsham, 1993).

Geographic information system (GIS) applications for FMD epidemiological exposition in Nigeria have not been reported by previous studies. Although, occurrence of FMD outbreaks in this study is similar with previous FMD report by Durojaiye, (1981) in Oyo state but lacked geo-spatial information despite the close boundary proximity. In this study, the inter-connectivity of towns, road, river networks and forest is seen as possible features (Plate VI and VII) that might enhance FMD virus spread manifesting in high disease morbidity and low mortality in adult cattle with high mortality in calves especially in Bode

I ward probably due to high viral contamination of neighboring water bodies as cattle graze around river moshi which shared boundary with Baruten forest (Plate VIII).

FMD serotype distribution indicates circulation of serotype O, A in Bode ward and serotypes O, A and SAT2 in Bukaru-rontuwa ward (Plate IX). Occurrence of SAT2 could be associated with un-controlled introduction of new cattle into the herd due to cross movements between herds and markets. The normadic/semi intensive management styles practiced (Olabode *et al.,* 2013) and fragmented structure of the land holdings in these areas may also play FMD spread where herdmen share their cattle on multiple fragmented lands.

Cattle locations in 500m-1km buffer zones (Plate X and XI) of the outbreaks further confirm road and river networks as potential facilitators of virus spread complex by the clustered (NNI Ratio=0.095054) nature of these herds (Plate XII), with boost from possible human traffic and activities. These observed features were similar to previously reported important risk factors of transhumance and herd mixing at watering points in Cameroon (Bronsvoort *et al*., 2004) and amongst Borona herds in Ethiopia (Rufeal *et al*., 2008). The common use of animal health personnel moving between farms (Olabode *et al*., 2014) wearing possibly the same clothes and using same equipment, without disinfection of these items between visits may also spread the disease.

Study area elevation data (Plate XIII) indicates that livestock locations were on higher elevations as this could facilitate FMD virus spread and transportation from outbreak locations through flowing rivers (water source) to other animals in the lower elevation

downstream particularly the livestock and possible wild animals in and around the Kwara National Park.

The wind may also have played a role in the spread of viral aerosols from infected cattle on the highlands to lowlands as previously reported by Lubroth, (2002) that states the capacity of wind to carry FMD aerosol droplets over a distance of 60 kms on land to susceptible population downwind as cattle were easily infected by aerosol virus generated by other animals requiring as little as 20 $TCID_{50}$ (tissues culture infective dose) of virus to establish infection (Kitching, 2002a).

Therefore, observed weak disease reporting system amongst the Fulani herdsmen and other stalkeholders in the absence of effective biosecurity and standard FMD management approach compels some herdsmen to seek services of quacks or indulge in self medication and un-approved ethno-veterinary practices (Olabode *et al*., 2014). The guarded prognostic consequences of the former on cattle population in the study area and in deed Nigeria could be attributed with absence of continuous monitoring Geographic Information System data based programmes which made speculative the exact point of disease entry/ casual factor.

Foot and Mouth Disease appraisal responses by Fulani herdsmen in Ilesha baruba district (Table 4.10) indicates a robust existing knowledge of the disease based on the indicators of assessment except for source of water, season of occurrence and medication. This appraisal reveals Yakana (white Fulani) and Bokoloji breeds were mostly affected with FMD, manifesting very severe forms of clinical signs. Other reported in-contact animals within the herds include sheep, goats and donkeys. Semi nomadic husbandry management is mostly practiced in herds with no history of FMD vaccination, this agrees with Megersa *et al.,* (2009) and Ishola *et al.,* (2011) as both reports states that FMD occur in both extensive

and intensive management system but more associated with extensively managed herd system.

Responses indicates herdsmen usually vaccinate their cattle against other reported diseases such as Hemorraghic Septicemia (Pasturellosis) (Sugun *et al.,* 2013), Contagious Bovine Pleuro-Pneumonia (CBPP) (Adamu, and Aliyu, (2006), Black leg (Sugun *et al.,* 2007) and a times Brucellosis (Cadumus *et al.,* 2006). The cattle usually graze around neighboring settlement (Bode) during dry season and Mosi Korfar during wet season while some herds do graze out during rainy season. Rivers Yepti and Janburku /lasa as well as neighboring available streams and water bodies may facilitate cattle contact with other herds but not with wildlife in spite of previous reports (Vosloo *et al*., 2002) of wildlife involvement in FMD epidemiology in Africa.

In depth discussion with all herdsmen indicates no recent history of animal introduction and or distant migration. However, trade cattle route (Paraku - Ilesha) proximity with Baukaru rontuwa ward maybe a predisposing factor to disease spread due to environmental contaminations. Further discussion, indicates their disease recognition capacity as some of them states signs like hair loss around the horns, upper regional swelling of the hoof which later results into sores, muzzle and tongue lesions as well as salivation. This signs were similar with earlier reports of Kitching, 2002a).

Herdsmen responses indicates all ages, sex, breeds of cattle were susceptible to FMD which is in line with reports of Ishola *et al*., (2011) Mekonen *et al*., (2011) and Olabode *et al.,* (2013) with the exception of donkeys. No particular season is associated with disease

occurrence in this study as it also agreed with report of Abdulkadir, (1989) in Nigeria and Rufael *et al*., (2008) in Ethiopia which states disease occurrence in both dry and wet seasons but attributed increased outbreaks to stress and cattle movement in dry season.

These herdsmen use local herbs, drug medication such as penicillin as palliative measures as they were unable to access robust veterinary services thus engaging a common service provider (Area livestock superintendent). The insufficient FMD vaccination due to high cost of limited imported FMD vaccines is also militating factor to intervention being desired by the herdsmen. Finally, animal that fails to respond to treatment were usually sold for human consumption to butchers at low market value as death of sick cattle is usually avoided, hence uncommon. This agrees with previous reports of Domingo *et al*., (2002) which states that FMD mortality is usually low, as disease decreases livestock productivity with serious impact on social and economic consequences of reduced milk and meat production due to high morbidity and loss of market value (Sangare *et al.*, 2004a).

Foot and Mouth Disease appraisal responses by the cattle marketers and butchers in Kwara state (Table 4.11) indicates the disease common names are Chabu or Boro. Disease is seen all sex and age of sick cattle brought to the market and slabs for sales and slaughter. Although more female (cows) and breed of White Fulani (Bunaji), Bokoloji (Sokoto gudali), and Red Bororo (Rahaji) were mostly associated with FMD. FMD cattle were seen in both dry and rainy season in the markets and slaughter slabs. Local treatment was usually attempted but un-responsive cattle are sold to the butchers. The marketer and butchers states no occurrence of human infection. Further discussion with cattle dealers reveals these

cattle were brought from within kwara state and neighboring states in the country other parts of Africa.

Foot and Mouth disease outbreaks in Ilesha Baruba district (Table 4.12) indicates overall incidence of 19.8% in this study which is higher than 9.8% reported by Abegunde, (1987). This could be due to poor control and vaccination efforts as documented by Ishola *et al.*, (2011), facilitating FMD outbreaks in the last 3-4 decades. Another likely reason could be the closed clustered nature of the Fulani herds and proximity of Baruten forest and river morshi where direct and indirect contact between infected and susceptible cattle sharing common grazing and watering points as previously documented by Abegunde *et al*., (1988) leading to continous cycle of exposure to the causative virus manifesting as increase observed sero-positivity.

The high sero-positivity obtained in this study confirmed insufficient control efforts over the years as NSP-ELISA kit detects FMD infected, non vaccinated cattle which have being exposed to infection within a minimum of 40 days prior to detection (Sorensen *et al*., 1998) as the kit targets antibodies to non-structural proteins in non vaccinated infected animals as against structural proteins for FMD vaccination differentiation.

Distribution of Bovine sero-positive cattle observed in FMD outbreaks according to sex (Table 4.13) as determined by NS-ELISA indicates female sex association in FMD outbreaks amongst the screened group.

Bovine sero-positive reactors observed in FMD outbreaks according to breed (Table 4.14) as determined by NS-ELISA indicates no breed association with FMD outbreaks as all breeds may possess equal risk of contracting the infection.

The distribution of Bovine sero-positive reactors observed in FMD outbreaks according to age (Table 4.15) as detected by NS-ELISA indicates no age association with FMD outbreaks as all ages may have equal risk of contracting FMDV.

The distribution of FMD with demographic factors of sex, breed and age indicated more young, white Fulani cows were positive to FMD, although not significant by chi square for breed and age as this observation may be due to small sampled size and sample technique used for outbreak sero-diagnosis, but confirms susceptibility of all sex, age and breeds to the disease. This is in line with previous report (Durojaiye, 1981) which states high morbidity in both exotic and indigenous breeds and all ages of cattle with high mortality in calves than adult breeds.

Hematological picture of FMD outbreak sero-positive cattle in Ilesha Baruba district (Table 4.16) indicates anemia, leucopenia and leucocytosis, as distinct changes in this study. The anemia is seen with characteristic anisocytosis and poikilocytosis. This is in line with earlier report (Radostitis *et al.,* 2000) that anemia in bovine FMD occurs as a sequel probably due to endocrine damage which leads to chronic syndrome. In addition, hemoparasitism and immunosupression that may arise from other management stressors could also a contributing factor to the anemia seen in this study.

The leucopenia seen in this study agrees with previous reports of Reece, (2009) that leucopenia is usually associated with early stages of viral infections as these blood samples were collected within 3-10days post outbreak reports. It also agrees with Mohan *et al*.,

(2008) reports on leucopenia seen in cattle and buffalo keratinized epithelium infected with FMD Asia1 type virus.

Leucocytosis is an increase in leukocyte number which usually occurs in bacterial infections (Reece, 2009). This increased leukocyte in FMD positive reactors could be due to ongoing bacterial infections. Although, differential white blood cell count and plasma biochemical parameters was not analyzed, these blood characteristic features show no significant changes ($P>0.05$) with FMD sero-positive cattle.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

This study provides information on the occurence, endemicity, serotypes, as well as demographic factors associated with FMD. The disease was detected in all sex, ages and breeds of trade cattle in Kwara state of Nigeria as well as those imported by cattle trading partners along the West African coast.

The use of geographic information system in elucidation of foot and mouth disease occurrence and spread provided preliminary indication that town and road networks, wind, elevated topography, water bodies and streams could facilitate FMD virus spread.

Participatory appraisal confirmed FMD is still a problem amongst the Fulani livestock pastoralist through their robust local exiting veterinary knowledge.

The hematological characteristics observed in FMD affected cattle under field exposure condition in this study provide preliminary hemogram for both researchers and clinicians.

ELISA especially SPC-ELISA test confirmed FMD outbreaks were associated with serotypes O, A, multiple serotype O, A, and O, SAT2 as well as evidence of vaccination against O and SAT2 sertypes in this study. rRT-PCR further associated Serotype A and SAT 2 with this outbreaks while molecular sequencing confirmed and named the serotype

isolates as A/NIG/1/2011 and SAT2/NIG/3/2011 respectively which were closely related with SAT2 isolates LIB/7/2003 and A/NIG/21/2009 linked to isolates of Libya and existing circulating Nigerian type A respectively.

Thus, FMD is a trans-boundary animal disease with high endemicity and morbidity in adults in affected Fulani herds in this Nigerian international border town with Benin Republic.

6.2 Recommendations

Base on the findings of this research, the following recommendations are therefore suggested:

1. Control strategy should in-coperate the use of vaccines produced from any of the four circulating serotypes to limit specific FMD outbreaks, early vaccination is thus recommended.

2. Strict border control and policing of animal movement following prescribed serological test in accordance with international standards.

3. National continuous sero-surveillance in conjunction with geographic information system application for data base formulation is advocated to enhance easy identification of exact disease entry point.

4. Base on the existing veterinary knowledge, these Fulani herdsmen should be trained as agents of FMD control to ensure early detection and disease reporting.

5. Biosecurity awareness campaign among Fulani herdsmen, field veterinarians and farm workers on the possible means of FMDV dissemination through watering points, human dissemination through clothing, shoes and hair is thus suggested.

6. Cleaning and disinfection procedures are strongly recommended before leaving FMD-infected premises using solutions of sodium carbonate at 4% concentration, sodium hydroxide at 2% or citric acid at 0.2%, as these are known to be effective for disinfecting contaminated surfaces (Blancou, 2002).

7. Capacity building programme for animal owners or manager, livestock owners are all levels of production, dealers and traders to familiarize them with basic FMD features such as recognition of the essential disease signs, the need for urgent action and how and where to seek for help if disease is suspected (FAO, 2002)

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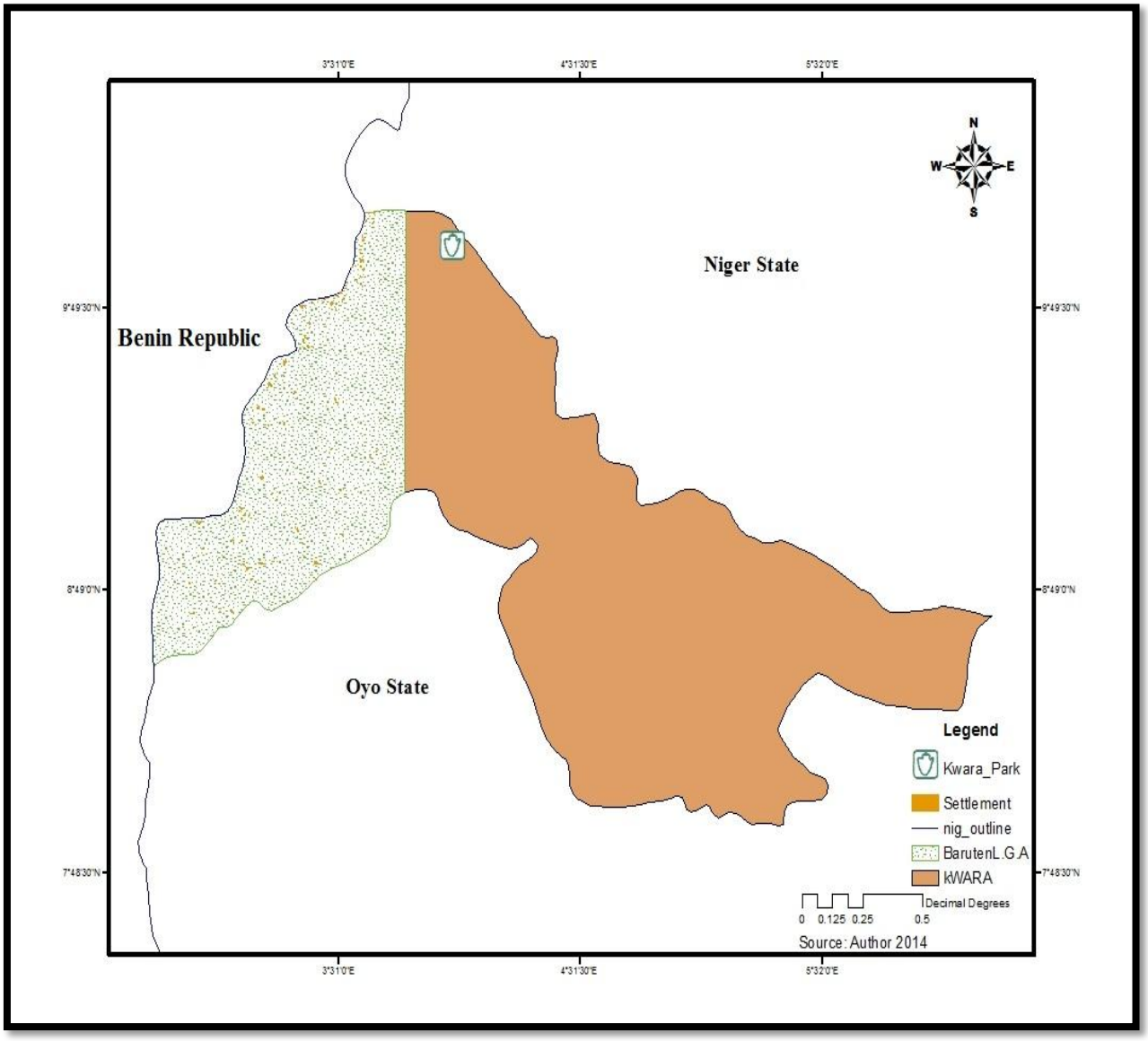
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APPENDIX

Appendix I: Map of Kwara State showing Study Area



Appendix II: Participatory appraisal form for herdsmen

S/No.	Questions	Response: (yes) or (No) and other remarks
1.	Type of husbandry management system	
2.	Grazing areas during the dry and wet seasons	
3.	What is the local name for FMD in cattle?	
4.	Can you recognize FMD infected cattle?	
5.	Give some signs of FMD	
6.	In which age group of cattle these signs are commonly seen?	
7.	In which breed of cattle these signs are commonly seen?	
8.	In which sex of cattle these signs are commonly seen?	
9.	In which season of the year these signs are commonly seen?	
10.	Do you have previous history of outbreaks?	
11.	What is the total number of your herd?	
12.	What is the total number of FMD affected?	
13.	What is the total number of death recorded?	
14.	Where is your source of water?	
15.	What is the history of your cattle migration?	
16.	Do your cattle have contact with other animals, and or wild animal?	
17.	Do you have a cattle route close by?	
18.	Do you vaccinate against FMD?	
19.	What is the medical intervention given in case of an outbreak?	
20.	Do you have service providers?	
21.	What do you do with unproductive animals?	

Appendix III: Participatory appraisal form for cattle traders and butchers

S/No.	Questions	Response: (yes) or (No) and other remarks
1.	What is the local name of FMD in cattle?	
2.	Give some signs of FMD	
3.	In which age group of cattle these signs are commonly seen?	
4.	In which breed of cattle these signs are commonly seen?	
5.	In which sex of cattle these signs are commonly seen?	
6.	In which season of the year these signs are commonly seen?	
7.	What is the medical intervention given in case of outbreak?	
8.	What do you do with unproductive animals?	
9.	Have noticed the infection in man?	
10	Name the source (states) of cattle brought to the market and slaughter slabs	

Appendix IV: Foot and mouth disease outbreaks showing GPS locations

S/No.	Location	GPS values	Month of infection	Total No. of animals	No. Sick	No. of dead animals	FMD serotype
1	Bode II	N09.02214 E003.51394 374m	21st December, 2010	300	50	3	O,OA
2	Bode I	N08.99479 E003.55410 356m	12th January, 2011	380	60	5	O
3	Bode I	N08.99526 E003.55782 367m	18th January, 2011	78	22	1	O
4	Bode II	N09.02155 E003.51631 384m	27th January, 2011	220	80	3	O
5	Bode II	N09.02214 E003.51394 374m	30th January, 2011	300	100	3	O
6	Bode II	N09.02437 E003.51848 382m	21st January, 2011	260	60	2	O
7	Bukaru-Rontuwa	N08.99626 E003.37350 409m	17th January, 2011	450	100	4	O,A
8	Bode I	N08.99649 E003.55794 372m	1st February, 2011	200	40	2	O
9	Bode II	N09.01692 E003.48110 410m	2th February, 2011	250	70	2	O
10	Bode II	N09.01952 E003.48270 414m	11th February, 2011	350	80	4	O
11	Bukaru-Rontuwa	N08.99821 E003.37374 421m	14th March, 2011	150	30	1	O, OA
12	Bukaru-Rontuwa	N08.99626 E003.37350 409m	19th March, 2011	450	60	3	O,SAT2
13	Bode I	N08.99649 E003.55794 372m	26th March, 2011	200	20	2	O,OA SAT_v
14	Bode I	N08.99475 E003.55900 363m	27th March, 2011	280	50	1	O,A O_v
15	Bode I	N08.99479 E003.55410 356m	31st March,	380	20	4	O,A

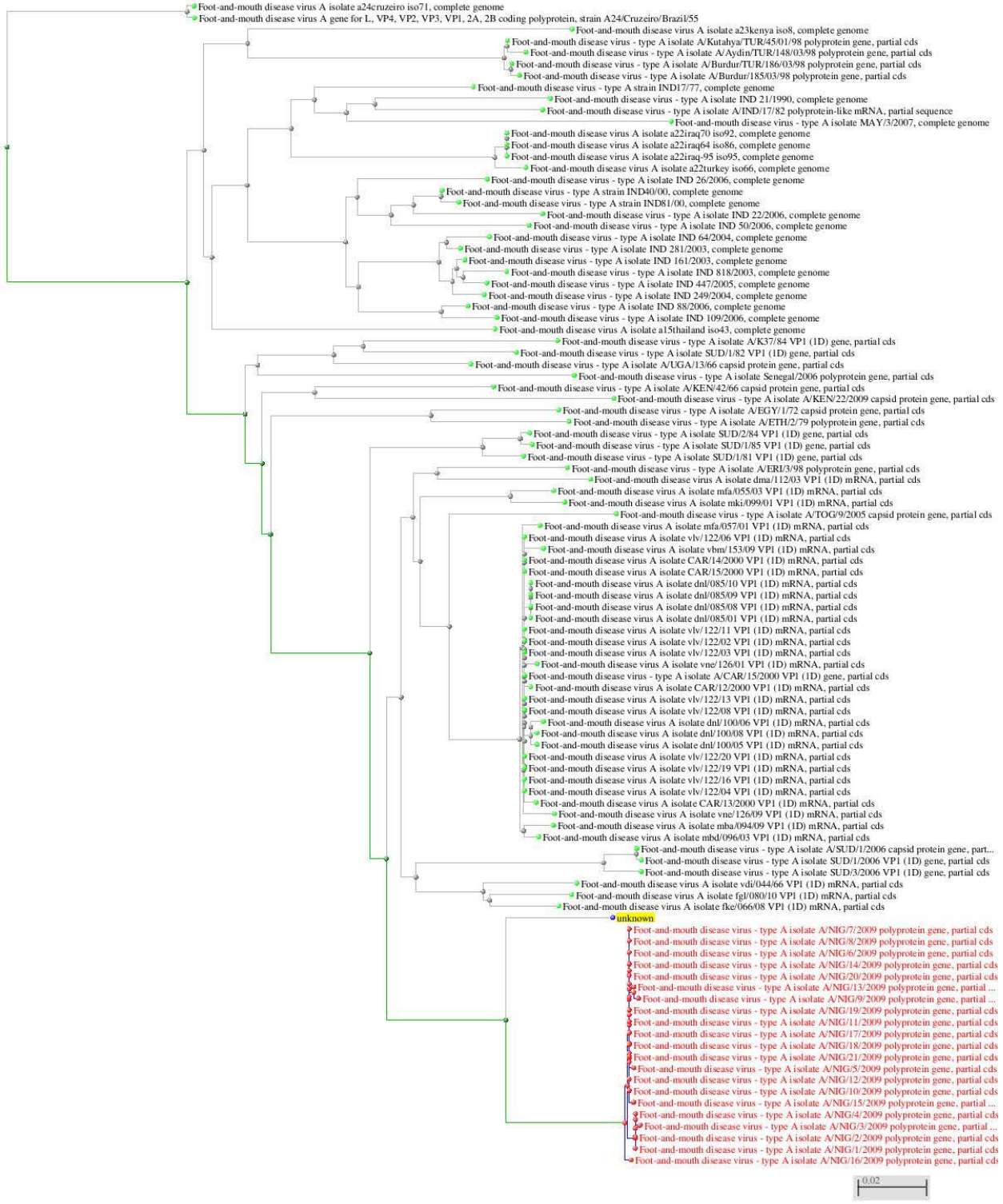
Appendix V: Statistical association of FMD sero-positive trade cattle sera according to sex, age, breed and location as detected by NS-ELISA

Demograph	Pearson Chi square value	Degree of freedom (df)	Level of significance (p value)
Sex	3.737	1	0.0532
Age	43.70	1	0.0001
Breed	57.16	2	0.0001
Location	17.80	4	0.0014

Appendix VI: Statistical association of FMD sero-positive outbreak cattle sera according to sex, age and breed as detected by NS-ELISA

Demograph	Pearson Chi square value	Degree of freedom (df)	Level of significance (p value)
Sex	53.0	1	0.001
Age	0.5	1	0.25
Breed	1.6	2	0.5

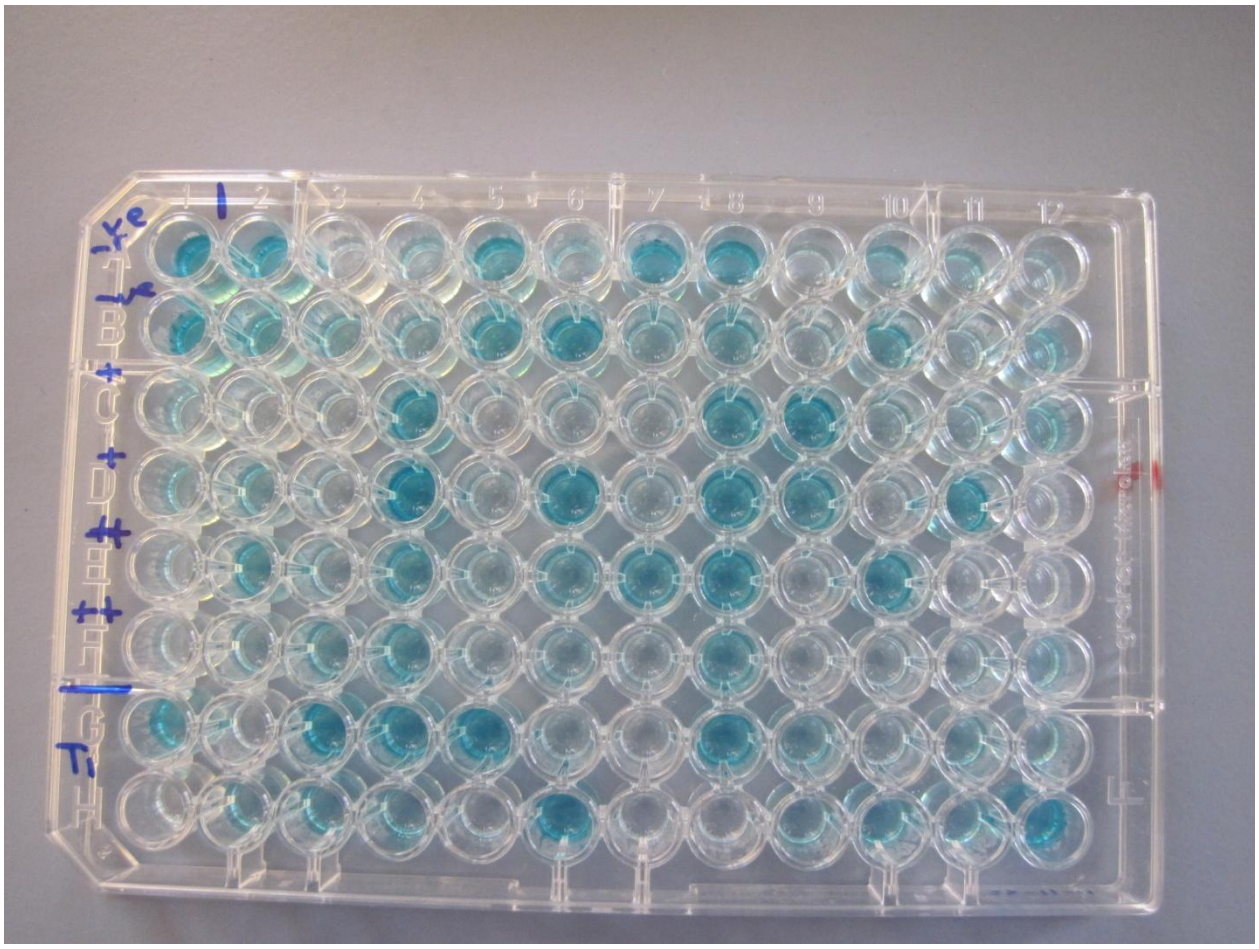
Appendix VII: Phylogenic Tree View of Nigerian Serotype A obtained in this study



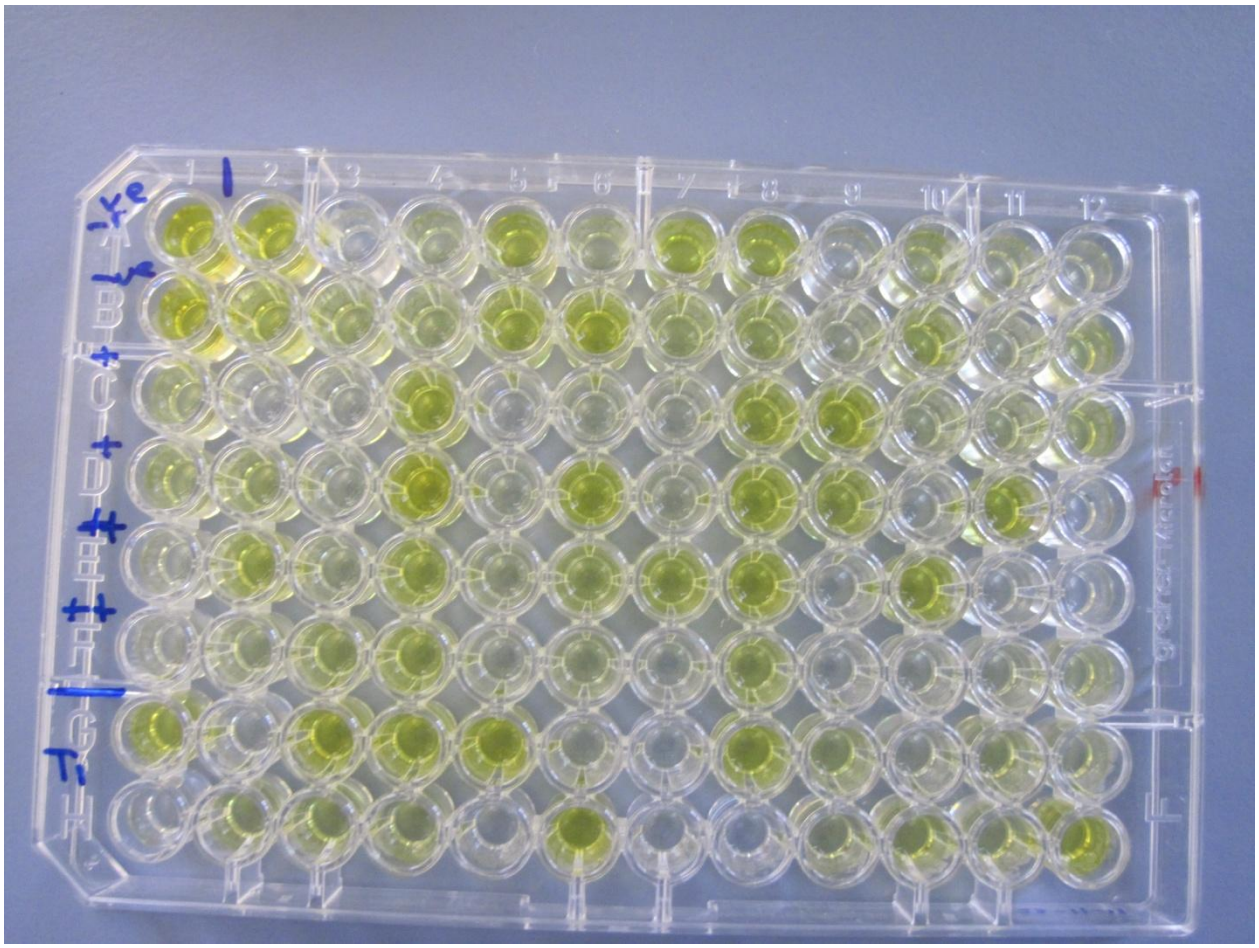
Appendix VIII: Phylogenic Tree View of Nigerian SAT2 obtained in this study



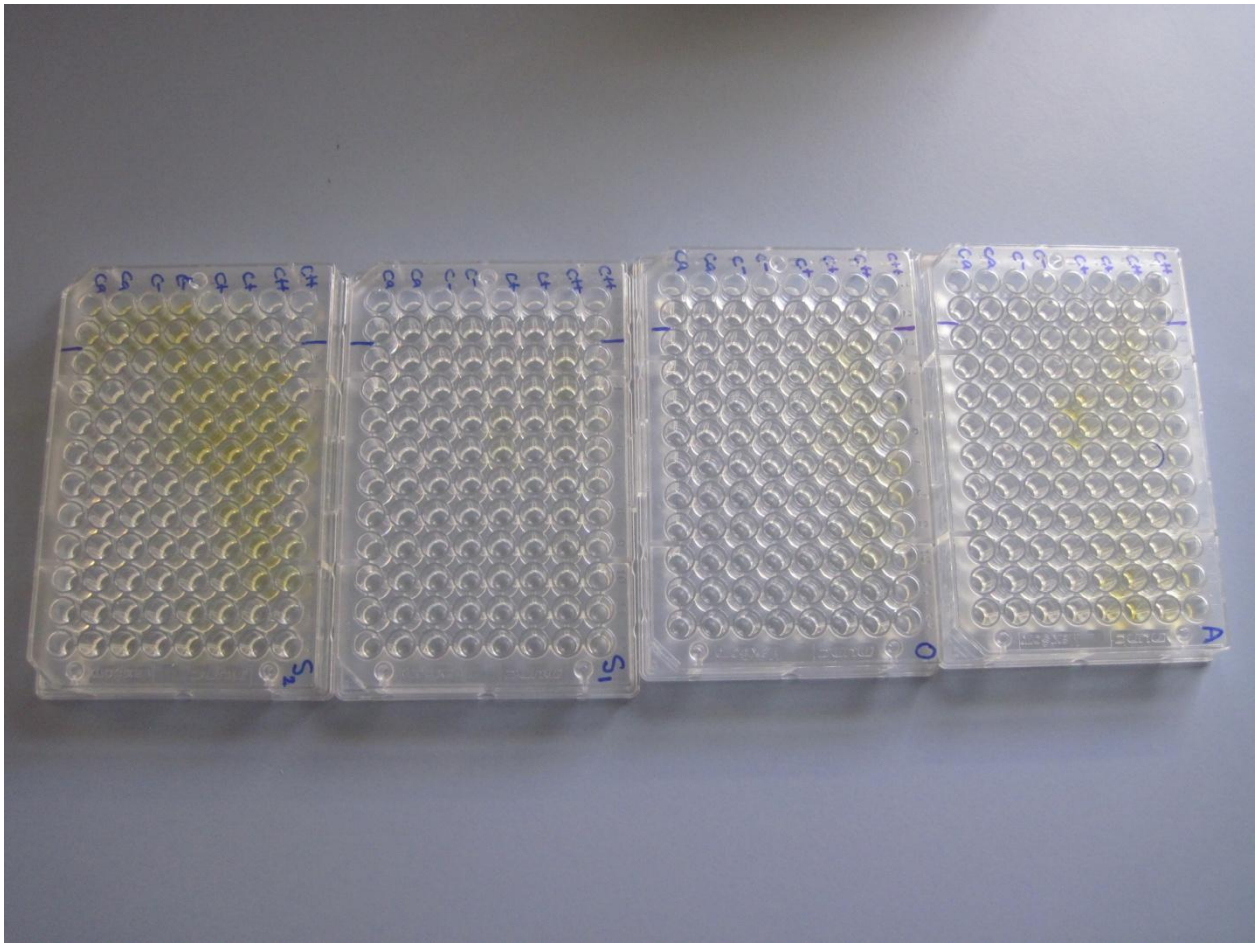
Appendix IX: ELISA micro-plate for NSP Positive FMD sera before addition of stop solution



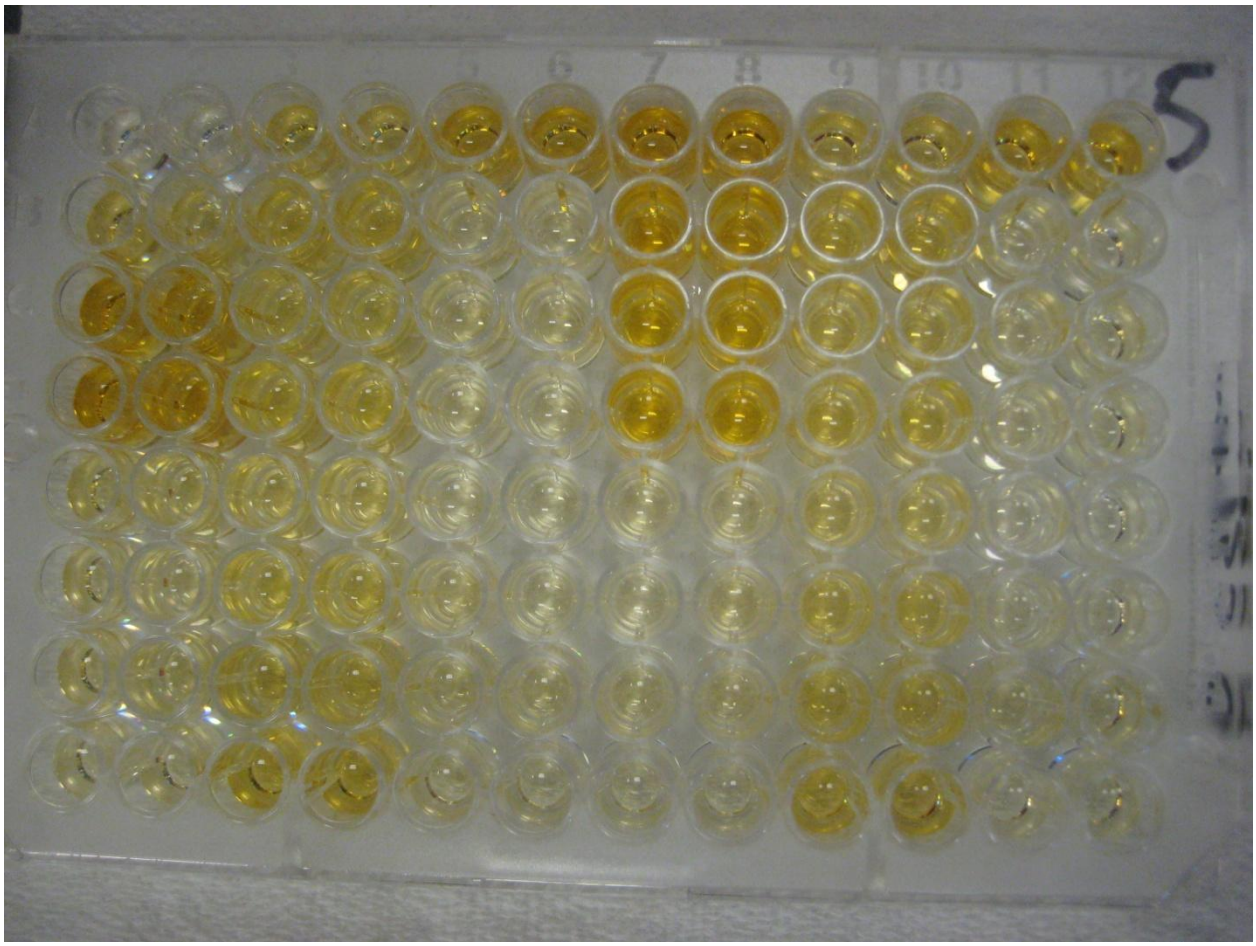
Appendix X: ELISA micro-plate of NSP Positive FMD sera after addition of stop solution



Appendix XI: Positive ELISA micro-plates for serotypes O, A, SAT1 and SAT2 obtained in this study



Appendix XII: ELISA micro-plate for $_C$ELISA Positive FMD sera obtained in this study



Serotype O positive ELISA plate

Appendix XIII: Optic density readings using ELISA reader at 450nm for trade cattle sera in Offa

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.711	1.437	0.127	0.360	1.078	0.330	1.204	1.005	0.142	0.513	0.355	0.224
B	1.689	0.754	0.468	0.404	0.984	1.281	0.400	0.566	0.187	0.704	0.224	0.533
C	0.435	0.071	0.133	1.212	0.063	0.188	0.137	0.980	1.264	0.249	0.299	0.603
D	0.500	0.513	0.174	1.882	0.181	1.329	0.204	1.188	0.852	0.085	1.090	0.114
E	0.222	0.868	0.250	0.850	0.258	0.591	0.816	1.109	0.097	1.021	0.117	0.110
F	0.207	0.355	0.537	0.723	0.168	0.343	0.144	0.696	0.103	0.177	0.233	0.444
G	1.021	0.108	1.338	1.108	1.364	0.217	0.081	1.147	0.478	0.192	0.438	0.366
H	0.111	0.483	0.583	0.443	0.118	1.400	0.117	0.069	0.382	0.669	0.467	1.381

Appendix XIV: Optic density readings using ELISA reader at 450nm for trade cattle sera in Bode Sadu

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.435	0.179	0.131	0.172	0.332	0.368	0.648	0.375	0.378	0.207	1.607	0.133
B	1.539	1.245	0.690	0.262	0.126	0.347	0.087	0.217	0.326	1.575	1.212	0.709
C	0.420	0.109	0.663	1.482	0.740	0.125	0.925	1.064	0.371	0.244	0.874	0.362
D	0.456	1.105	0.148	0.490	0.135	0.213	0.335	1.023	0.264	1.607	0.252	0.627
E	0.212	0.263	0.153	0.574	0.326	0.097	0.463	1.428	0.568	0.068	0.158	0.102
F	0.226	0.886	0.111	1.053	0.193	0.669	1.009	0.498	0.247	0.113	1.409	0.661
G	0.644	0.192	0.337	0.093	0.965	0.347	1.579	1.118	0.107	0.298	0.180	1.474
H	1.201	1.451	0.102	0.173	0.651	1.263	0.892	0.121	0.054	1.651	0.212	0.096

Appendix XV: Optic density readings using ELISA reader at 450nm for trade cattle sera in Ilesha Baruba

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.800	1.042	0.188	0.625	0.168	0.478	0.760	0.366	0.306	0.060	0.180	0.470
B	1.804	2.918	0.325	0.096	0.132	0.409	1.476	0.688	0.140	0.520	0.172	0.733
C	0.408	0.245	0.216	0.082	0.072	0.071	0.936	0.466	0.211	0.258	0.170	0.216
D	0.501	0.615	0.180	0.068	0.628	0.237	0.316	0.698	0.451	0.218	0.352	0.869
E	0.204	0.220	0.143	0.292	0.306	0.567	0.076	0.178	0.171	0.262	0.690	0.807
F	0.216	0.943	0.192	0.166	0.647	0.438	0.407	0.122	0.233	0.169	0.861	1.055
G	2.056	0.388	0.211	0.339	0.312	0.137	0.143	0.638	1.176	0.100	0.660	0.689
H	0.286	0.447	0.323	1.506	0.324	0.334	0.241	1.091	1.086	1.910	0.149	0.412

Appendix XVI: Optic density readings using ELISA reader at 450nm for trade cattle sera in Ilorin

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.446	1.434	0.839	1.173	0.434	0.828	0.123	0.620	0.458	0.169	0.152	1.874
B	1.710	0.353	1.505	0.189	1.228	0.461	0.400	0.294	0.484	0.588	0.564	0.183
C	0.536	0.056	0.227	1.632	0.228	1.357	0.093	1.016	0.234	1.185	0.134	0.131
D	0.549	0.328	0.257	0.125	0.148	0.067	0.271	1.125	0.182	1.061	0.353	0.294
E	0.213	0.272	0.646	0.532	1.626	0.458	0.956	0.215	0.110	0.483	0.301	1.008
F	0.224	0.420	0.162	0.548	0.415	0.432	0.504	0.161	0.641	0.468	0.170	1.241
G	0.728	0.145	0.125	0.370	0.946	0.132	0.467	0.332	1.037	0.214	0.399	0.625
H	0.212	0.391	0.747	2.484	0.608	0.122	0.985	0.294	0.275	1.287	0.422	0.383

Appendix XVII: Optic density readings using ELISA reader at 450nm for trade cattle sera in Ajasse

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.415	0.172	0.429	0.139	0.441	0.800	1.030	0.193	1.313	0.196	0.071	0.345
B	1.904	1.624	0.989	2.034	0.077	0.374	0.152	1.431	1.150	0.400	0.252	1.221
C	0.564	1.319	0.160	1.662	0.366	0.106	0.440	0.469	1.242	1.245	0.183	0.062
D	0.569	0.407	0.081	0.090	0.273	0.255	0.143	0.765	0.104	1.427	0.422	1.428
E	0.218	0.394	0.805	0.252	0.459	1.388	1.014	0.331	1.230	0.746	1.757	1.386
F	0.231	0.599	1.252	1.545	0.215	0.079	0.477	0.566	1.082	0.191	0.256	1.444
G	0.095	0.113	1.525	0.235	0.666	1.193	0.440	0.399	1.246	0.128	0.535	0.390
H	0.318	0.355	0.069	0.426	0.095	1.227	0.570	0.200	1.807	2.085	1.407	0.151

Appendix XVIII: Optic density readings using ELISA reader at 450nm for outbreak cattle sera

Measurement count: 1 Filter: 450

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.370	0.409	1.196	1.875	1.056	0.370	0.979	1.022	0.285	0.380	1.161	1.535
B	2.295	1.006	0.782	0.837	0.492	0.136	1.254	0.839	0.988	1.316	1.258	0.358
C	1.678	0.421	0.302	2.033	0.824	2.071	1.637	1.493	1.627	1.025	0.405	1.722
D	1.728	0.570	0.419	1.763	0.400	0.568	0.627	0.405	0.513	1.005	1.306	0.289
E	1.099	1.505	0.340	1.222	1.160	1.628	0.188	0.843	0.093	0.185	0.947	0.552
F	1.088	0.327	0.521	0.168	1.702	1.415	1.821	0.295	0.181	1.564	0.119	1.045
G	0.914	0.897	1.563	1.816	0.349	0.877	1.157	0.305	0.924	0.192	1.838	0.483
H	0.159	0.781	0.493	1.089	0.775	0.904	1.205	0.630	0.332	0.639	0.785	2.074
gative Con	2.3325											
Positive C	1.703											
Positive C	1.0935											

PERCENTAGE INHIBITION RESULT

	1	2	3	4	5	6	7	8	9	10	11	12
A	CONTROL	82.465	48.725	19.614	54.727	84.137	58.028	56.184	87.781	83.708	50.225	34.191
B	CONTROL	56.870	66.474	64.116	78.907	94.169	46.238	64.030	57.642	43.580	46.066	84.652
C	CONTROL	81.951	87.053	12.840	64.673	11.211	29.818	35.991	30.247	56.056	82.637	26.174
D	CONTROL	75.563	82.036	24.416	82.851	75.648	73.119	82.637	78.006	56.913	44.009	87.610
E	CONTROL	35.477	85.423	47.610	50.268	30.204	91.940	63.859	96.013	92.069	59.400	76.334
F	CONTROL	85.981	77.663	92.797	27.031	39.335	21.929	87.353	92.240	32.947	94.898	55.198
G	60.815	61.543	32.990	22.144	85.038	62.401	50.397	86.924	60.386	91.768	21.200	79.293
H	93.183	66.517	78.864	53.312	66.774	61.243	48.339	72.990	85.766	72.605	66.345	11.083

	1	2	3	4	5	6	7	8	9	10	11	12
A	CONTROL	1	0	0	1	1	1	1	1	1	1	0
B	CONTROL	1	1	1	1	1	0	1	1	0	0	1
C	CONTROL	1	1	0	1	0	0	0	0	1	1	0
D	CONTROL	1	1	0	1	1	1	1	1	1	0	1
E	CONTROL	0	1	0	1	0	1	1	1	1	1	1
F	CONTROL	1	1	1	0	0	0	1	1	0	1	1
G	1	1	0	0	1	1	1	1	1	1	0	1
H	1	1	1	1	1	1	0	1	1	1	1	0

Appendix XIX: Optic density readings using ELISA reader at 450nm and 490nm for trade cattle sera

Sample ID	%Inhib NSP	Serostatus	FMD A CELISA	Serostatus	FMD O	Serostatus	Sat 1	Serostatus	SAT 2		SAT 3	Serostatus	Asia 1	**Final Interpretation**
1	98.00	P	90.51	P	90.30	P	45.10	N	71.69	N	38.15	N	75.27	O , A
2	96.95	P	88.14	P	89.99	P	46.63	N	39.20	N	17.21	N	48.10	O , A
3	5.72	N	69.67	P	48.03	N	12.01	N	9.80	N	9.38	N	17.08	A
4	90.63	P	41.77	P	59.34	P	24.05	N	22.20	N	20.61	N	32.72	O
5	65.24	P	72.61	P	79.73	P	43.62	N	46.61	N	20.87	N	26.25	O
6	99.67	P	78.54	P	71.92	P	42.30	N	17.19	N	22.53	N	50.60	A
7	38.74	N	44.21	N	14.62	N	7.18	N	44.57	N	9.27	N	6.30	NEG
8	46.57	N	39.17	N	88.29	P	38.09	N	67.15	P	20.67	N	34.97	Vaccine O
9	23.29	N	33.72	N	79.73	P	13.55	N	62.94	P	11.38	N	8.27	Vaccine O
10	82.94	P	73.22	P	86.33	P	56.00	P	24.72	N	29.81	N	31.42	O
11	88.76	P	63.75	P	84.47	P	11.66	N	18.86	N	10.25	N	18.30	O
12	98.26	P	93.37	P	38.07	P	52.44	P	80.07	P	31.44	N	71.27	A
13	36.26	N	61.67	P	79.76	N	5.97	N	19.04	N	-12.04	N	3.89	O
14	95.33	P	29.77	P	15.06	N	18.15	N	66.19	P	11.82	N	-1.97	SAT2
15	69.84	P	68.04	P	69.52	P	46.41	N	49.40	N	30.44	N	41.30	O
16	76.71	P	76.15	P	92.36	P	37.98	N	22.70	N	27.05	N	45.75	O
17	92.35	P	43.56	N	27.35	N	16.58	N	75.54	P	18.59	N	18.90	SAT2
18	97.93	P	17.57	N	50.18	P	2.06	N	9.65	N	-97.06	N	-34.88	O
19	12.13	N	91.80	N	30.13	N	46.76	N	14.45	N	19.23	N	23.84	Vaccine O
20	-1.05	N	44.81	N	15.73	N	-1.41	N	3.96	N	3.16	N	-2.30	NEG
21	16.58	N	17.33	N	82.60	P	-22.08	N	-4.60	N	2.65	N	18.47	Vaccine O
22	17.75	N	20.19	N	62.99	P	22.18	N	19.05	N	11.34	N	13.19	Vaccine O
23	70.57	P	32.24	P	93.53	P	48.60	N	28.05	N	26.48	N	27.75	O
24	64.35	P	84.65	P	13.42	N	12.15	N	9.96	N	20.16	N	61.24	A
25	55.67	P	50.07	P	15.79	N	22.54	N	39.97	N	21.78	N	15.92	A
26	99.12	P	84.95	P	90.05	P	43.19	N	48.30	N	28.03	N	24.43	O

27	19.73	N	20.00	N	15.73	N	12.48	N	16.59	N	8.34	N	-0.56	NEG
28	96.38	P	2.53	N	3.17	N	-7.06	N	32.94	N	-7.86	N	64.24	?
29	3.24	N	37.27	N	-11.42	N	-25.01	N	27.91	N	-30.87	N	-4.02	NEG
30	70.91	P	18.20	N	77.03	P	46.77	N	17.35	N	26.76	N	29.99	O
31	90.66	P	45.23	N	80.78	P	23.43	N	10.71	N	12.91	N	4.06	O
32	34.41	N	13.30	N	20.96	N	33.72	N	34.89	N	19.38	N	7.00	NEG
33	98.42	P	75.54	P	91.92	P	53.26	P	15.82	N	28.32	N	55.43	SAT2
34	94.68	P	19.29	N	18.51	N	29.52	N	71.83	P	20.96	N	6.03	SAT2
35	98.26	P	21.80	N	44.55	N	20.46	N	14.57	N	4.42	N	8.10	?
36	32.08	N	4.14	N	29.80	N	0.32	N	4.50	N	6.69	N	-0.73	NEG
37	71.78	P	52.00	P	74.62	P	14.26	N	7.72	N	14.38	N	18.98	O
38	56.61	P	79.42	P	20.84	N	31.60	N	15.76	N	6.78	N	19.63	A
39	97.96	P	77.03	P	94.11	P	65.39	P	85.13	P	32.24	N	25.52	O,A
40	84.70	P	74.76	P	43.93	N	59.11	P	54.52	P	38.02	N	25.74	A
41	40.62	N	83.06	P	90.73	P	58.40	P	14.42	N	35.30	N	59.76	Vaccine O,A
42	94.06	P	43.67	N	85.65	P	50.70	P	47.29	N	37.87	N	21.92	O
43	94.01	P	15.77	N	88.62	P	57.30	P	56.70	P	29.72	N	11.94	O
44	80.59	P	28.63	N	84.15	P	27.51	N	53.47	P	18.93	N	24.32	O
45	96.71	P	75.38	P	88.55	P	58.77	P	58.60	P	24.10	N	36.49	O
46	98.97	P	23.89	N	96.09	P	85.33	P	94.20	P	54.00	P	44.83	O ,SAT2
47	96.43	P	75.98	P	93.14	P	48.54	N	66.91	P	32.50	N	nd	O
48	94.92	P	63.50	P	89.16	P	39.73	N	72.80	P	23.63	N	nd	O
49	99.33	P	89.33	P	50.42	N	62.22	P	90.37	P	46.43	N	nd	A ,SAT2
50	15.67	N	33.74	N	11.32	N	4.24	N	17.72	N	21.07	N	nd	NEG
51	25.01	N	10.23	N	79.44	P	6.29	N	10.44	N	4.56	N	nd	Vaccine O
52	98.88	P	53.32	P	84.18	P	14.31	N	11.32	N	15.94	N	nd	O
53	97.40	P	84.81	P	86.02	P	45.28	N	81.78	P	41.84	N	nd	O,A
54	96.95	P	71.28	P	91.65	P	69.20	P	66.66	P	42.41	N	nd	O
55	98.54	P	13.09	P	89.25	P	51.45	P	46.96	N	27.14	N	nd	O
56	95.80	P	89.79	P	79.74	P	58.99	P	72.55	P	29.04	N	nd	A
57	29.77		9.92	N	20.92	N	1.07	N	8.07	N	15.09	N	nd	NEG
58	56.16	P	7.26	N	49.75	N	20.35	N	77.53	P	27.45	N	nd	SAT2

59	45.32	N	75.12	P	29.56	N	38.34	N	23.38	N	18.16	N	nd	Vaccine A
60	36.50	N	35.37	N	84.24	P	21.92	N	13.90	N	24.88	N	nd	Vaccine O
61	84.88	P	36.05	N	52.22	P	26.97	N	43.49	N	7.28	N	nd	O
62	97.02	P	85.46	P	94.51	P	64.90	P	66.85	P	7.21	N	nd	O, A
63	78.33	P	13.64	N	32.33	N	-17.31	N	2.89	N	-19.28	N	nd	?
64	99.46	P	83.29	P	88.99	P	78.72	N	83.44	P	39.96	N	nd	O ,A, SAT2?
65	43.21	N	20.27	N	93.14	P	24.21	N	13.17	N	28.32	N	nd	Vaccine O
66	68.26	P	76.50	P	53.93	P	19.16	N	18.93	N	26.20	N	nd	A
67	83.01	P	68.45	P	21.74	P	32.20	N	84.85	P	26.01	N	nd	SAT2
68	85.43	P	89.95	P	87.37	P	43.91	N	16.36	N	26.97	N	nd	A
69	98.71	P	35.12	N	72.41	P	50.32	P	62.42	P	23.62	N	nd	O
70	97.91	P	13.24	N	86.70	P	35.80	N	29.68	N	24.87	N	nd	O
71	96.43	P	85.90	P	91.66	P	44.50	N	16.39	N	23.30	N	nd	O
72	71.34	P	84.36	P	44.69	N	9.99	N	11.73	N	14.57	N	nd	A
73	94.84	P	64.72	P	87.66	P	34.53	N	10.59	N	21.54	N	nd	O
74	98.93	P	67.00	P	15.51	N	39.35	N	43.79	N	30.88	N	nd	A
75	57.01	P	53.04	P	85.21	P	51.16	P	67.35	P	33.33	N	nd	O
76	70.21	P	67.21	P	14.70	N	26.25	N	57.39	P	23.92	N	nd	A
77	99.13	P	24.79	N	79.40	P	27.11	N	34.48	N	22.39	N	nd	O
78	98.10	P	36.42	N	22.44	N	44.06	N	74.55	P	26.02	N	nd	SAT2
79	99.17	P	85.97	P	92.90	P	72.29	N	76.65	P	48.24	N	nd	O
80	58.65	P	8.37	N	21.93	N	12.95	N	61.84	P	8.67	N	nd	SAT2
81	80.40	P	20.49	N	54.36	P	15.08	N	25.23	N	16.60	N	nd	O
82	41.28	N	22.80	N	90.34	P	47.54	N	41.37	N	20.57	N	nd	O?
83	95.70	P	51.95	P	92.99	P	62.90	P	55.70	P	40.90	N	nd	O
84	44.80	N	50.64	P	40.33	N	53.29	P	32.34	N	24.39	N	nd	Vaccine A
85	75.59	P	62.75	P	91.89	P	38.28	N	68.96	P	33.00	N	nd	O
86	92.68	P	80.63	P	93.10	P	74.19	N	66.27	P	42.45	N	nd	O
87	85.32	P	54.47	P	17.60	N	7.94	N	17.13	N	15.81	N	nd	A
88	94.49	P	17.45	N	94.30	P	65.38	P	87.62	P	40.17	N	nd	O
89	69.87	P	12.60	N	72.84	P	29.34	N	38.60	N	8.12	N	nd	O

90	97.17	P	90.10	P	95.55	P	81.50	N	85.80	P	54.98	P	nd	O, A
91	100.18	P	48.80	N	89.96	P	70.70	N	37.90	N	40.49	N	nd	O
92	97.68	P	57.56	P	80.58	P	55.03	P	37.63	N	42.97	N	nd	O
93	95.19	P	66.32	P	71.20	P	20.34	N	37.36	N	18.08	N	nd	O
94	98.79	P	60.91	P	15.25	N	25.67	N	82.00	P	14.56	N	nd	SAT2
95	59.35	P	10.54	N	66.73	P	39.48	N	38.52	N	21.51	N	nd	O
96	41.16	N	15.48	N	49.01	N	9.87	N	35.89	N	8.48	N	nd	NEG
97	29.21	N	17.57	N	36.57	N	16.41	N	20.52	N	-4.78	N	nd	NEG
98	66.86	P	13.11	N	87.21	P	31.09	N	46.33	N	30.10	N	nd	O
99	91.74	P	29.57	N	81.96	P	61.29	P	49.34	N	29.06	N	nd	O
100	17.24	N	20.41	N	46.11	N	46.78	N	8.12	N	18.61	N	nd	NEG
101	98.64	P	63.98	P	67.51	P	56.37	P	65.95	P	39.78	N	nd	O
102	91.92	P	75.62	P	93.73	P	63.35	P	49.97	N	40.30	N	nd	O
103	96.59	P	60.57	P	47.48	N	34.85	N	33.16	N	24.72	N	nd	A
104	99.33	P	51.84	P	91.35	P	49.27	N	58.49	P	27.65	N	nd	O
105	94.57	P	28.77	N	92.26	P	34.08	N	46.83	N	17.67	N	nd	O
106	85.13	P	25.98	N	16.27	N	13.08	N	12.68	N	15.95	N	nd	?
107	97.97	P	44.44	N	92.95	P	24.85	N	20.18	N	28.72	N	nd	O
108	68.83	P	7.45	N	26.45	N	17.67	N	48.94	N	13.74	N	nd	?
109	2.16	N	50.73	P	67.47	P	29.79	N	21.71	N	24.27	N	nd	Vaccine O
110	59.72	P	24.90	N	87.49	P	30.48	N	20.06	N	22.41	N	nd	O
111	3.99	N	40.78	N	69.82	P	53.65	P	21.25	N	8.51	N	nd	Vaccine O
112	37.00	N	7.21	N	18.15	N	20.84	N	47.58	N	0.98	N	nd	NEG
113	99.28	P	65.02	P	93.05	P	62.70	P	17.17	N	32.03	N	nd	
114	1.29	N	33.95	N	15.14	N	-0.91	N	1.85	N	8.04	N	nd	NEG
115	98.21	P	85.39	P	71.77	P	58.96	P	82.21	P	29.54	N	nd	
116	14.86	N	9.67	N	23.91	N	-14.98	N	9.68	N	3.13	N	nd	NEG
117	-15.04	N	3.90	N	6.35	N	-7.33	N	0.17	N	-13.79	N	nd	NEG
118	29.90	N	3.93	N	5.71	N	-5.02	N	-1.24	N	-5.22	N	nd	NEG
119	95.39	P	37.85	N	91.04	P	71.39	N	76.99	P	25.84	N	nd	O
120	90.91	P	19.99	N	41.72	N	59.09	P	63.53	P	23.87	N	nd	SAT2
121	52.11	P	49.58	N	62.08	P	54.87	P	24.50	N	20.05	N	nd	O
122	27.95	N	-1.16	N	6.50	N	-1.44	N	13.89	N	-6.56	N	nd	NEG

123	16.44	N	13.72	N	15.25	N	8.93	N	13.80	N	17.01	N	nd	NEG
124	82.88	P	57.51	P	90.98	P	52.23	P	23.62	N	35.30	N	nd	O
125	94.89	P	89.21	P	93.81	P	74.53	N	24.38	N	42.89	N	nd	O
126	95.58	P	45.88	N	87.25	P	45.13	N	29.17	N	27.59	N	nd	O
127	86.15	P	88.25	P	41.81	N	17.00	N	18.61	N	17.72	N	nd	A
128	-15.78	N	5.98	N	41.36	N	10.37	N	5.82	N	10.45	N	nd	NEG
129	48.92	N	52.17	P	91.75	P	-9.66	N	66.28	P	45.70	N	nd	Vaccine O
130	21.35	N	1.77	N	47.35	N	-1.21	N	0.20	N	14.75	N	nd	NEG
131	6.63	N	7.87	N	4.69	N	-3.55	N	0.95	N	5.45	N	nd	NEG
132	98.41	P	64.23	P	83.50	P	53.77	P	60.44	P	27.28	N	nd	O
133	82.40	P	69.93	P	92.02	P	53.20	P	70.40	P	32.00	N	nd	O
134	34.49	N	7.84	N	27.86	N	21.94	N	24.92	N	20.37	N	nd	NEG
135	87.04	P	7.26	N	60.77	P	58.10	P	50.25	P	27.99	N	nd	O
136	17.83	N	40.21	N	84.61	P	38.90	N	65.99	N	14.73	N	nd	O
137	94.24	P	69.71	P	87.29	P	58.59	P	42.39	N	39.56	N	nd	O
138	48.17	N	13.98	N	65.08	P	43.49	N	49.74	N	23.56	N	nd	Vaccine O
139	93.34	P	11.86	N	25.30	N	29.93	N	82.23	P	33.23	N	nd	SAT2
140	95.96	P	86.33	P	87.53	P	46.18	N	73.22	P	38.77	N	nd	O,A
141	87.45	P	55.75	P	74.58	P	31.75	N	32.28	N	21.67	N	nd	O
142	15.57	N	63.11	N	87.50	P	60.12	P	16.83	N	39.82	N	nd	Vaccine O
143	58.54	P	22.01	N	69.56	P	10.95	N	69.07	P	17.33	N	nd	O,SAT2
144	20.97	N	61.98	P	75.82	P	0.76	N	29.30	N	1.63	N	nd	Vaccine O
145	4.96	N	52.44	N	53.14	N	20.29	N	35.08	N	7.88	N	nd	NEG
146	95.76	P	77.87	P	93.87	P	60.08	P	78.86	P	42.13	N	nd	O
147	73.82	P	19.22	N	84.35	P	34.75	N	48.37	N	25.25	N	nd	O
148	98.86	P	84.57	P	83.40	P	54.65	P	64.60	P	37.83	N	nd	A
149	96.83	P	87.44	P	90.86	P	60.25	P	61.58	P	44.90	N	nd	O
150	94.70	P	78.26	P	84.48	P	69.12	P	67.41	P	40.43	N	nd	O
151	86.24	P	62.82	P	71.28	P	46.51	N	67.84	P	30.05	N	nd	O
152	93.06	P	79.59	P	67.52	P	49.80	N	83.97	P	39.48	N	nd	SAT2
153	55.36	P	53.90	P	95.36	P	64.34	P	68.00	P	26.79	N	nd	O
154	98.14	P	80.96	P	91.72	P	70.97	P	67.66	P	39.07	N	nd	O

155	97.58	P	32.79	N	91.85	P	64.79	P	76.19	N	36.15	N	nd	O
156	71.27	P	40.17	N	79.17	P	37.16	N	37.49	N	33.48	N	nd	O
157	92.27	P	76.03	P	93.78	P	41.66	N	30.80	N	29.51	N	nd	O
158	94.46	P	62.07	P	89.08	P	72.63	P	78.28	P	37.44	N	nd	O
159	33.17	N	20.54	N	92.59	P	44.47	N	63.52	P	31.65	N	nd	Vaccine O
160	97.73	P	84.35	P	94.01	P	49.67	N	62.35	P	28.32	N	nd	O
161	97.84	P	47.67	N	66.95	P	48.76	N	62.27	P	30.10	N	nd	O
162	61.76	P	74.49	P	91.38	P	43.16	N	63.99	P	38.96	N	nd	O
163	-10.87	N	14.81	N	91.81	P	33.43	N	57.31	P	17.36	N	nd	O
164	90.33	P	93.04	P	88.56	P	56.52	P	45.27	N	40.24	N	nd	A
165	52.86	P	27.23	N	89.11	P	35.98	N	35.30	N	34.86	N	nd	O
166	31.22	N	48.30	N	18.06	N	48.97	N	36.28	N	16.77	N	nd	NEG
167	89.80	P	46.16	N	25.13	N	18.43	N	54.68	P	22.65	N	nd	SAT2
168	92.24	P	74.24	P	78.95	P	53.18	P	76.23	P	39.69	N	nd	O
169	98.61	P	38.63	N	95.09	P	72.79	N	88.85	P	45.82	N	nd	O
170	NO SAMPLE		NO SAMPLE		NO SAMPLE		No Sample	N			No Sample	N	No Sample	No Sample
171	63.80	P	10.75	N	93.34	P	36.53	N	56.79	P	38.40	N	nd	O
172	59.51	P	23.89	N	85.59	P	35.83	N	38.75	N	28.23	N	nd	O
173	72.50	P	13.53	N	76.08	P	48.95	N	67.49	P	34.00	N	nd	O
174	98.42	P	86.05	P	94.97	P	76.31	N	85.21	P	50.38	P	nd	O
175	79.20	P	69.92	P	88.59	P	22.47	N	67.80	P	23.51	N	nd	O
176	78.00	P	61.10	P	88.15	P	51.84	P	71.10	P	43.33	N	nd	O
177	95.73	P	78.12	P	24.28	N	53.10	P	70.40	P	44.96	N	nd	A
178	81.59	P	63.43	P	91.07	P	69.62	N	24.92	N	41.80	N	nd	O
179	11.77	N	20.85	N	63.62	P	32.21	N	50.25	P	22.32	N	nd	O
180	74.57	P	52.03	P	60.71	P	43.73	N	65.99	P	30.52	N	nd	SAT2
181	64.73	P	88.07	P	92.07	P	54.80	P	42.39	N	38.91	N	nd	O
182	97.99	P	91.12	P	85.31	P	74.33	P	49.74	N	52.86	P	nd	A
183	59.23	P	31.56	N	49.97	N	-4.98	N	82.23	P	24.73	N	nd	SAT2
184	37.11	N	33.66	N	71.75	P	38.97	N	73.22	P	33.71	N	nd	SAT2
185	89.02	P	54.95	P	94.52	P	83.47	P	32.28	N	51.48	P	nd	O
186	91.96	P	73.64	P	88.57	P	28.89	N	16.83	N	33.13	N	nd	O
187	92.67	P	17.41	N	91.43	P	60.79	P	69.07	N	43.80	N	nd	O

188	47.64	N	65.37	P	64.05	P	50.37	P	29.30	N	37.21	N	nd	A
189	59.84	P	62.65	P	84.36	P	46.65	N	35.08	N	34.49	N	nd	O
190	87.16	P	87.62	P	84.32	P	49.26	N	78.86	P	34.43	N	nd	A
191	42.02		44.59	N	75.09	P	71.22	P	48.37	N	36.12	N	nd	Vaccine O, A
192	99.30	P	69.79	P	78.31	P	41.99	N	64.60	P	39.81	N	nd	O
193	74.20	P	17.42	N	25.84	N	18.72	N	61.58	P	25.33	N	nd	SAT2
194	91.99	P	83.61	P	90.29	P	33.93	N	67.41	P	34.11	N	nd	O
195	100.12	P	77.04	P	28.86	N	54.27	P	67.84	P	29.80	N	nd	A
196	99.03	P	83.04	P	93.64	P	59.37	P	83.97	P	44.81	N	nd	O
197	51.34	P	15.65	N	53.87	P	38.09	N	68.00	P	25.25	N	nd	SAT2
198	100.37	P	80.45	P	86.95	P	87.77	P	67.66	P	56.33	P	nd	O
199	61.04	P	31.19	N	75.09	P	31.32	N	76.19	P	27.33	N	nd	SAT2
200	96.88	P	30.49	N	84.49	P	34.07	N	37.49	N	31.60	N	nd	O
201	74.51	P	57.34	P	86.85	P	45.09	N	30.80	N	43.99	N	nd	O
202	73.94	P	18.43	N	91.47	P	30.69	N	78.28	P	31.51	N	nd	O
203	95.97	P	76.80	P	87.33	P	45.09	N	63.52	P	32.72	N	nd	O
204	99.33	P	55.27	P	93.83	P	57.49	P	62.35	P	47.90	N	nd	O
205	59.99	P	17.18	N	33.30	N	36.82	N	62.27	P	30.64	N	nd	SAT2
206	100.40	P	78.98	P	95.68	P	85.88	P	63.99	P	49.51	N	nd	O
207	96.91	P	53.29	P	89.93	P	29.31	N	57.31	P	19.05	N	nd	O
208	31.23		18.01	N	30.11	N	28.40	N	45.27	N	20.96	N	nd	NEG
209	92.76	P	12.20	N	95.13	P	80.43	P	35.30	N	43.74	N	nd	O
210	84.09	P	68.25	P	92.94	P	67.18	P	36.28	N	43.10	N	nd	O
211	78.55	P	76.83	P	80.82	P	38.15	N	54.68	P	27.21	N	nd	O
212	46.34	N	17.97	N	29.68	N	69.13	P	76.23	P	35.86	N	nd	SAT2
213	65.58	P	20.97	N	92.52	P	29.73	N	88.85	P	36.21	N	nd	O, sat2?
214	78.46	P	50.06	P	81.90	P	40.02	N	-3.19	N	35.40	N	nd	O
215	94.11	P	54.77	P	94.06	P	70.40	P	56.79	P	47.50	N	nd	O
216	96.94	P	34.46	N	89.21	P	62.24	P	38.75	N	33.77	N	nd	O
217	97.25	P	23.82	N	44.95	N	46.39	N	67.49	P	10.83	N	nd	SAT2
218	95.02	P	76.59	P	93.01	P	72.50	P	85.21	P	52.69	P	nd	O
219	31.72	N	46.74	N	85.44	P	42.39	N	67.80	P	24.16	N	nd	Vaccine O
220	42.63	N	31.61	N	94.00	P	53.51	P	71.10	P	42.89	N	nd	Vaccine O

221	79.35	P	78.51	P	89.47	P	68.17	P	26.34	N	39.47	N	nd	O
222	49.69	N	31.52	N	78.30	P	38.66	N	55.75	P	12.41	N	nd	O
223	41.85	N	89.00	P	94.77	P	82.00	P	68.82	P	50.74	P	nd	Vaccine O,A
224	62.12	P	36.79	P	84.78	P	41.42	N	60.33	P	38.03	N	nd	O
225	78.67	P	26.78	P	52.98	P	75.47	P	84.60	P	36.66	N	nd	SAT2
226	44.62	N	71.92	P	90.35	P	80.08	P	77.66	P	43.93	N	nd	Vaccine O
227	99.84	P	66.82	P	68.52	P	5.83	N	48.28	N	17.22	N	nd	O
228	11.60	N	17.79	N	8.57	N	6.25	N	27.98	N	17.94	N	nd	NEG
229	74.52	P	16.75	N	84.69	P	21.82	N	23.04	N	24.65	N	nd	O
230	75.58	P	19.59	N	94.98	P	52.45	P	36.97	N	40.13	N	nd	O
231	18.98	N	63.77	P	58.62	N	56.31	P	64.62	P	19.30	N	nd	Vaccine A, SAT2 ?
232	97.24	P	76.76	P	92.93	P	76.02	P	79.05	N	37.98	N	nd	O
233	94.48	P	10.55	N	41.02	N	37.74	N	90.52	P	18.62	N	nd	SAT2
234	98.45	P	86.99	P	44.78	N	71.89	P	76.06	P	47.58	N	nd	A
235	95.27	P	43.43	N	76.32	P	43.37	N	79.62	P	31.60	N	nd	SAT2
236	99.93	P	72.07	P	72.55	P	62.38	P	84.77	P	36.92	N	nd	SAT2
237	13.83	N	24.15	N	91.54	P	54.13	P	36.78	N	17.26	N	nd	O
238	29.24	N	13.16	N	86.93	P	41.18	N	53.28	P	26.25	N	nd	O
239	68.55	P	32.29	N	90.64	P	66.56	P	77.18	P	39.45	N	nd	O
240	67.62	P	76.98	P	66.40	P	33.89	N	26.95	N	13.95	N	nd	A
241	43.72	N	10.56	N	71.54	P	33.19	N	48.59	N	21.24	N	nd	Vaccine O
242	91.87	P	65.62	P	93.86	P	75.78	P	83.84	P	6.03	N	nd	O
243	99.00	P	51.08	P	83.48	P	84.26	P	86.55	P	46.83	N	nd	O
244	95.50	P	55.28	P	83.15	P	62.03	P	75.79	P	35.79	N	nd	O
245	94.71	P	85.73	P	95.77	P	69.95	P	69.02	P	38.71	N	nd	O
246	97.51	P	61.12	P	91.91	P	68.76	P	74.31	P	43.70	N	nd	O
247	98.02	P	85.61	P	95.26	P	77.79	P	80.77	P	47.01	N	nd	O
248	98.05	P	86.17	P	19.17	N	51.04	P	83.35	P	27.37	N	nd	A, SAT2
249	96.33	P	53.77	P	70.40	P	38.43	N	76.07	P	31.66	N	nd	O, SAT2
250	44.73	N	41.50	N	68.64	P	31.59	N	58.70	P	24.65	N	nd	Vaccine O

251	73.89	P	36.54	N	75.75	P	39.26	N	48.67	N	20.03	N	nd	O
252	97.40	P	58.54	P	89.54	P	38.51	N	49.19	N	24.11	N	nd	O
253	5.22	N	13.93	N	92.17	P	36.10	N	15.94	N	15.76	N	nd	Vaccine O
254	85.75	P	75.97	P	93.90	P	45.34	N	25.64	N	37.09	N	nd	O

Optic density readings using ELISA reader at 450nm and 490nm for Outbreak cattle sera

1	66.98	P	27.90	N	87.21	P	52.88	P	70.55	P	29.16	N	nd	O
2	99.31	P	80.26	P	93.94	P	67.43	P	39.56	N	28.97	N	nd	O
3	99.38	P	90.32	P	90.35	P	59.83	P	71.18	P	37.23	N	nd	O
4	97.93	P	11.88	N	84.54	P	34.79	N	66.80	P	36.95	N	nd	O,A
5	88.90	P	9.83	N	76.51	P	15.74	N	47.99	N	-109.16	N	nd	O
6	80.03	P	6.62	N	88.58	P	36.90	N	42.80	N	2.72	N	nd	O
7	99.44	P	89.27	P	93.19	P	62.28	P	72.60	P	47.09	N	nd	O
8	67.27	P	13.63	N	79.79	P	26.30	N	49.68	N	12.15	N	nd	O
9	90.44	P	67.06	P	95.12	P	82.49	P	84.98	P	44.29	N	nd	O
10	82.63	P	68.29	P	90.88	P	35.47	N	47.60	N	26.32	N	nd	O
11	97.90	P	53.17	P	90.46	P	53.60	P	33.79	N	36.64	N	nd	O
12	87.92	P	45.52	N	89.61	P	67.34	P	57.03	P	34.30	N	nd	O
13	96.06	P	16.66	N	91.41	P	74.24	P	81.35	P	39.59	N	nd	O
14	77.78	P	16.77	N	70.32	P	29.17	N	45.08	N	16.57	N	nd	O
15	97.87	P	47.94	N	90.11	P	64.15	P	57.02	P	43.83	N	nd	O
16	92.83	P	13.27	N	66.58	P	43.11	N	29.07	N	-5.55	N	nd	O
17	96.11	P	41.46	N	71.47	P	20.78	N	21.83	N	26.24	N	nd	O
18	96.49	P	76.05	P	93.95	P	32.83	N	65.11	P	26.53	N	nd	O
19	61.38	P	11.48	N	91.53	P	49.08	N	39.37	N	36.40	N	nd	O
20	76.34	P	52.36	P	80.95	P	57.85	P	60.66	P	26.77	N	nd	O
21	87.52	P	59.86	P	91.77	P	64.73	P	44.87	N	26.53	N	nd	O
22	83.15	P	53.77	P	86.83	P	79.00	P	82.11	P	44.05	N	nd	O
23	98.38	P	21.81	N	78.13	P	47.49	N	57.63	P	27.46	N	nd	O
24	27.40	N	42.57	N	84.47	P	57.02	P	61.92	P	8.43	N	nd	O
25	98.69	P	53.88	P	92.26	P	69.85	P	63.56	P	44.44	N	nd	O
26	80.36	P	71.42	P	43.80	N	48.71	N	39.31	N	28.12	N	nd	A
27	87.36	P	86.00	P	93.32	P	74.16	P	62.44	P	42.38	N	nd	O

28	99.71	P	51.45	P	93.62	P	84.31	P	88.47	P	41.56	N	nd	O
29	92.40	P	74.98	P	83.40	P	46.07	N	81.34	P	31.40	N	nd	O
30	41.74	N	25.93	N	88.21	P	36.49	N	22.95	N	23.19	N	nd	O
31	55.28	P	30.81	N	50.53	P	32.85	N	32.30	N	16.57	N	nd	O
32	92.86	P	79.25	P	82.40	P	41.38	N	63.86	P	32.47	N	nd	O
33	64.78	P	66.99	N	79.65	P	31.75	N	45.66	N	26.24	N	nd	O
34	97.40	P	38.97	N	80.92	P	20.35	N	47.48	N	22.77	N	nd	O
35	99.91	P	34.43	N	86.80	P	41.15	N	74.01	P	28.19	N	nd	O
36	91.47	P	54.56	P	58.27	P	16.55	N	37.27	N	27.62	N	nd	O
37	26.08	N	39.57	N	60.52	P	21.12	N	11.61	N	11.25	N	nd	O
38	88.51	P	68.97	P	91.55	P	46.18	N	53.89	P	32.82	N	nd	O
39	97.55	P	60.48	P	92.85	P	48.62	N	62.49	P	38.94	N	nd	O
40	52.00	P	12.18	N	78.76	P	42.76	N	37.17	N	24.52	N	nd	O
41	98.44	P	83.70	P	92.54	P	63.12	P	43.57	N	36.54	N	nd	O
42	99.07	P	55.72	P	90.16	P	60.46	P	38.91	N	44.41	N	nd	O
43	97.36	P	28.31	N	73.68	P	53.74	P	51.46	P	-94.68	N	nd	O
44	99.17	P	91.26	P	94.31	P	54.56	P	35.29	N	43.75	N	nd	O,A
45	75.32	P	59.86	P	85.77	P	49.86	N	72.72	P	32.27	N	nd	O
46	87.57	P	35.97	N	82.49	P	41.45	N	60.02	P	27.30	N	nd	O
47	99.45	P	83.99	P	85.31	P	71.57	P	85.58	P	49.83	N	nd	O,SAT2
48	98.46	P	73.97	P	89.98	P	32.92	N	72.62	P	22.07	N	nd	O
49	61.75	P	48.18	N	86.96	P	62.80	P	51.70	P	30.15	N	nd	O
50	99.10	P	52.96	P	90.07	P	61.26	P	40.30	N	30.79	N	nd	O
51	99.05	P	65.24	P	94.36	P	61.81	P	38.17	N	30.18	N	nd	O
52	93.91	P	61.82	P	84.02	P	56.75	P	72.74	P	29.67	N	nd	O
53	98.14	P	25.25	N	90.98	P	55.65	P	61.69	P	21.79	N	nd	O
54	21.15	N	38.84	N	61.25	P	21.65	N	70.26	P	5.18	N	nd	Vaccine SAT2
55	99.33	P	95.93	P	93.07	P	69.05	P	72.35	P	49.50	N	nd	O, A
56	97.42	P	87.29	P	92.07	P	55.78	P	55.40	P	33.14	N	nd	O
57	92.44	P	71.04	P	60.99	P	20.43	N	44.35	N	11.70	N	nd	A
58	99.61	P	68.41	P	58.90	P	61.48	P	37.22	N	35.38	N	nd	O
59	26.92	N	8.68	N	65.60	P	42.68	N	49.54	N	29.64	N	nd	Vaccine O
60	96.56	P	84.95	P	66.98	P	36.90	N	75.48	P	23.71	N	nd	A

61	94.66	P	80.76	P	71.61	P	34.33	N	74.08	P	33.01	N	nd	A
62	72.51	P	42.40	N	90.78	P	52.13	P	73.30	P	37.37	N	nd	O
63	93.88	P	49.79	N	91.70	P	49.88	N	55.04	N	31.82	N	nd	O
64	56.80	P	80.38	P	82.19	P	33.41	N	39.22	N	18.92	N	nd	O
65	99.62	P	92.06	P	56.68	P	53.57	P	56.36	p	39.17	N	nd	A
66	86.88	P	68.20	P	93.88	P	74.37	P	56.37	p	23.83	N	nd	O

	All Negative
	NSP negative, but positive for at least one serotype
	Positive for at least one serotype (highest PI)
	Weak positive, but considered as negative when compared to the highest level of PI
	NSP antibody positive but SP antibody negative
cELISA Cuttoff	Equivalent to or greater than 50% PI