

Epidemiology of peste-des-petits ruminants virus in sheep, goats and camels in Nigeria

by

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DEDICATION

To Christ Jesus my Lord, who gave me the privilege to live and the strength to come this far.
To the king eternal, immortal, invisible, the only God, be honour and glory forever and ever.

I Timothy 1:12, 17.

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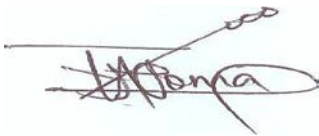
My loving children, Ngainu, Numachuchun, Tehila and Nupoyah for the patience, understanding and support during my long absence from home. I love you more and will always do as long as I live. I promise to make you proud in all my endeavours.

My parents, Mr and Mrs Timothy W Woma; my sisters Dr (Mrs) Ruth Adi, Mrs Rautha James and my brothers, Engr Yakubu, Rev Musa and master Nkenu, Uncle I I Yaboro and family.

The many prayers and moral support from all of you is gratefully acknowledged. I will continue to uphold the family tradition of the dignity of labour daddy instilled in me.

DECLARATION

I declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy (PhD) Veterinary Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.



Candidate: T Y Woma

ETHICS STATEMENT

The author, whose name appears on the title page of this thesis, has obtained, for the research described in this work, the applicable research ethics approval (Appendix 1).

The author declares that he has observed the ethical standards required in terms of the University of Pretoria's *Code of ethics for researchers* and the *Policy guidelines for responsible research*.

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LIST OF ABBREVIATIONS

aa = Amino acid
aas = Amino acids
AGID = Agarose gel immunodiffusion
Ap = Apparent prevalence
AU-IBAR = African Union Inter-African Bureau for Animal Resources
BDSL = Biological Diagnostic Supplies Limited
BLAST = Basic local alignment search tool
bp = Base pairs
CCPP = Contagious caprine pleuropneumonia
CDV = Canine distemper virus
cELISA = Competitive enzyme linked immunosorbent assay
CI = Confidence interval
CIEP = Counter immunoelectrophoresis
CIRAD-EMVT = Centre de coopération internationale en recherche agronomique pour le développement. Département d'élevage et de médecine vétérinaire.
CM² = Centimetre square
CMV = Cetacean morbillivirus
CP = Competition percentage
d = Confidence limits as $\pm$ %
DAMBE = Data Analysis in Molecular Biology Evolution
DEFF = Design effect for cluster surveys
DIVA = Differentiating infected from vaccinated animals
DMEM = Dulbecco's modified eagle medium
DMV = Dolphin morbillivirus
DNA = Deoxyribonucleic acid
DRC = Democratic Republic of Congo
EDI = ELISA data interchange
EDTA = Ethylenediaminetetraacetic acid
ELISA = Enzyme linked immunosorbent assay
FAO = Food and Agricultural Organization (of the United Nations)
FmoPV = Feline morbillivirus
H₂SO₄ = Tetraoxosulphate six (sulphuric) acid
IAEA = International Atomic Energy Agency
IAH = Institute for Animal Health

iELISA = Immunocapture enzyme linked immunosorbent assay

ISO = International Organization for Standardization

KM = Kilometre

Km² = Kilometre square

LINII = Lineage II PPRV full genome sequenced in this study

LINIV = Lineage IV PPRV full genome sequenced in this study

MAb = Monoclonal antibody

MEGA = Molecular Evolutionary Genetics Analysis

MeV = Measles virus

Min = Minute

ml = Millilitre

mRNA = Messenger ribonucleic acid

N = Population size

nt = Nucleotide

NVRI = National Veterinary Research Institute, Vom, Nigeria

OD = Optical density

ODNc = Optical density of the negative control

ODPc = Optical density of the positive control

°C = Degree centigrade

OIE = World Organisation for Animal Health

ORF = Open reading frame

p = Hypothesized % frequency of outcome factor in a population

PAUP* = Phylogenetic analysis using parsimony (*and other methods)

PCR = Polymerase chain reaction

PDV = Phocine distemper virus

PI = Percentage inhibition

PPR = Peste-des-petits ruminants

PPRV = Peste-des-petits ruminants virus

RdRp = RNA-dependent RNA polymerase

RNA = Ribonucleic acid

RNAi = Ribonucleic acid interference

RNP = Ribonucleoprotein

RP = Rinderpest

RPV = Rinderpest virus

RT-PCR = Reverse transcription polymerase chain reaction

SADC = Southern African Development Community

Se = Sensitivity

SLAM = Signalling lymphocyte activation molecule

Sp = Specificity

STAT1 = Signal transducer and activator of transcription 1

USA = United States of America

USD = United State dollars

UK = United Kingdom

UTR = Untranslated region

μ = Micron

μ l =Microlitre

VN = Virus neutralization

WAD = West African Dwarf

w/v = weight by volume

ABSTRACT

Peste-des-petits-ruminants (PPR), caused by the peste-des-petits ruminants virus (PPRV) is endemic in Nigeria and is a major constraint to the improvement of small ruminant production, thereby affecting the rural poor who depends on these livestock species as their source of livelihoods.

The major objectives of this work were: 1) to conduct a seroprevalence of PPRV in camels, sheep and goats in Nigeria; 2) to isolate PPRV from naturally infected animals; and 3) to characterize the isolates using molecular techniques and compare the profile of the current isolates with the worldwide vaccine (Nig75/1) and other existing strains from other parts of the world.

The major contribution of this work to current knowledge were: finding that more than one lineage of PPRV was circulating in Nigeria, the “emerging” Asian lineage IV gradually replacing the “traditional” lineage II in the country; the isolation in cell cultures and full genome sequencing of current field viruses which was last done in 1976; the unique amino acid residues in the individual proteins of the various lineages of PPRV were identified and used to develop a lineage-specific diagnostic test for the virus; and the current seroprevalence report of PPR in camels, sheep and goats from all the agro-ecological zones of the country. This work has also increased the sequences of PPRV available in GenBank.

A total of 6,065 field serum samples from camels (1,517), goats (3,489) and sheep (1,059) were collected from all the six agro-ecological zones of Nigeria. The study subjects cut across all ages and sexes. The samples were subjected to both the N and H protein based c-ELISA.

In addition, 140 clinical samples from 16 sheep and 63 goats with symptoms suggestive of PPRV infection were collected from different (15) states of Nigeria during a four year period (2010 – 2013). Published primers were used to amplify a 351 bp segment of the PPRV nucleoprotein (N) gene and a 370 bp segment of the fusion (F) gene. Monkey CV 1 cell line expressing the sheep-goat SLAM protein was used to isolate PPRV from RT-PCR positive samples. Thirty primer pairs were used to amplify and sequence two full genome isolates of the current circulating PPRV in Nigeria. Primer express software was used to develop a lineage-specific real time PCR for PPRV.

The overall prevalence estimate of serum positive results for PPRV in sheep and goats was 23.16 % ($n = 1,018/4,548$, 95 % confidence interval [CI]: 21.79 - 24.57). There were significant differences in prevalences between the states ($p = 0.001$), zones ($p = 0.058$) and age ($p = 0.032$). Taraba State had the highest seroprevalence rate of 27.97 % while the lowest rate of 14.76 % was observed in Cross River State. There were no significant differences in the PPRV prevalences between male and female animals ($p = 0.571$) and between species ($p = 0.639$). The overall prevalence in camels was 3.36 % ($51/1517$, 95 % CI: 2.51 – 4.39). There were no significant differences in prevalences between states ($p = 0.892$) and between male and female camels ($p = 0.742$). The prevalence differed significantly ($p < 0.001$) by body condition scores - camels with poor body condition score have a higher (16.67 %) antibody seroprevalences to PPRV compared to those with fair and good body condition scores. There was a statistically significant difference between camels aged ≤ 5 years and those > 5 years ($p = 0.004$).

The clinical samples subjected to RT-PCR showed that 33 (42 %) animals were positive for PPRV nucleic acid, which included two sheep (13 %) and 31 goats (49 %). The amplicons were sequenced and phylogenetic analysis using the neighbour joining, maximum parsimony and Bayesian analysis of the sequences with those available in GenBank showed that isolates from the current study belonged to lineage II and lineage IV. The lineage II viruses from this study grouped into two clades, one closely related to the vaccine virus (Nigeria75/1) and the other clade group with other wild-type viruses from Mali, Senegal and Sierra Leone. The lineage IV isolates also grouped into two sub-clades, one closely related to a 2011 strain from Gabon and the other closely related to a 1997 strain from Cameroon.

Analysis of two full genomes of PPRV from Nigeria representing the two lineages of the virus circulating currently in the country showed that the lineage II representative isolated in 2012 has an identity of 96 % at the nucleotide level with the lineage II Nigeria75/1 virus isolated in 1975 and used as a vaccine. The lineage IV representative isolated in 2013 revealed an identity of 96 % with Sungri/96 from India. The unique amino acid residues in the individual proteins of the various lineages of PPRV were identified and used to develop a one-step TaqMan® real-time RT-PCR assay targeting the H gene of PPRV.

This study reports for the first time, the presence of PPRV lineage IV, the Asian lineage in Nigeria. As part of the study, a lineage-specific assay was also developed, which once validated, could be included in the panel of assays recommended by the World Organization for Animal Health (OIE). The seroprevalence studies showed occasional transient PPRV

infection of camels and the current antibody seroprevalence to PPRV in the small ruminants' population in Nigeria. There is the need to include camels among species to be studied in elucidating the epidemiology of the disease in sheep and goats. The seroprevalence studies may be helpful in reaching a decision on the vaccination strategy for the control of the disease in the country.

CHAPTER

1. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. General Introduction

Peste des petits ruminants (PPR) is a severe, fast – spreading disease of mainly domestic small ruminants (sheep and goats). It is characterized by sudden onset of depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing, coughing, foul-smelling diarrhoea and death (FAO, 2009). The virus which causes PPR, peste-des-petits-ruminants virus (PPRV), is a member of the genus *Morbillivirus* in the family *Paramyxoviridae*. It is closely related to rinderpest virus that infects cattle and buffaloes, measles virus that infects humans, distemper virus that infects dogs and some wild carnivores, and the morbilliviruses that infects aquatic mammals (Barrett *et al.*, 2006).

In Nigeria, the name given to PPR during the first description of the disease was “goat plague” because goats were the only species involved in the reported outbreaks. Across Nigeria, viral pathogens such as PPRV place a huge burden on small ruminant production and are responsible for increasing poverty in some of the poorest parts of the country. Hamdy *et al.*, (1976) evaluated the annual loss incurred due to PPR in Nigeria at USD 1.5 million, equivalent to approximately USD 500 million in the current economy.

The virus is highly contagious and transmitted easily by direct contact with the secretions and/or excretions of infected animals (Ezeibe *et al.*, 2008). A study conducted between 1995 and 2000 reported the PPR seroprevalence rates in some states of Nigeria at 49.26 % in sheep and 38.34 % in goats (Shamaki, 2002). The study also confirmed the presence of PPR in camel and warthog. In 2008, PPR field outbreaks were recorded in sheep and goats across different ecological zones of Nigeria (Kazeem *et al.*, 2009).

Small ruminants play a very important role in Nigeria’s livestock production system, helping to meet the rising demand for animal protein in Nigeria’s population of over 150 million people. They provide smallholder farmers with a source of income (Adamu *et al.*, 2005), contributing manure for crop production and are part of the food and cash security available to many Nigerians (Obi *et al.*, 1988). The animals are also used for sacrifices during religious and traditional festivals. Sheep and goats in Nigeria provide approximately 35 % of

the meat consumed and their skins support the leather industry and earn foreign exchange (Ibu, 2005), a more efficient management and disease control in small ruminants is therefore warranted. To plan an effective strategy for the control of PPR, greater understanding of the current situation in the field is necessary. The pattern of outbreaks needs to be thoroughly investigated and the offending viral strains isolated and characterized.

The role of camels and donkeys in the epidemiology of PPR in Nigeria needs to be investigated because camels and donkeys share grazing land and drinking points with small ruminants. Camels and donkeys are known to carry PPRV antigens and antibodies, without showing clinical disease (Daneji *et al.*, 1997; Shamaki, 2002). PPRV antibodies in 4 % of 250 camel sera collected from Sokoto central abattoir have been documented (Daneji *et al.*, 1997) and the detection of PPR infections of camels in Borno and Kano states has also been reported (Ibu *et al.*, 2008).

Nigeria share borders with Cameroon and Chad to the East, Niger Republic to the North and Benin Republic to the West. The land borders are porous and there is constant movement of pastoralists and their animals into and out of Nigeria. Due to the movement of small ruminants in and out of Nigeria, it is imperative to carry out continuous molecular and sero-surveillance of the circulating lineages of PPRV in Nigeria.

This study is aimed at determining the seroprevalence in camel, sheep and goats, isolation of PPRV from current outbreaks of the disease and comparing them with earlier isolates made in Nigeria and other parts of the world. The study provides genomic data on the PPRVs circulating in Nigeria and information on the movement of small animals and their trade across the West African sub-region.

1.2. Hypotheses

- More than one lineage of PPRV is circulating in Nigeria.
- The current seroprevalence of PPR in Nigeria is higher than reported previously.

1.3. Literature Review

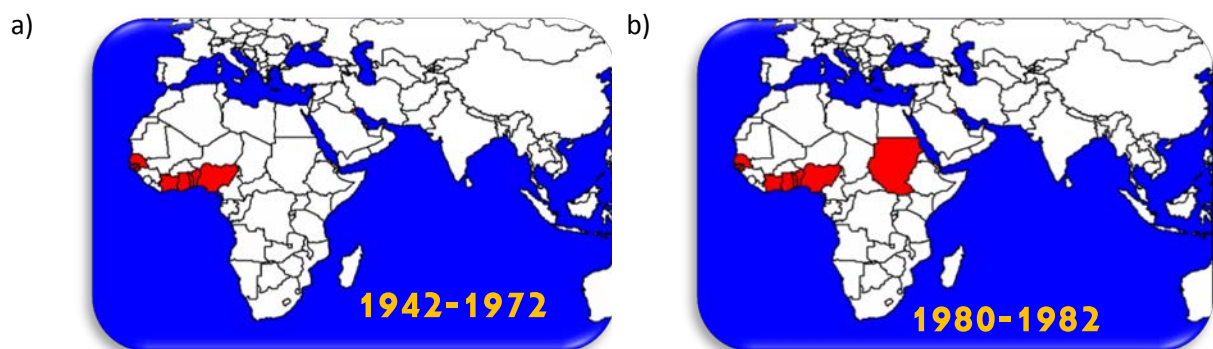
“Peste des petits ruminants” is the French name given by Gargadennec & Lalanne (1942), who were the first to describe a syndrome in small ruminants in Côte d’Ivoire in the 1940s,

in reference to the disease's clinical similarity with rinderpest. PPR is also known as stomatitis-pneumoenteritis-complex, pest of sheep and goats, kata, contagious pestular stomatitis, pseudorinderpest, and goat plague. However, stomatitis-pneumoenteritis-complex is the designation that fits best with the clinical signs (Diallo, 2000).

PPR is widespread throughout some of the poorest developing regions of the world. It is a transboundary animal disease that causes significant economic losses due to its high morbidity and mortality, and has therefore been classified as a disease that must be reported to the World Organization for Animal Health (OIE) (Diallo, 2000; 2006). The disease poses a serious threat to the livelihood of sheep and goat farmers in regions where it is endemic and its control and eradication is a priority in order to alleviate poverty (Banyard *et al.*, 2010).

The risk of transboundary spread of PPR is high because sheep and goats are easily transported and trade across borders is difficult to control. In 2011 alone, 27 African countries reported PPR outbreaks to the African Union - Interafrican Bureau for Animal Resources (AU-IBAR). In total, 1,185 epidemiological units were affected in 27 countries, causing 101,016 cases and 62,388 deaths with a case fatality rate of 61.8 % (AU-IBAR, 2013).

PPR was thought to be a West African problem in the early 1970s. However, the disease has steadily evolved in an apparent eastward manner from West Africa up to the Middle East and then Asia (Figure 1.1). The report of PPR outside of West Africa was made in Sudan in 1972 – 1973. In 1983, it was reported in the Arabian Peninsula, it expanded its endemicity into the Middle East and South Asia (China, Bhutan, Vietnam) by the 1980s. PPR expanded into uninfected parts of Africa dramatically between 2005 – 2012 by spreading both northwards and southwards extending from North Africa to Tanzania, Democratic Republic of Congo and Angola (Diallo & Libeau, 2014).



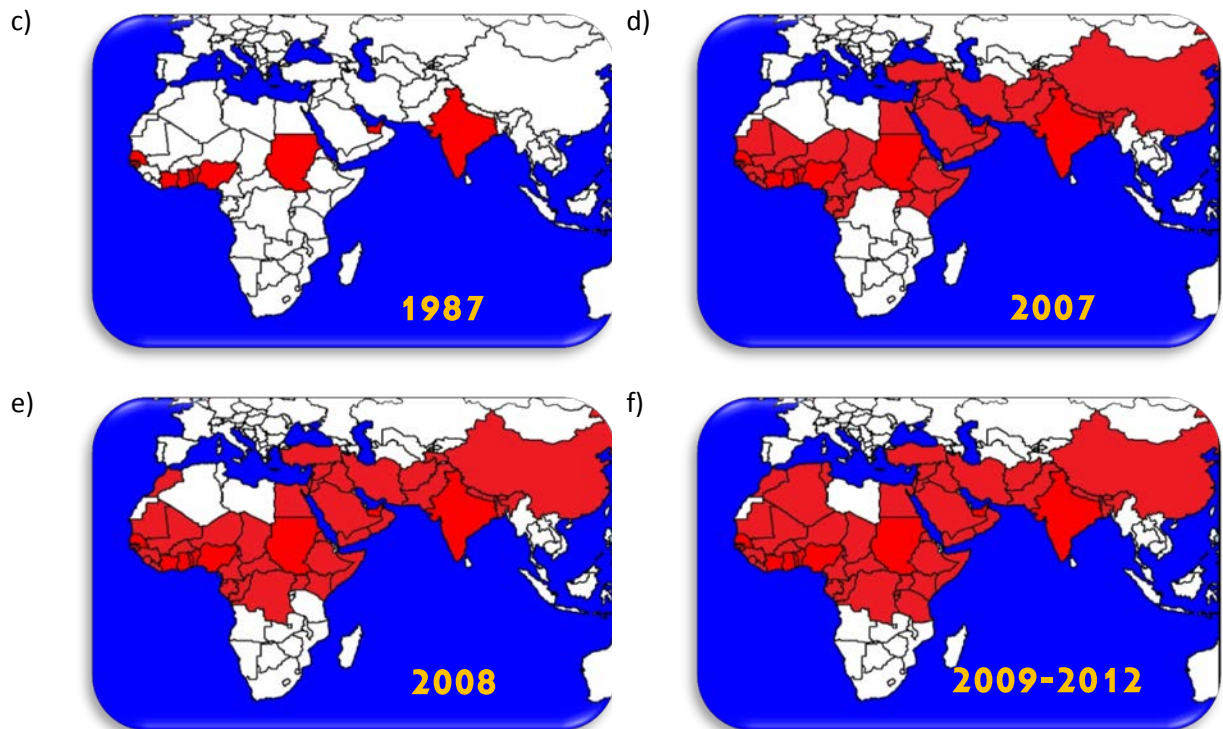


Figure 1.1: Evolution of PPRV in the world. Areas in red are regions from which PPRV were reported in: a) 1942 - 1972; b) 1980 - 1982 c) 1987; d) 2007; e) 2008 and f) 2009 - 2013 as adapted from a presentation by W.G. Dundon (IAEA). Although a whole country is coloured, the presence of PPR may be limited to only one or few regions.

1.3.1. Aetiology

The causal agent of PPR is the peste-des-petits-ruminants virus (PPRV), a virus which was thought to be a rinderpest mutant adapted to grow in sheep and goats due to the similarity in clinical symptoms and from serological analyses and cross-protection experiments (Diallo, 2000). Epidemiological and molecular studies have confirmed the difference between the causal agents of the two diseases. PPRV is classified in the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*. Other members of the genus *Morbillivirus* include: rinderpest virus (RPV), the cause of cattle plague; measles virus (MeV), of man and monkey; canine distemper virus (CDV), affecting animals of the family *Canidae*; feline morbillivirus (FmPV) of domestic cats; phocine distemper virus (PDV) of seals; and cetacean morbillivirus (CeMV), which affect marine mammals (Gibbs *et al.*, 1979; Barrett *et al.*, 2006; Woo *et al.*, 2012; King *et al.*, 2012).

PPRV is composed of a nucleocapsid surrounded by an envelope. It is a pleomorphic particle with a size varying between 150 and 700 nm, with a mean size of about 500 nm. PPRV is a fragile heat-sensitive virus; it is inactivated by detergents and liquid solvents such as ether

and chloroform. It survives for 2.2 min at 56 °C, 3.3 hours at 37 °C, 9.9 days at 4 °C, and 24.2 days at -20 °C. The virus is stable in liquid suspension at pH values ranging from 5.8 to 9.5 (Rossiter, 2004).

The PPRV genome is organized into six contiguous, non-overlapping, transcriptional units. PPRV is a non-segmented, single, negative sense RNA virus with a genome of approximately 15 – 16 kb in size (with a total 15,948 nucleotides); it contains six transcription units encoding eight proteins (Figure 1.2). These are the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H), large polymerase (L) and two non-structural proteins (V and C) which are translated from messenger RNAs transcribed from the P gene. The L protein is the viral RNA-dependent RNA polymerase (RdRp) (Barrett *et al.*, 1993; Bailey *et al.*, 2005; Chard *et al.*, 2008; Bao *et al.*, 2012; Dundon *et al.*, 2014). The N, P and L proteins form the minimal replicative unit of the virus, along with the negative sense RNA, and are required for both transcription and replication of the viral RNA. The F and H proteins are embedded in the host cell-derived envelope and are required for attachment to cellular receptors and fusion of the viral envelope with host cell membranes. The M protein is found inside the virion and is believed to be vital for virus particle assembly. The two non-structural proteins, C and V are thought to be involved in viral evasion of the host immune responses, specifically by blocking interferon production during infection (Nanda & Baron 2006; Chard *et al.*, 2008).

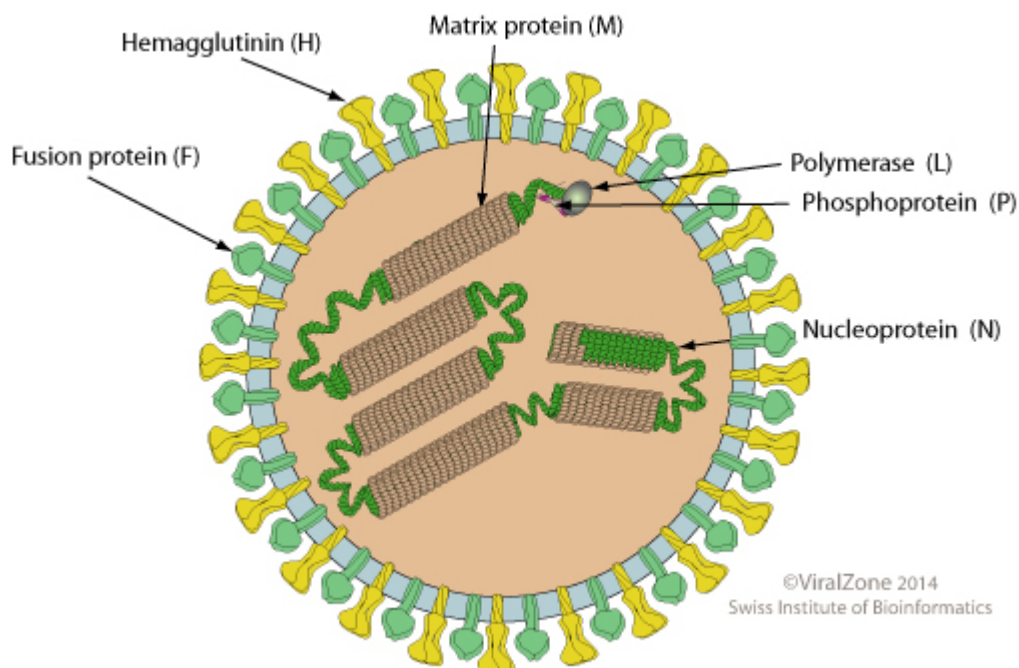


Figure 1.2: PPRV structure. (Source: ViralZone:www.expasy.org/viralzone, SIB Swiss Institute of

Bioinformatics) 

The N gene is preceded by a small fragment of 52 nucleotides called the leader while at the 5' end, the L gene is followed by a trailer that is 37 nucleotides long (Figure 1.3). The leader and the trailer serve as attachment points of the RNA polymerase to the RNA. PPRV transcription unit ends in sequences rich in U residues, followed by an intergenic motif (CTT) and a conserved sequence (UCCU/C) at the start of the next. The 3' leader region is also separated from the N gene start by an intergenic trinucleotide, usually GAA in the genome sense. This GAA is conserved at each junction of the gene except at the H/L junction (GCA) and at the L/trailer junction (GAU) (Baron & Barrett 1995; Diallo, 2000; Bailey *et al.*, 2005).

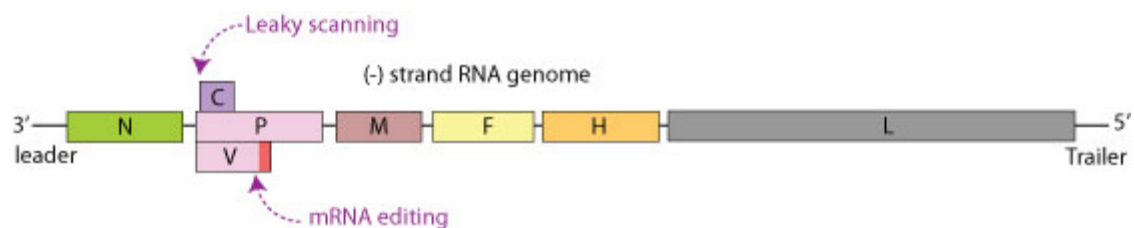


Figure 1.3: PPRV genome organisation (Source: ViralZone:www.expasy.org/viralzone, SIB Swiss

Institute of Bioinformatics)



The N has multiple roles in the virus lifecycle, it is required for encapsidation of RNA, associated with the P–L polymerase complex during replication and transcription, and it is also associated with the M protein during virus assembly. The N protein is the principal component of the helical capsids enwrapping the negative-sense genomic RNA of morbilliviruses, and is essential for packaging the genome into a ribonucleoprotein complex that serves as template for transcription, replication and packaging into progeny virions (Huber *et al.*, 1991; Bailey *et al.*, 2005; Chard *et al.*, 2008).

The P protein is an essential component of the viral polymerase complex and is also believed to be a key determinant of cross-species morbillivirus pathogenicity (Yoneda *et al.*, 2004). The P protein of morbilliviruses interacts with both the N and L proteins to form the viral polymerase; it has a highly variable N-terminus but a conserved C-terminus. For measles virus (MeV) and rinderpest virus (RPV), three serine residues (Ser 86, Ser 151 and Ser 180) and (Ser 49, Ser 88 and Ser 151), respectively, have been shown to be involved in the regulation of viral transcription through alterations in its phosphorylation status (Dundon *et al.*, 2014).

The V (a non-structural) protein is produced as a result of RNA editing across a conserved site of the P located at nt 1807–2702 (Mahapatra *et al.*, 2003). This yields a 298 aa

protein. The V protein is the least conserved of the proteins. A tyrosine at position 110 (Y110) of the V protein has been shown in both MeV and CDV to be responsible for specific binding of the signal transducer and activator of transcription I (STAT1) binding (Devaux *et al.*, 2007; Röthlisberger *et al.*, 2010). Activated STAT1 is involved together with other transcription and regulatory factors in the expression of proteins responsible for the antiviral effect of interferon (Randall & Goodbourn, 2008).

The C protein ORF is located at nt 1829–2362. This is also a non-structural protein and is 177 aa in size with a predicted molecular weight of 20.11 kDa. C is produced as a result of the ribosome scanning past the first initiation codon and initiating translation at a second initiation codon, 22 nt downstream in a different frame (Chard *et al.*, 2008). The region of 102 – 124 residues of the C protein is highly conserved in all morbilliviruses (Muthuchelvan *et al.*, 2006). The C protein has seven conserved cysteine residues that are thought to interact to form a motif for binding metal ions (especially zinc). The C and V proteins of paramyxoviruses, although essentially non-structural, have been shown to have critical roles in infection (Bailey *et al.*, 2005). In RPV they were shown to be important for replication (Baron & Barrett, 2000). The C and V proteins of paramyxoviruses also act as interferon antagonists, modifying the cellular immune response to infection (Bailey *et al.*, 2005).

The F protein enables the virus to penetrate the cell membrane and enter the cytoplasm by effecting fusion of the virus and host cell membranes. This phenomenon is also responsible for virus spread from cell to cell without formation of free viral particles, and this protein is critical for the induction of an effective protective immune response (Choppin & Scheid 1980; Merz *et al.*, 1980; Lamb, 1993). The F and H proteins together form the enveloped glycoproteins of the morbilliviruses. In PPRV, they are required for attachment and fusion of the virus particle with the host cells (Chard *et al.*, 2008).

The matrix (M) proteins are basic membrane associated molecules that interact with surface glycoproteins in the lipid envelope as well as the virion RNP. They are the most highly conserved proteins in the paramyxovirus family. This high conservation is probably due to the pivotal role the M protein plays in virion morphogenesis. M interacts with both the nucleocapsid and the cytoplasmic tails of the F and H glycoproteins. A small protein with such a critical role is likely to be intolerant to variation, especially within a genus whose members are antigenically similar (Bailey *et al.*, 2005).

The hemagglutinin (H) protein is the outermost protein of PPRV and is responsible for viral attachment. The H protein induces a strong, highly protective neutralizing antibody response. The H protein is responsible for the attachment of the virus to the host cell and it is also known to induce a strong neutralizing antibody response, which is highly protective. As it is a surface protein, it is subjected to immunological pressure (Lamb & Kolakofsky, 2001; Dhar *et al.*, 2006). The H protein of PPRV has been shown to exhibit neuraminidase and haemadsorption functions (Seth & Shaila, 2001). In MeV, H appears to be a major determinant of tissue tropism (Duprex *et al.*, 1999) and experiments using lapinised RPV indicated that, like the P protein, H is also involved in cross-species pathogenesis and is also an important antigenic determinant of the morbilliviruses (Yoneda *et al.*, 2002). The predicted molecular weight of the H protein is approximately 68 kDa, similar to that reported for the H protein of rinderpest virus (RPV) (Dhar *et al.*, 2006).

The large (L) protein is the final and longest ORF in the PPRV genome. This is the enzymatic component of the viral transcriptase and replicase. The L proteins of paramyxoviruses are multi-functional with polymerase activity, methylation, capping and polyadenylation activities (Lamb & Kolakofsky, 2001). Morbillivirus L proteins have three highly conserved domains separated by two hinge regions (McIlhatton *et al.*, 1997).

Transcription and replication of PPRV is controlled by untranslated regions (UTRs) at the 3' and 5' ends of the genome, known as the genome promoter (GP) and antigenome promoter (AGP). The UTRs are at the start of the N gene and antigenome sense RNA at the end of the L (Bailey *et al.*, 2005). A conserved stretch of 23 – 31 nucleotides at the 3' terminus of both the GP and AGP has been shown to be an essential domain required for PPRV promoter activity. The sequence of the GP and AGP is highly conserved in PPRV (Bailey *et al.*, 2005). Experiment with RPV strains shows that swapping the GP and AGP of virulent strains led to attenuation of the virus in cattle, suggesting that nucleotides within this region are important in virus attenuation (Banyard *et al.*, 2005). The GP promotes the synthesis of virus mRNA and the production of full length positive sense antigenome RNA copy. The AGP promotes the production of full length negative sense genome RNA at the 5' end after the L protein and it may be essential for viral replication (Barrett *et al.*, 2006).

1.3.2. Epidemiology

The biology and epidemiology of PPRV has been the subject of reviews (Banyard *et al.*, 2010; Sen *et al.*, 2010; Baron *et al.*, 2011; Libeau *et al.*, 2014).

PPRV lineage differentiation is determined by the sequence comparison of a small region of the F gene (Forsyth & Barrett, 1995) or N gene (Couacy-Hymann *et al.*, 2002). The F and N primer sets typed PPR viruses into four lineages numbered I to IV (Figure 1.4). Lineage typing differs depending on the gene used for comparison. Earlier Nigerian isolates (Taylor & Abegunde, 1979) grouped in lineage II according to the N gene used by Couacy-Hymann *et al.*, (2002) or lineage I according to the F gene used by Forsyth & Barrett (1995).

West Africa has experienced significant outbreaks of PPRV. Recent evidence of PPRV lineage I and II circulation within western Africa from samples from Burkina Faso (2008), Ghana (2010), Nigeria (2007) and Senegal (2010) has been documented by Banyard *et al.* (2010).

PPRV was isolated from samples from Nigeria and Cote d'Ivoire (Adombi *et al.*, 2011). Cases of PPRV in camels and a warthog from a zoo in Nigeria were reported (Shamaki, 2002). Other cases of PPRV in sheep, goat and camel populations have also been described in Nigeria (Ibu *et al.*, 2008; El-Yuguda *et al.*, 2010). Haemagglutination tests were used to test faecal matter for PPRV excretion and it was suggested that healthy animals may serve as carriers for PPRV (Obidike *et al.*, 2006). PPR outbreaks in various states of Nigeria occurred during 2008 and a higher mortality rate was observed in states in the southern parts of the country (Kazeem *et al.*, 2009). Antibody prevalence to PPRV has been reported to be 49.26 % in sheep and 38.34 % in goats (Shamaki, 2002) in some states of Nigeria. Ezeibe *et al.*, (2008) described transmission pattern in Nigeria. Obi (1983), Opasina (1983) and Durojaiye *et al.*, (1983), reported outbreaks of PPR during the dry and cold months of the year and Ojo (1983), also reported outbreaks within 48 hours of rainfall in Ondo state, Nigeria.

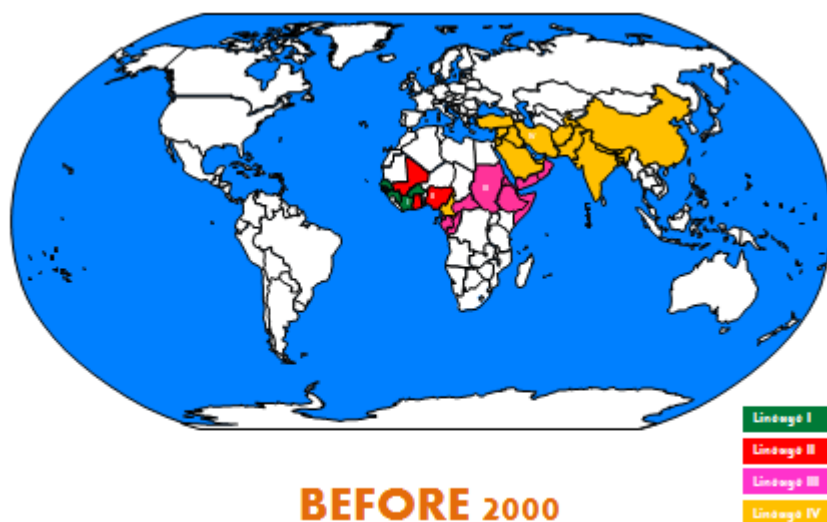


Figure 1.4: Distribution of PPRV lineages before year 2000. Adapted from a presentation by W.G.Dundon (IAEA).

PPRV is endemic across many of the countries in East Africa. Genetic typing of the virus in 1996 determined that the virus circulating in Ethiopia belonged to lineage III (Figure 1.4). Confirmation of the endemicity of PPRV in East Africa was shown through the detection of antibodies to PPRV in Kenya (1999 and 2009), Uganda (2005 and 2007) Sudan (2000), Somalia (2006), and Tanzania (2010) (Osman *et al.*, 2009; Nyamweya *et al.*, 2009; Saeed *et al.*, 2010). Both lineages III and IV are circulating in Sudan. In Tanzania, a high overall PPRV seroprevalence of 39.8 % and 49.5 % was reported in sheep and goats, respectively (Swai *et al.*, 2009; Banyard *et al.*, 2010).

Regular epizootic activities across the Sahara-Sahel belt in Africa resulted in the spread of PPR into new uninfected areas, particularly from 2004 to 2013, as reported to the OIE (OIE, 2013). PPR stretched northwards to Tunisia in 2006 (Ayari-Fakhfakh *et al.*, 2011), Morocco in 2008 (Kwiatek *et al.*, 2011) and Algeria in 2011. At the same period, the disease extended southwards when it was diagnosed in Tanzania in 2008, the Democratic Republic of Congo, Angola and Comoros in 2012 (Libeau *et al.*, 2014) and Zambia in 2015 (OIE, 2015). This also holds true for the Asian continent. PPRV spread from the enzootic areas in the Middle East towards the edge of European Union, with sporadic outbreaks that occurred in both Anatolia (Asian Turkey) and Thrace (European Turkey) in 2004 (Yesilbağ *et al.*, 2005). In southwest Asia, it spread to China (Tibet autonomous region) in 2007 (Bao *et al.*, 2008), the Maldives in 2009, and Bhutan in 2010 (Libeau *et al.*, 2014).

Serological surveillance have identified PPRV in the central African countries of Congo Brazzaville (2006), Central African Republic (1999, 2005, and 2006), Chad (1999 and 2006), Cameroon (2009) (Awa *et al.*, 2000) and Gabon (2007). Phylogenetic analysis has shown that lineage IV viruses are circulating predominantly across Central Africa, although lineage I viruses were detected in the Congo in 2006 (Banyard *et al.*, 2010). Cameroon has an extensive land border with Nigeria, which poses a risk for the introduction of lineage IV PPRV into Nigeria.

PPRV has been detected in North Africa, in Egypt in 1987 and 1990 (Ismail & House, 1990), and in an outbreak in 2006, which was characterized as lineage IV (El-Hakim, 2006). During 2008, local veterinary services reported 257 distinct outbreaks in Morocco's 61 provinces. Despite low mortality and morbidity, these outbreaks were of great significance due to the commercial trade between Morocco and both Algeria and Spain. Genetic characterization of

the Moroccan virus classified it as a lineage IV virus (FAO, 2009). Studies have presented serological evidence for the presence of PPRV in Tunisia (Ayari-Fakhfakh *et al.*, 2011). The virus may be present, but undetected across other regions of North Africa (Banyard *et al.*, 2010).

The Democratic Republic of Congo (DRC), Tanzania and Zambia reported PPR to the OIE in 2005, 2008 and 2015 respectively. In view of the above, PPR remains a significant threat to southern Africa especially countries directly neighbouring DRC and Tanzania (Angola, Malawi and Mozambique). In June 2011, a two day meeting of senior veterinary officials, organised by the Southern Africa Development Community (SADC), was held to deliberate and agree on a common approach to halt and eventually eradicate PPR from the region. The regional strategy agreed upon includes: a comprehensive programme of epidemiological surveillance, establishment of a protection zone along the borders with DRC and Tanzania, development of minimal laboratory capacity in all six states based on OIE-recommended competition-ELISA test, upstream advocacy of politicians and downstream awareness-raising of farmers and rural communities (OIE, 2011). Subsequent regional meetings have been held along this line.

In April 2002, Saudi Arabia reported an outbreak of PPRV with a case fatality rate of 100 % in sheep and goats (Housawi *et al.*, 2004). Further serological surveys and outbreaks have been reported in that country (Abu-Elzein *et al.*, 2004; El-Rahim *et al.*, 2005; Al-Dubaib 2008; 2009). Seroprevalence of both sheep and goats to PPRV has been reported in North Jordan (Al-Majali *et al.*, 2008) and in Lebanon (Attieh, 2007). Lineage IV PPRV was detected in a game reserve in the United Arab Emirates (Kinne *et al.*, 2010). In Qatar, both lineages III and IV were isolated from goats in 2010 and are circulating currently in the country (Figure 1.5). The situation in Qatar is further complicated with the detection of PPRV in wild deer populations. In Yemen and Oman, lineage III virus of PPRV continues to circulate (Banyard *et al.*, 2010).



Figure 1.5: Distribution of PPRV lineages after year 2000. Adapted from a presentation by W.G.Dundon (IAEA).

PPRV belonging to lineage IV has been reported in different parts of Pakistan since 1991 (Amjad *et al.*, 1996; Ahmad *et al.*, 2005; Abubakar *et al.*, 2008; Rashid *et al.*, 2008; Mehmood *et al.*, 2009; Zahur *et al.*, 2009).

PPRV was first reported in India in 1987 (Shaila *et al.*, 1989) and the disease is endemic across much of the country. Molecular characterization of virus isolates from India show that virtually all isolates analysed belong to lineage IV, except for a virus detected in the Tamil Nadu region in 1992 which belonged to lineage III (Banyard *et al.*, 2010).

Outbreaks of PPRV in Iraq dates back to 2000 where a virus causing high morbidity and low mortality rates was characterized (Barhoom *et al.*, 2000). In Iran, PPRV has been circulating for many years, with initial detection in 1995 (Bazarghani *et al.*, 2006; Abdollahpour *et al.*, 2006). More recently, PPRV from Iran has been characterized as lineage IV (Banyard *et al.*, 2010).

PPRV was reported in association with rinderpest virus (RPV) in Tajikistan (Kwiatek *et al.*, 2007). PPRV is also believed to be present in Kazakhstan, although very few seropositive animals have been identified (Lundervold *et al.*, 2004). The detection of PPRV in the Ngari region of western Tibet in China in July 2007 has been documented (Wang *et al.*, 2009). PPRV has also been detected in Bangladesh (2000 and 2009). All recent isolates from Asia and the Middle East belong to lineage IV including the Tibetan isolate (Banyard *et al.*, 2010). Serological detection of PPRV antibodies have been reported in samples from Vietnam

(Maillard *et al.*, 2008) and samples from Bhutan have been typed as lineage IV virus (Banyard *et al.*, 2010).

PPRV was first reported in (European) Turkey in 1996, which highlighted the potential threat of the spread of PPRV to the rest of Europe (Ozkul *et al.*, 2002). Since then, there have been numerous reports of PPRV outbreaks in different parts of Turkey (Toplu, 2004; Yesilbag *et al.*, 2005; Tufan, 2006; Kul *et al.*, 2007; Albayrak & Alkan, 2009). These reports suggested that PPRV is present across much of Turkey and World Reference Laboratories have confirmed Turkish PPRV isolates as belonging to lineage IV. The outbreaks in Morocco and other North African countries have increased the threat of the virus moving into southern Europe. PPRV is currently circulating on the trans-continental edges of the European Union, with recent outbreaks in Morocco and Tunisia in 2008 and antibodies to PPRV reported in an Algerian sheep in 2011 (Banyard *et al.*, 2010; Baron *et al.*, 2011).

Outbreaks of PPR affecting sheep and goats have been reported (Taylor & Abegunde, 1979; Roeder *et al.*, 1994; Taylor *et al.*, 2002; Singh *et al.*, 2004a; Chauhan *et al.*, 2009; Wang *et al.*, 2009). In some outbreaks goats appear not to be affected, while sheep succumb with high rates of mortality and morbidity (Yesilbag *et al.*, 2005). The reason for this variability is unclear, but both virus sequences and host species are thought to be important. PPRV have exhibited varied virulence when the same breed of goat were experimentally infected with the same isolates (Couacy-Hymann *et al.*, 2007) and different breeds of goats have responded differently to infection with the same virus (Diop *et al.*, 2005). Asymptomatic infections are also known to occur in some species (Bidjeh *et al.*, 1995).

The existence of sylvatic reservoirs for PPRV has been reported with infections and deaths in captive wild ungulates (Furley *et al.*, 1987; Ogunsanmi *et al.*, 2003; Abu-Elzein *et al.*, 2004; Kinne *et al.*, 2010). PPRV has also been reported in a warthog kept in a zoological garden, in water buffalo and in diseased camels. Experimentally, a white-tailed deer has been infected. Cattle undergo subclinical infections and pigs develop a viraemia (Govindarajan *et al.*, 1997; Shamaki, 2002; Khalafalla *et al.*, 2010; Kwiatak *et al.*, 2011; Baron *et al.*, 2011). These outbreaks, often described in wildlife collections living in semi free-range conditions, have shown that many species are susceptible to PPRV. The role wildlife species play in the epidemiology of PPRV remains unclear (Banyard *et al.*, 2010).

1.3.2.1. Pathogenesis

PPRV has a direct life cycle, maintained by transmission of the virus from infected to susceptible animals, without involvement of carrier animals or vectors. Risk factors for transmission include extensive husbandry, rapid turnover of small ruminants' populations (host density and birth rate), in addition to the season of the year. There is one serotype of the virus, immunity is long lasting and colostral antibodies protect young animals. Infected animals excrete the virus for at least seven days after the onset of hyperthermia. Infectious oculo-nasal secretions, faeces and urine contaminate the environment. The main route of infection is through the inhalation of contaminated aerosols from the environment and infection of the respiratory tract. Infection can also occur through the oral route, by licking infected material and entry through the oropharynx, subsequent multiplication in the draining lymph nodes, and thereafter in lymph nodes through-out the body. The virus enters the circulatory system and is transported to the epithelium, where it multiplies in susceptible cells, resulting in the development of lesions and clinical signs associated with damage to the target organs (Diallo, 2006).

Morbilliviruses are not only lymphotropic but are also epitheliotropic. During infection, aerosols containing the viruses enter the upper respiratory tract and target dendritic cells and macrophages. The infected cells release the virus to the local lymph nodes where it can infect new populations of T and B cells expressing signalling lymphocyte activation molecule (SLAM). Infected lymphocytes disseminate the virus throughout the host via peripheral blood and lymphatic systems and then PPRV infects the epithelial cells of the respiratory, gastrointestinal, urinary, and endocrine systems via the Nectin-4 epithelial receptor (Barrett *et al.*, 2006). Both SLAM and Nectin-4 receptors are required for the full pathogenesis of the virus. Mortality rates in endemic areas are low, 4 – 5 % while rates as high as 20 – 90 % have been reported under epidemic conditions (Rossiter & Taylor, 1994; Sato *et al.*, 2012; Sawatsky *et al.*, 2012).

1.3.2.2. Evolutionary genetics

Like all RNA viruses, PPRV is genetically variable as result of the inherently high mutation rate associated with RNA-dependent RNA polymerase (Drake & Holland, 1999). This genetic variability is evidenced by the subdivision of PPRV strains into four lineages. A key question is whether geographical distribution and expansion observed for PPRV lineage II and IV may be attributed to some natural selection providing viruses with more epidemiologic potential, or whether they are a result of intensified animal movement and trade. Unlike RPV strains, distinct classes of virulence cannot be described for PPRV strains. To date, the evolution of virulence in PPRV has received little attention so far (Libeau *et al.*, 2014).

1.3.2.3. Economic impact of PPRV

In 2001, an international study for animal diseases prioritization in developing countries, rated PPR in the list of the top ten priority diseases to be considered for poverty alleviation in sub-saharan Africa and South Asia. Control of a disease such as PPR is considered as an essential element in the fight for global food security and poverty alleviation (Perry *et al.*, 2002).

The annual economic cost of the disease in Nigeria was estimated to be USD 1. 5 million in 1976, and a reduction in mortality of 24 % (compared to controls) was reported in Nigeria following a combined PPR vaccination and dipping programme (Hamdy *et al.*, 1976; Reynolds & Francis, 1988). Stem (1993) estimated a USD 24 million return on an investment of USD 2 million in PPR control in Niger over a 5 year period. Awa *et al.*, (2000) indicated a benefit-to-cost ratio of 2.26 to 4.23 for a PPR vaccination and strategic anthelmintic programme in Cameroon. Estimates for the economic cost of PPR in India was thought to be 1800 million Indian Rupees (USD 39 million) per year (Singh *et al.*, 2004a). The annual loss attributed to PPR in Kenya is thought to be in excess of 1 billion Kenya shillings (AU-IBAR, 2013).

Small ruminants' populations at risk of PPR are estimated to be 63 % by the FAO (FAO, 2009). There is a risk of transmission to more than one billion sheep and goats. The huge impact on small ruminant production has resulted in PPRV emerging as an essential global animal health concern. Hence, PPR has been proposed as the next animal disease to be eradicated after rinderpest (FAO, 2013).

1.3.3. Clinical signs

The incubation period of PPR is between two to seven days. Clinical signs include fever (40 – 41 °C), restlessness, depression, rough hair coat, dry muzzle, loss of appetite, profuse salivation, respiratory distress, serous nasal and ocular discharges which becomes mucopurulent with a fetid odour, congested conjunctiva and matting of the eyelids, necrosis and inflammation of the lower lip and gum, coughing due to bronchopneumonia, profuse diarrhoea leading to dehydration and loss of weight. In the terminal stage, animals become hypothermic and death occurs between 3 – 20 days, depending on the severity of the signs, the immune status of the host and the presence or absence of a secondary bacterial infection. Post mortem lesions include an emaciated carcass, inflamed conjunctiva, necrosis

and erosive lesions in buccal cavity and pharynx, abomasums, small intestine, large intestine and rectum. The organs may be congested and haemorrhagic. Congestive lesions are also seen in the respiratory tract, which may show lesions of bronchopneumonia. Lesions may have a “zebra stripes” appearance in the colon. The trachea, lungs and larynx may be inflamed. Leukopaenia, eosinopaenia and an increase in packed cell volume has been reported. Staining may reveal giant cells with eosinophilic intracytoplasmic and sometimes intranuclear inclusion bodies (Obi *et al.*, 1988; Rossiter & Taylor 1994; Ibu, 2005).

PPRV has been identified in the brain even though no neurological disorders have yet been linked to this virus in small ruminants (Toplu, 2004; Toplu *et al.*, 2012).

1.3.4. Diagnosis

PPR have been misdiagnosed in the past as pasteurellosis (due to the frequent bronchopneumonia) or rinderpest (due to the clinical resemblance). However, tests are now available (from the late 1980s) for the routine diagnosis of PPR. The virus causes a profound immunosuppression that leads to the development of secondary infections which most times causes death. Virulence of PPRV infection appears to vary from strain to strain. Although there is only one serotype, the disease symptoms are often confused with, and exacerbated by, secondary infections making PPR a difficult disease to characterize, diagnose and treat, with differential diagnoses including pasteurellosis, contagious ecthyma, contagious caprine pleuropneumonia (CCPP), bluetongue, heartwater, coccidiosis, mineral poisoning and foot-and mouth disease (Couacy-Hymann *et al.*, 2005). Samples required for laboratory diagnosis of PPR include: whole blood in heparin or EDTA, serum, lymph nodes, tonsils, spleen, lung and swabs of secretions (Rossiter & Taylor, 1994).

PPRV induces a transient but severe immunosuppression, which favours secondary opportunistic bacterial and parasitic infections (Obi *et al.*, 1983; Ugochukwu & Agwu, 1991).

Clinical diagnosis of PPR is confirmed by various methods. The most commonly used are: counterimmunoelectrophoresis (CIEP), agar gel immunodiffusion (AGID), indirect or passive haemagglutination, immunofluorescence or immunoperoxidase staining, single radial haemolysis, immunocapture ELISA, competitive ELISA (c-ELISA), latex agglutination, virus neutralisation (VN), complement-fixation, and virus isolation in cell cultures. (Libeau *et al.*, 1994; 1995; Ismail *et al.*, 1995; Diallo *et al.*, 1995; FAO, 1996; Dhinakar Raj *et al.*, 2000; 2008; Choi *et al.*, 2005; Saravanan *et al.*, 2008; Ezeibe *et al.*, 2008; Keerti *et al.*, 2009). A

genetically engineered cell line expressing goat SLAM that facilitates the isolation of PPRV has been reported recently (Adombi *et al.*, 2011).

For molecular detection of PPRV, conventional RT-PCR tests (Forsyth & Barrett, 1995; Couacy-Hymann *et al.*, 2002) are available in addition to non-radioactive probes using either biotinylated DNA (Pandey *et al.*, 1992) or digoxigenin-labelled oligonucleotides (Diallo *et al.*, 1995), real-time RT-PCR assays (Bao *et al.*, 2008; Kwiatak *et al.*, 2010) and loop-mediated isothermal amplification techniques (Wei *et al.*, 2009) have also been developed. However, the gold standard for definitive diagnosis is virus isolation from tissue samples.

The OIE recommends the use of a competitive PPRV-specific anti-H monoclonal based ELISA (Anderson & McKay, 1994) and virus neutralization tests (FAO, 1996). The virus neutralisation test is time consuming (requiring 10 to 12 days for a result), requires tissue culture and sterile test samples; is labour intensive and of low throughput. Meanwhile, the competitive ELISA for PPR has a sensitivity of 94.5 % and a specificity of 99.4 % (Libeau *et al.*, 1992; 1995; Saliki *et al.*, 1993; Anderson & McKay, 1994). The ease with which ELISA can be performed, the large number of samples that can be tested in a short time and the high sensitivity of the test, have all contributed to its adoption as a routine technique in disease diagnosis.

1.3.5. Control

In endemic areas PPR is controlled through administration of a live-attenuated PPRV vaccine such as the Nigeria 75/1 strain (Diallo, 2003). In India three live-attenuated vaccines are currently licensed for use: Sungri 96, Arasur 87 and Coimbatore 97 (Saravanan *et al.*, 2010). Historically, live-attenuated rinderpest vaccines were also used to protect small ruminants against PPRV due to the antigenic cross-reactivity between the two viruses (Taylor, 1979). However, use of the latter was halted to avoid false-positive detection of RPV during the final stages of the eradication campaign and to enable a country to seek OIE recognition for freedom from rinderpest (FAO, 2007). Several studies have involved the development of a new generation of marker vaccines that will enable the differentiation of infected from vaccinated animals (Mahapatra *et al.*, 2006). Vaccines also exist based on the incorporation of PPRV immunogens into vectors such as sheep and goat pox (Diallo *et al.*, 2002; Berhe *et al.*, 2003; Chaudhary *et al.*, 2009; Chen *et al.*, 2010,) and attempts are also being made to develop new vaccines based on recombinant DNA technology (Diallo *et al.*, 2007). However, all these efforts are still awaiting OIE approvals or where tenable, not commercially

available due to disinterest from companies to buy the patent as a result of the limited market. The vaccine in current use in Nigeria is thermolabile, and it is necessary to maintain it in an effective cold chain (Berhe *et al.*, 2003), a condition that is sometimes difficult to achieve in many of the rural areas where the majority of susceptible animals are kept. It is envisaged that this vaccine could be improved upon by using the Xerovac technology (Worrwal *et al.*, 2001) to increase its thermostability.

The most effective control and or eradication of PPRV are the stamping out policy in addition to movement restriction. These eradication measures may be very difficult to implement in countries like Nigeria, where PPR is endemic. There is at present no international programme for PPR control; the situation differs in each country according to the level of state subsidy, if any, for disease control programmes and the degree of emphasis on the private sector to undertake vaccination under a demand and supply system (AU-IBAR, 2013).

1.4. Purpose of the project

To increase small ruminant production in Nigeria through reduced mortality due to better disease control by continued serosurveillance and mass vaccinations, isolation and molecular characterization of PPRV isolates.

1.5. Specific research aims

- Characterize current field PPRV from Nigeria using molecular techniques and thus compare the profile of the current isolates with existing strains from other parts of the world (available in GenBank).
- Isolate PPRV from naturally infected animals and to sequence the full genome of representative current viral isolates from Nigeria and compare them with historical isolates being used worldwide as vaccine strain (Nigeria75/1).
- Serosurvey PPRV in sheep and goats in Nigeria; continued surveillance is important for effective control of the disease.
- Serosurvey PPRV in camels in Nigeria; PPR antibody status of camels and other species that share grazing land and drinking points with small ruminants will help determine their role in the epidemiology of the disease.

CHAPTER

2. MOLECULAR PHYLOGENY OF CURRENT PPRV OUTBREAKS IN NIGERIA¹

2.1. Introduction

Peste des petits ruminants (PPR) is a serious infectious disease of sheep and goats. It is endemic in large parts of Africa (from North Africa to Tanzania), the Middle East, Turkey, Iran and many countries in Asia (Libeau *et al.*, 2014). The disease is characterized by pyrexia, depression, anorexia, diarrhoea, respiratory distress, mucopurulent oculo-nasal discharges with matting of the eyelids, necrotic oral lesions with a fetid smell and sometimes abortion in pregnant animals. In Nigeria most outbreaks occur between February and May, the dry harmattan season and also between July and September, mostly in the south-eastern and south-western parts of the country. This disease is caused by a virus, the peste-des-petits ruminants virus (PPRV), which belongs to the genus *Morbillivirus* within the family *Paramyxoviridae*, along with rinderpest virus, measles virus, canine distemper virus, marine mammals and feline morbilliviruses (Gibbs *et al.*, 1979; Osterhaus *et al.*, 1995; Woo *et al.*, 2012). They are enveloped viruses with a genome composed of a non-segmented single-stranded RNA of negative sense. The genome of PPRV is 15 948 nucleotides in size and encodes for two non-structural proteins C and V, and six structural proteins arranged in the order: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin protein (H) and viral RNA-dependent polymerase (L) (Diallo 2003; Bailey *et al.*, 2005; Truong *et al.*, 2014). Based on partial sequences of the N and F genes, PPRV has been classified into four genetically distinct lineages (I, II, III and IV) even though the virus is serologically monotypic (Banyard *et al.*, 2010; Kwiatak *et al.*, 2011). Viruses that were first identified in Africa belong to lineages I, II and III while viruses belonging to lineage IV, also referred to as the Asian lineage, have been isolated in many Asian countries including Iran, Turkey and the Middle East (Banyard *et al.*, 2010; Kwiatak *et al.*, 2011). However, over the last one to two decades, there have been reports of the presence of the Asian lineage of PPRV in African countries such as Sudan, Morocco, Egypt, Algeria, Uganda, Gabon, Cameroon and Central African Republic (Banyard *et al.*, 2010; Kwiatak *et al.*, 2011; Sharawi & Abd-El-Rahim, 2011; De Nardi *et al.*, 2012; Luka *et al.*, 2012; Magana *et al.*, 2013).

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PPRV identified in Nigeria from the 1970s up until 2000 all belonged to lineage II (Shamaki, 2002; Kwiatek *et al.*, 2007; Banyard *et al.*, 2010). However, following reports of the circulation of lineage IV in Cameroon and the Central African Republic, it has become necessary to re-assess the epidemiological situation of PPRV in Nigeria especially in states at the border with neighbouring countries. Indeed, Cameroon has a long porous border with Nigeria and many inhabitants of north eastern Nigeria (Taraba, Adamawa, Borno States) have families in that country, thus facilitating animal trade and smuggling and taking advantage of the limited control over animal movements at the border.

2.2. Materials and methods

2.2.1. Samples

A total of 140 clinical samples consisting of spleen, trachea, lung, liver and lymph nodes were collected between 2010 – 2013 from 16 sheep and 63 goats with symptoms suggestive of PPRV infection. These animals were from different states representing the six different agro-ecological zones of Nigeria. Samples were transported on ice to the laboratory at the National Veterinary Institute in Vom where they were stored at -80 °C until shipment with dry ice to Vienna, Austria, for further analysis at the FAO/IAEA Animal Production and Health Laboratory. The location of each sampling was recorded using the GPSMAP® 76 versatile navigator (Garmin).

2.2.2. RNA extraction and RT-PCR

A 10 % w/v homogenate was made by grinding tissue with sterile glass in DMEM medium and the suspension was then clarified at 1200 x *g* for 5 min. Total RNA was extracted from the sample supernatant using a Qiagen RNeasy Plus RNA extraction kit (Qiagen, Germany) according to the manufacturers' instructions. Reverse transcription PCR (RT-PCR) was performed with 2 µl extracted RNA using the Qiagen® one step RT-PCR kit (Qiagen, Germany) to amplify a 351 bp segment of the PPRV N gene with the NP3/NP4 diagnostic primers (Couacy-Hymann *et al.*, 2002). The F1/F2 primers (Forsyth & Barrett, 1995) were used to amplify a 370 bp of the F gene segment using the same one step RT-PCR kit from samples that were positive for the N gene segment amplification. PCR products were resolved on a 1.2 % agarose gel stained with ethidium bromide to reveal the expected band size.

2.2.3. Sequencing and phylogenetic analysis

Amplified products were purified for sequencing using the Wizard® SV gel and PCR clean-up system (Promega®, USA) according to the manufacturer's instructions. The purified DNA was quantified by spectrophotometer (NanoDrop® ND-1000, PeQlab Biotech GmbH). Sequencing was carried out in both forward and reverse directions by LGC Genomics (Germany).

The nucleotide sequences of the N and F gene amplicons were aligned using the Molecular Evolutionary Genetics Analyses (MEGA) software version 5 (Tamura *et al.*, 2011). Phylogenetic trees were constructed by comparisons with unique published sequences available (i.e. in peer review journals) in GenBank. TIM+G and K80+I substitution models were determined by Modeltest v3.7 (Posada & Crandall, 1998) for the N and F genes respectively. Distance (neighbour-joining) and character (Bayesian, maximum parsimony) based phylogenies of the nucleotide sequences were explored using PAUP* 4.0b10 (Farris *et al.*, 1994) and Mr Bayes v3.2.2 (Ronquist & Huelsenbeck, 2003). The reliability of each tree was estimated using 1,000 bootstrap replicates and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Felsenstein, 1985). Character congruence between the N and F genes of PPRV was tested using the partition homogeneity test in PAUP* 4.0b10 (Farris *et al.*, 1994). Sequences have been deposited in GenBank under accession numbers KF479408 – KF479444, KJ124726 – KJ124773 for the N gene and KJ124774 – KJ124805 for the F gene.

2.3. Results

During this study, we observed that most of the outbreaks of PPR occurred during February to May, which is part of the dry harmattan season, in northern states and July to September, part of the rainy season, in southern states. Most of the affected animals were between 3 and 12 months of age. We also observed that more goats than sheep were affected in areas where they grazed together and shared the same drinking sources.

A total of 140 samples from 79 animals were tested by RT-PCR. Samples for 33 animals were positive, i.e. 2 out of 16 sheep (13 %) and 31 out of 63 goats (49 %). The details of the positive samples are summarized in Table 2.1.

Table 2.1: Details of animals positive for PPRV by RT-PCR in Nigeria.

State	Location	Species	Breed	Age*	Anamnesis	Collection date	Sample type	Sample name	Sub-Clade
Adamawa	Njobli	Ca	WAD	7	Diarrhoea, matted eyelids, oral lesions, nasal discharges, anorexia	12.04.2010	Lung	NGAD2010-NJ13LN	IV-NigB
Anambra	Adazi-Ani	Ca	WAD	6	Cough, fever, anorexia, raised hair coat, diarrhoea	21.09.2011	Lung	NGAN2011-2151LG	IV-NigB
Cross River	Akpet Central	Ca	WAD	4	Oculo-nasal discharges, oral lesions, diarrhoea	23.09.2011	Liver	NGCR2011-1807	IV-NigB
Cross River	Ikot-Omin	Ca	WAD	4	Oculo-nasal discharges, oral lesions, diarrhoea	23.09.2011	Liver	NGCR2011-1806	IV-NigB
Cross River	Mariam	Ca	WAD	4	Oculo-nasal discharges, oral lesions, diarrhoea	23.09.2011	Liver	NGCR2011-1808	IV-NigB
Cross River	Biase	Ca	WAD	4	Oculo-nasal discharges, oral lesions, diarrhoea	23.09.2011	Liver	NGCR2011-1810	IV-NigB
Cross River	Ikot-Eneobong	Ca	WAD	4	Oculo-nasal discharges, oral lesions, diarrhoea	23.09.2011	Liver	NGCR2011-1811	IV-NigB
Anambra	Umuchi	Ca	WAD	7	Cough, fever, anorexia, raised hair coat, diarrhoea	23.09.2011	Lymph node	NGAN2011-2154	IV-NigB
Anambra	Amada	Ca	WAD	5	Oculo-nasal discharges, oral lesions, diarrhoea	22.09.2011	Liver	NGAN2011-2152	IV-NigB
Osun	Iregba	Ca	WAD	4	Blood tinged diarrhoea, pneumonia, vesicular stomatitis	11.09.2011	Lung	NGOS2011-110GYM14	II-NigB
Ogun	Ijebu-Ode	Ca	WAD	9	Oral lesions, diarrhoea, cough,	29.09.2011	Lung	NGOG2011-31GYM	II-NigA
Oyo	Bodija	Ca	WAD	11	Soiled hindquarters, oral lesions with putrid smell, anorexia, sneezing	29.09.2011	Kidney	NGOY2011-127GYM31	II-NigB
Adamawa	Gulak	Ca	WAD	6	Raised hair coat, depression, diarrhoea	12.04.2012	Lymph node	NGAD2012-2167	IV-NigA
Taraba	Kassa	Ov	WAL	6	Fever, emaciated, pneumonia, anorexia,	27.03.2012	Liver	NGTA2012-17LV	IV-NigA

State	Location	Species	Breed	Age*	Anamnesis	Collection date	Sample type	Sample name	Sub-Clade
Taraba	Jalingo	Ca	WAD	15	diarrhoea				
Taraba	Wukari	Ca	WAD	11	Weak, depressed, fever, cough, anorexia	01.04.2012	Spleen	NGTA2012-367	IV-NigA
					Oculo-nasal discharges, oral lesions, diarrhoea, fever	30.03.2012	Lymph node	NGTA2013-23	IV-NigA
Taraba	Maihula	Ov	WAL	5	emaciated, pneumonia, diarrhoea, fever, matted eyelids	17.02.2012	Lung	NGTA2012-250A	IV-NigA
Taraba	Garbabi	Ca	WAD	6	Matted eyelids, emaciated, pneumonia, diarrhoea	31.03.2012	Lung	NGTA2012-253	IV-NigA
Taraba	Kassa	Ca	WAL	17	Oral lesions, matted eyelids, diarrhoea	29.03.2012	Lymph node	NGTA2012-355	IV-NigA
Kano	Kano Municipal Council	Ca	WAL	12	Pneumonia, depressed, fever, oral lesions	15.04.2012	Lung	NGKA2012-2159	IV-NigA
Kano	Dogongora	Ca	WAL	5	Anorexia, emaciated, diarrhoea, weakness	19.04.2012	Lymph node	NGKA2012-2160	IV-NigA
Ondo	Akure	Ca	WAD	14	Diarrhoea, cough, oral lesions, fever, pneumonia	27.04.2012	Liver	NGON2012-832LV	IV-NigA
Ondo	Idanre	Ca	WAD	7	Diarrhoea, cough, oral lesions, fever, pneumonia	27.04.2012	Lung	NGON2012-832KD	IV-NigA
Kwara	Baruten	Ca	WAD	5	Emaciation, diarrhoea, vesicles on feet, weakness	09.05.2012	Lymph node	NGKW2012-1111MSLN	II-NigA
Kwara	Illorin	Ca	WAD	5	Emaciation, diarrhoea, vesicles on feet, weakness	09.05.2012	Lung	NGKW2012-LG	II-NigA
Imo	Eziama-Obaire	Ca	WAD	13	Cough, fever, anorexia, raised hair coat, diarrhoea	16.07.2012	Liver	NGIM2012-2149LV	IV-NigB
Imo	Iho	Ca	WAD	4	Oculo-nasal discharges, oral lesions, diarrhoea	18.07.2012	Lung	NGIM2012-2150LG	IV-NigB
Yobe	Yusufari	Ca	WAL	9	Raised hair coat, weak, depressed, fever, anorexia	15.02.2013	Lymph node	NGYO2013-2161	IV-NigA
Yobe	Yusufari	Ca	WAL	10	Raised hair coat, weak, depressed, fever, anorexia	15.02.2013	Lung	NGYO2012-2162	IV-NigA
Plateau	Angwa Kurma	Ca	WAD	6	Oral lesions, diarrhoea, cough,	19.03.2013	Lung	NGPL2013-2170	IV-NigB

State	Location	Species	Breed	Age*	Anamnesis	Collection date	Sample type	Sample name	Sub-Clade
Plateau	Jos	Ca	WAD	8	emaciation Matted eyelids, oculo-nasal discharges, depression, soiled vent	19.03.2013	Liver	NGPL2013-2171	IV-NigB
Akwa Ibom	Uyo	Ca	WAD	14	Matted eyelids, oculo-nasal discharges, depression, soiled vent, emaciated	03.05.2013	Lymph node	NGAK2013-2205	II-NigB
Sokoto	Sokoto	Ca	WAD	14	Matted eyelids, oculo-nasal discharges, depression, soiled vent, emaciated	21.05.2013	Lymph node	NGSO2013-2201	II-NigB

* Age in months, Ca - Caprine, Ov - Ovine, WAD - West African Dwarf, WAL - West African Long-leg.

The amplified products were sequenced and the data were analysed together with PPRV N and F gene sequences available in GenBank (Kwiatek *et al.*, 2007, 2011; Banyard *et al.*, 2010). Three phylogenetic analysis methods were used and all generated trees with similar topology. The phylogeny inferred with the distance methods was consistent with those of the character based analysis. Based on the partial N gene sequence, most ($n = 26$) of the PPRV strains collected in Nigeria between 2010 and 2013, unexpectedly, clustered in lineage IV and only a few ($n = 7$) belonged to lineage II (Figure 2.1). Lineage IV isolates from Nigeria grouped into two clades (i.e. IV-NigA and IV-NigB) supported by bootstrap value of 90 %. Samples from the two positive sheep belonged to IV-NigA. Samples in IV-NigA were all collected between February and April 2012 and February 2013. PPRV lineage IV-NigA was distributed across the entire north eastern part of the country and present only in one southern state (e.g. Ondo) (Figure 2.2). In contrast, in the north east of the country, only the Plateau and Adamawa states were infected with viruses of the second lineage IV clade (IV-NigB) (Figure 2.2). The majority of the lineage IV-NigB viruses were present in three southern states: Cross River, Imo and Anambra identified in samples from goats collected in April 2010, September 2011, July 2012 and March 2013. Lineage IV-NigB viruses showed 94 % identity with IV-NigA and were most closely related to viruses identified in Cameroon and Sudan in 1997 and 2008, respectively.

Only seven sequences from the present study were from lineage II viruses similar to earlier isolates from Nigeria collected in the 1970s and 1990s (Kwiatek *et al.*, 2007; Shamaki, 2002) and were collected from goats in September 2011, May 2012 and May 2013. The lineage II sequences from this study also formed two separate clades, II-NigA and II-NigB (Figure 2.1). Lineage II-NigA samples had a high identity with PPRV strains identified in Mali obtained in 1999 (DQ840198), while those in lineage II-NigB showed 98 % identity with the Nigerian 1975/1 virus (DQ840160) that has been attenuated and is commonly used as a vaccine (Diallo *et al.*, 1989; Kwiatek *et al.*, 2007).

The partial F gene based phylogenetic tree (Figure 2.3) also grouped current Nigerian isolates into lineage IV (with 2 separate clades) and lineage II and is similar to the N-based phylogenetic tree. However, fewer F gene sequences are currently available in the GenBank database compared to the N gene. The partition homogeneity test in PAUP* gave a character congruence p-value of 0.0001 suggesting incongruence between the N and the F genes of PPRV. A combined N and F gene phylogenetic analysis was therefore not performed.

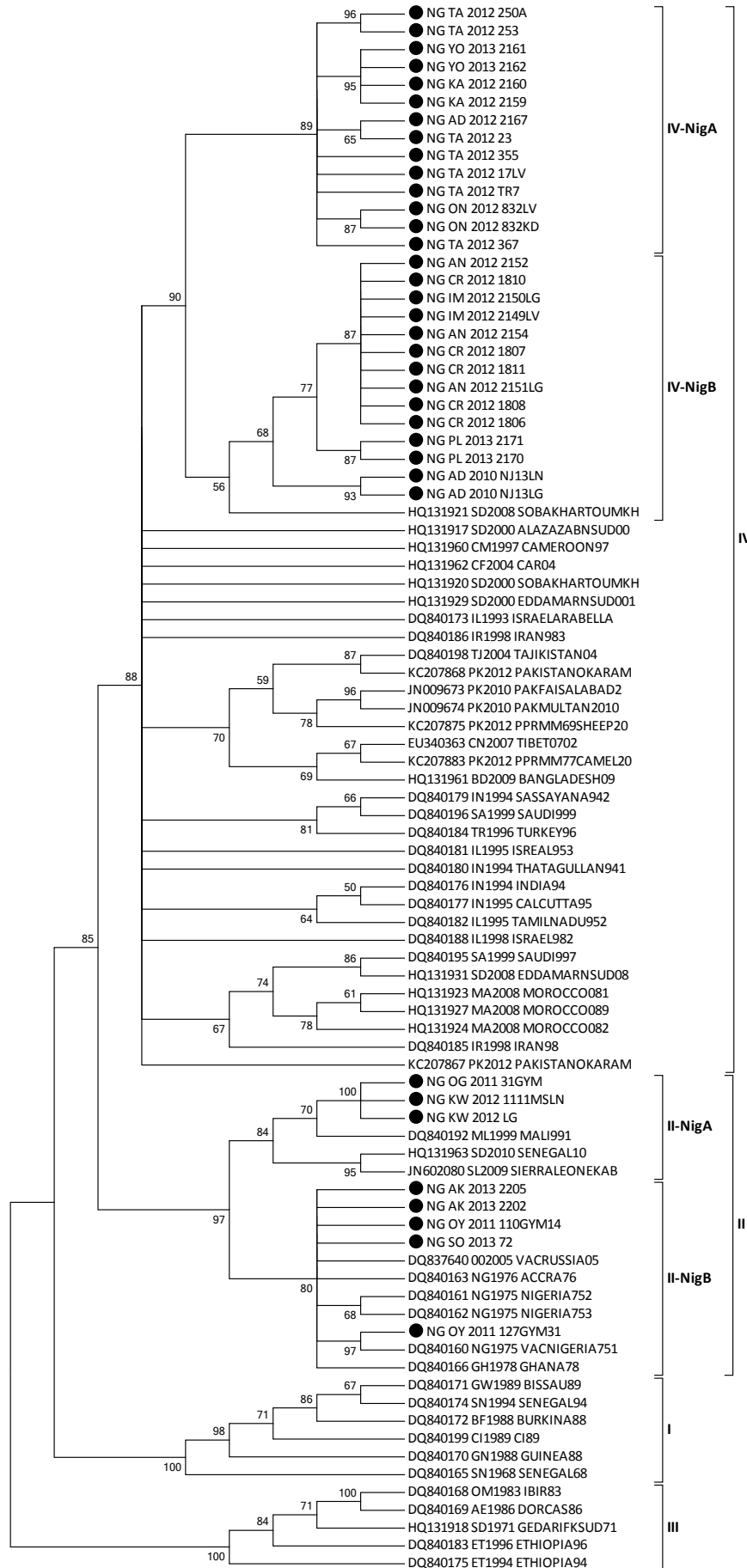


Figure 2.1: PPRV N gene phylogenetic analysis. Neighbor-joining unrooted cladogram showing the relationship between the N gene sequences from this study (indicated by black circles, ISO 3166 country code and state code, year of sample collection and sample laboratory number) with unique published sequences obtained from GenBank (indicated by accession number, ISO 3166 country code, year of isolation and name of isolate). The numbers at the nodes are bootstrap values obtained from 1000 re-samplings.

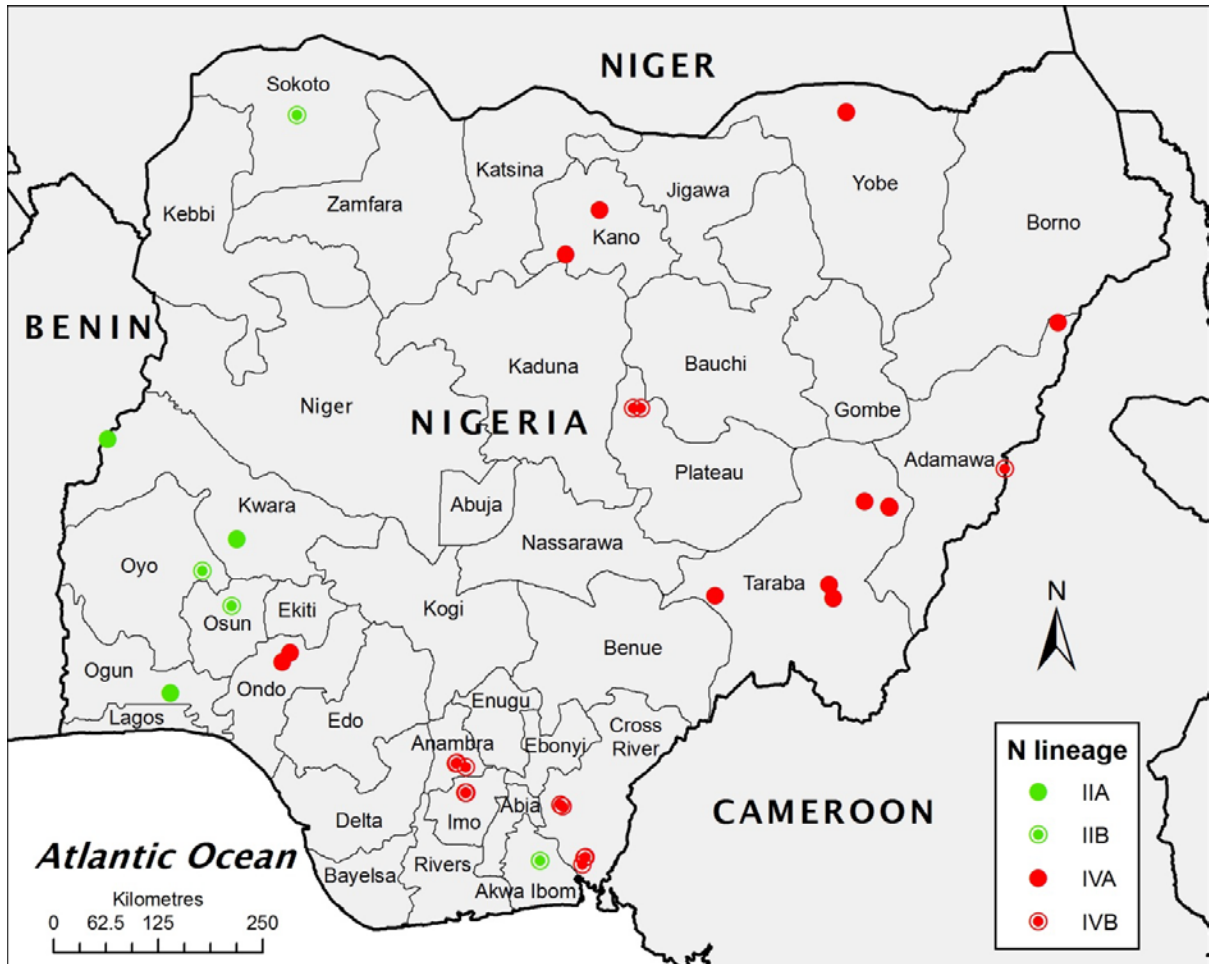


Figure 2.2: The distribution of PPRV lineage II (green circles) and IV (red circles) in different states of Nigeria. The map shows the location of villages where positive PPR samples were collected (see Table 2.1).

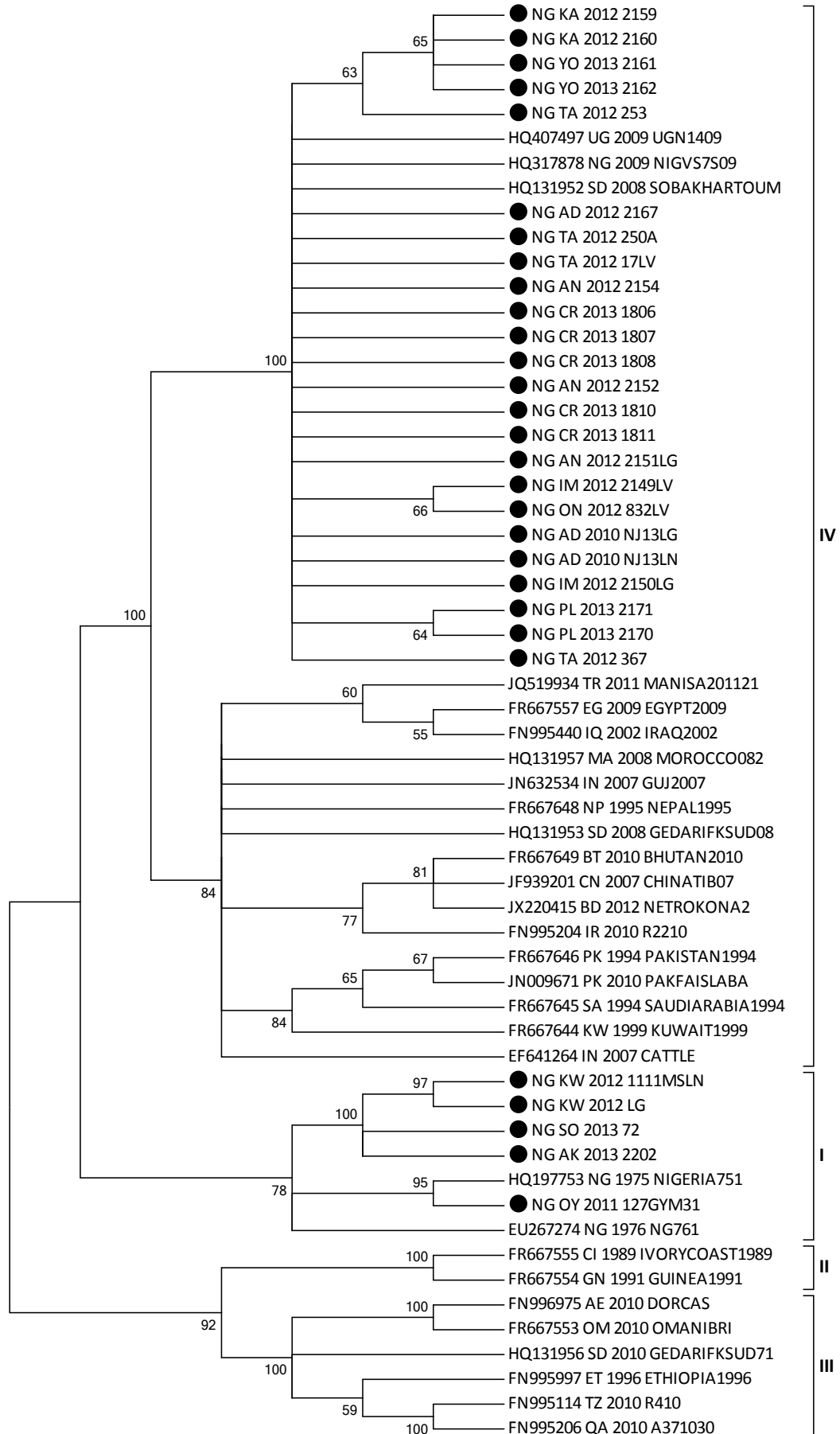


Figure 2.3: PPRV F gene phylogenetic analysis. Neighbour-joining unrooted cladogram showing the relationship between the F gene sequences from this study (indicated by black circles, ISO 3166 country code and state code, year of sample collection and sample laboratory number) with unique published sequences obtained from GenBank (indicated by accession number, ISO 3166 country code, year of isolation and name of isolate). The numbers at the nodes are bootstrap values obtained from 1000 re-samplings.

2.4. Discussion

The findings of this study relating to PPR surveillance in Nigeria over a four year period (2010 – 2013) have confirmed the widespread distribution of PPRV throughout Nigeria, as has been described since the early 1970s (Isoun & Mann, 1972) and also revealed the presence of PPRV lineage IV in the country for the first time.

Analysis of the sequence data obtained from the samples showed the presence of viruses belonging to two lineages, namely, PPRV Asian lineage IV and PPRV lineage II, the so-called “indigenous” lineage given that it is the lineage to which PPR viruses isolated in Nigeria between 1975-1976 and also in the late 1990’s (Shamaki, 2002; Kwiatak *et al.*, 2007) belong. Surprisingly, the number of samples that belonged to this “indigenous” group was small compared to those of the “emerging” lineage IV. Some earlier Nigerian samples collected between 2008 and 2009 were also previously classified as lineage IV (A. Diallo, unpublished data). The Nigerian lineage IV viruses from this study formed two distinct clades, IV-NigA and IV-NigB which were clearly distinct from other lineage IV viruses except for the sequence of a Sudanese isolate (HQ131921) (Figure 2.1). Of particular interest are the samples collected in Adamawa state in April 2010. This state borders Cameroon and in 1997 lineage IV virus (HQ131960, Figure 2.1) was reported (Kwiatak *et al.*, 2011). Therefore it is tempting to think that viruses of lineage IV-NigB were first introduced into Nigeria from Cameroon and then evolved into lineage IV-NigA viruses due to “genetic drift” following in-country transmission. Similar processes have been suggested for measles viruses by different authors (Santibanez *et al.*, 2002; Alla *et al.*, 2006; Waku-Kouomou *et al.*, 2010). Further in-depth genetic analysis using next generation sequencing is planned in order to confirm this hypothesis. The presence of a lineage IV-NigA virus (NG_ON_2012_832LV) in Ondo State, a region where lineage II and lineage IV-NigB viruses are predominant may be the result of an importation of infected animals from Northern Nigeria. A lineage II PPRV (NG_AK_2013_2202) was identified in Akwa Ibom state in the far south of the country which is a region where lineage IV-NigB viruses predominate. This may be also as a result of

importation from states bordering Benin where PPRV lineage II is predominant e.g. Ogun, Osun, Oyo and Kwara States.

The lineage II isolates subdivided into two distinct clades. One clade was closely related to the Nigeria 75/1 vaccine strain while the second clade was closely related to viruses found in Mali, Senegal and Sierra Leone. At present, there is no information on the PPRV lineages currently circulating in Niger so the isolates collected in Yobe and Sokoto states are of particular interest. These two states have borders with Niger and while the sample collected in Sokoto (NG_SO_2013_72) was identified as belonging to lineage II-NigB the samples collected in Yobe (NG_YO_2013_2161/2162) were of lineage IV-NigB.

This study has shown that Nigerian sheep and goats are being infected by viruses of two PPRV lineages: lineage II, the “indigenous lineage”, which is being replaced by the Asian lineage. Kwirotek *et al.*, (2011) reported a similar situation in Sudan where lineage IV PPRV is now dominant and has replaced lineage III PPRV that was present in the country until 2000. The authors suggested that the lineage IV viruses which were present historically in Asia and parts of the Middle East may have become more virulent and so have spread more easily to Africa. It is possible that these more virulent lineage IV PPRV strains have a selective advantage over lineage III viruses in the case of Sudan and lineage II viruses in the case of Nigeria. This view is supported by the remarkably rapid spread of the lineage IV viruses over the north-eastern part of Nigeria bordering Cameroon. We have yet to understand whether this change in PPRV lineage distribution in Nigeria has any relationship to pathogenicity or is just a result of geographical speciation. A more widespread analysis of PPRV in other African countries would help to understand the geographic extent of the spread of the Asian lineage within the continent.

CHAPTER

3. ISOLATION AND FULL GENOME SEQUENCES OF TWO CO-CIRCULATING LINEAGES OF PPRV IN NIGERIA²

3.1. Introduction

PPRV strains have been classified into four genetic lineages based on the comparison of a sequence fragment for the N (351 bp) and/or F (370 bp) genes. Serologically, only one serotype of PPRV exists. Until very recently, lineage I and II isolates were confined to Western Africa and lineage III to Eastern Africa, Yemen and Oman. Lineage IV is the predominant PPRV lineage globally and recent reports have now indicated the spread of lineage IV viruses in the African continent (Banyard *et al.*, 2010; Dundon *et al.*, 2014; Woma *et al.*, 2015).

Viral genomes display a higher mutation rate than cellular organisms, because RNA viruses such as PPRV are replicated by RNA-dependent RNA polymerase that lack proofreading ability, they have the highest mutation rates per base pair. The mutation rates proposed for double stranded DNA viruses is 10^{-8} ; single stranded DNA viruses is $10^{-7} - 10^{-6}$; double stranded RNA viruses is 10^{-5} while single stranded RNA viruses like PPRV is 10^{-4} (Barrett *et al.*, 2006).

The numbers of full genome sequences of PPRV available in public databases have increased within the last two years. It is evident that more complete genome sequence data need to be generated in order to understand completely the evolution and transboundary spread of this important virus, and to assist in reverse genetics studies. Here we present the full genome sequences of two field strains of PPRV representing two different lineages co-circulating currently in Nigeria, the NGYO2013-2161 isolate (lineage IV) and NGKW2012-1111MSLN (lineage II).

3.2. Materials and methods

² Sequencing was done at the Animal Production and Health Laboratory of the International Atomic Energy Agency (IAEA) in Siebersdorf, Austria.

Two isolates suitable for whole genome amplification were identified from a bank of tissue samples from 79 animals that died with clinical signs compatible with PPR outbreaks in different states of Nigeria. The samples were collected between January 2010 and May 2013. The samples were processed as reported earlier (Woma *et al.*, 2015). The partial N gene based phylogenetic tree grouped current Nigerian isolates into lineage II and IV. KR828814 NG2012 (NGKW2012-1111MSLN) (LINII), a lineage II and KR828813 NG2013 (NGYO2013-2161) (LINIV), a lineage IV PPRV were selected for virus isolation and full genome sequencing. The two viruses were selected because of their geographical origin neighbouring Benin Republic (lineage II) and Niger (lineage IV).

LINII was obtained from the mesenteric lymph node of a female West African dwarf goat that died of classical signs of PPR (emaciated carcass, soiled anal region due to profuse diarrhoea, vesicles on the feet and matted eyelids) on the 9th May 2012, in Baruten (9.557222 °N, 3.458611 °E), Kosubosu Local Government Area of Kwara state that borders Benin Republic. LINIV was obtained from the lung tissue of a male West African long legged goat that died on the 15th February 2013, after showing signs of raised hair coat, fever, anorexia and oculo-nasal discharges in Yusufari town (13.087920 °N, 11.211080 °E) of Yobe state, north eastern Nigeria bordering Niger Republic (Figure 3.1).



Figure 3.1: Map of Nigeria showing the locations of where LINII and LINIV isolates were collected.

Homogenates were made of the lung and mesenteric lymph node tissues by grinding them together with sterile quartz in a mortar and pestle to give a 10 % (w/v) suspension in serum-free DMEM media. The suspensions were clarified by centrifugation at $1,200 \times g$ for 5 min. For virus isolation, 0.5 ml of the sample homogenate was used to infect CHS-20 cells, a monkey CV1 cell line expressing the sheep/goat SLAM receptor for PPRV (Adombi *et al.*, 2011) and grown as a cell monolayer at 80 % of confluence in a 25 cm^2 tissue culture flasks. The homogenate was allowed to adsorb for one hour at 37°C with shaking of the flasks every 15 min for one hour. Five ml of cell growth medium supplemented with 2 % antibiotics was added to the flask and replaced daily. The cells were examined daily by microscopic observation for the detection of cytopathic effect.

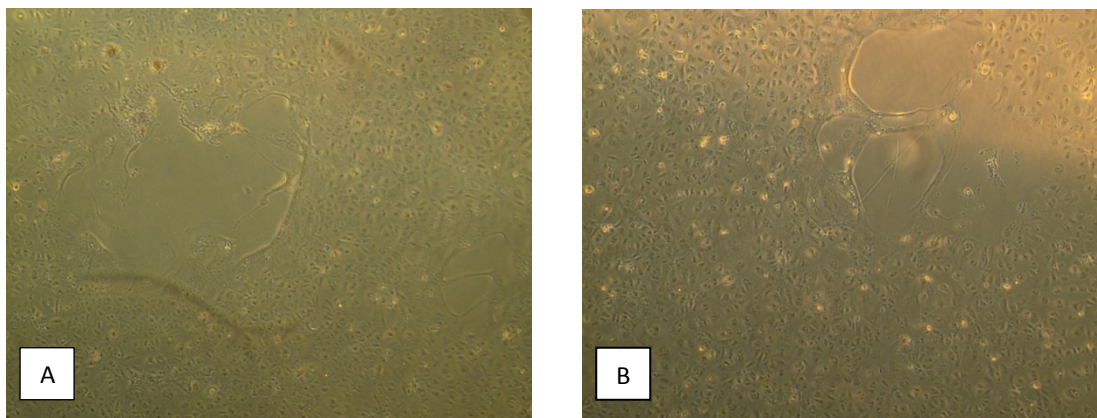
To sequence the full genome of the selected samples, total RNA was extracted from the sample supernatant using a Qiagen RNeasy Plus RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RT-PCR reactions were performed using the OneStep RT-PCR kit (Qiagen) and 30 primer pairs, as described by Dundon *et al.*

(2014). PCR amplicons were purified directly using the Wizard® SV gel and PCR Clean-Up System (Promega) and sent for sequencing using standard Sanger methods at LGC genomics. The full genomes of the two isolates were sequenced twice from two separate RNA extractions resulting in a four-fold coverage per nucleotide. Genome assembly was performed using the Staden package (Staden *et al.*, 1998). Contiguous assembly of the sequence data was accomplished in the Gap4 programme using the Phred assembly algorithm. The full genome sequence of LINII and LINIV are available from GenBank under accession numbers KR828814 and KR828813 respectively.

A general time reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites (GTR+I+Γ) substitution model was determined by Modeltest v3.7 (Posada & Crandall, 1998) and MrModeltest2.3 (Nylander, 2004). PAUP* v4b10 (Swofford, 2003) was used to explore distance and parsimony phylogenetic analyses of nucleotide sequences; MRBAYES v3.1.2 (Ronquist & Huelsenbeck, 2003; Huelsenbeck & Ronquist, 2001) was used to perform a Bayesian phylogenetic analysis. The phylogram was edited in MEGA v6 (Tamura *et al.*, 2013).

3.3. Results

A cytopathic effect, as evidenced by syncytial formation (Figure 3.2) was detected in the cell culture during isolation. The full genome sequences of LINII and LINIV were compared with the NG1975 (X74443) (isolated in Nigeria in 1975) and IN1996 (KJ867542) (isolated in India in 1996) respectively.



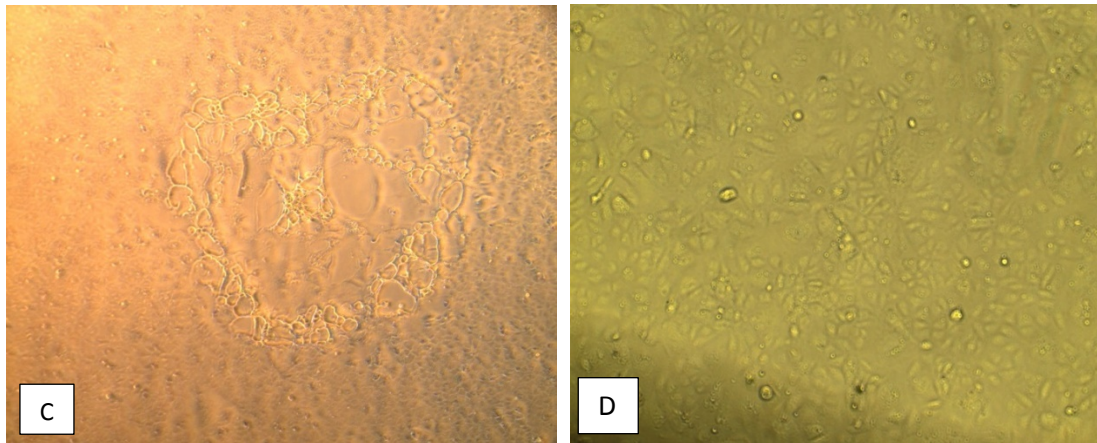


Figure 3.2: Cytopathic effect seen on PPRV samples inoculated with CHS 20 cells (2 days post inoculation) during virus isolation. A – C = positive isolation, D = not inoculated negative control.

A Basic Local Alignment Search Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) analysis revealed that the LINII genome showed the highest identity (97 %) with recent PPRV lineage II viruses from Ghana (KJ466106_GH2010) and Senegal (KM212177_SN2013), isolated in 2010 and 2013 respectively. LINII showed a 96 % identity with an earlier Nigerian lineage II isolate (X74443_NG1775) of 1975, which is the isolate used to produce a vaccine manufactured by NVRI. The LINIV isolate had the highest identity (96 %) with two Indian lineage IV PPRV isolated in 1994 (KR140086_IN1994) and 1996 (KJ867542_IN1996) respectively (Table 3.1). The 1996 isolate is also being used as a vaccine in that country.

Table 3.1: Primers (Dundon *et al.*, 2014) used to sequence the full genome of PPRV LINII and LINIV.

Name	Sequence 5'- 3'	Position on genome (bp)
PPRRG-1F	CCAAACAAAGTTGGGTAACCA	2-22
PPRRG-1R	AGRAGGATCCARACTTGTGC	620-640
PPRRG-2F	ARGGRCCCTCGAGTGGATGT	508-528
PPRRG-2R	CCCATTGAGTTCTCCARC	1133-1150
PPRRG-3F	AGGTTGCACCYTACATGGTAA	1030-1050
PPRRG-3R	TCYTTGTCGTTGTRGAYCT	1653-1672
PPRRG-4F	CTCGTGAGGCTCAAAGATCG	1561-1581
PPRRG-4R	TYTGTTGAACCACGAGAGAC	2187-2207
PPRRG-5F	CAACCAAYCATGCTCAYCAG	1986-2006
PPRRG-5R	GGATCGTCTTTGCACTCCYC	2634-2653
PPRRG-6F	ATAARTGGTGCAACCCAAGC	2548-2567
PPRRG-6R	ATTGCAGAGCTTGCTGYTT	3136-3155
PPRRG-7F	TCAAGAAACCYGCTGTKGAT	3035-3054
PPRRG-7R	TTGACCTACCGACCCCTAGA	3668-3687
PPRRG-8F	AGGGTCAATTGCYCSCATMG	3482-3421
PPRRG-8R	TTGCCACGTGTACCATAAA	4086-4105
PPRRG-9F	AATGYTGGARTTCCGCTCAG	3965-3984
PPRRG-9R	GCCTCTTGGGGGYKTCRR	4643-4660
PPRRG-10F	CCACTCGATACTCCCCAGAG	4377-4397

Name	Sequence 5'- 3'	Position on genome (bp)
PPRRG-10R	GGAGTCTTGGGGTGTGTGTT	4970-4988
PPRRG-11F	ACCRCCRGGGRAGGCYCY	4788-4805
PPRRG-11R	GGYTGRCTCTCDGKGGYTYT	5375-5394
PPRRG-12F	AGACCGAGRGAGGACCAC	5233-5250
PPRRG-12R	CGCCCAGGYGTYAGRGTYTG	5817-5836
PPRRG-13F	TGTTAAARCCTGTAGAGGATGC	5861-5882
PPRRG-13R	CTGTGCCCCAATGTTGTATG	6420-6440
PPRRG-14F	ATAAAGGCYCGRGTACATA	6306-6325
PPRRG-14R	AGCTCCTTTGCATTCTCCAG	6921-6940
PPRRG-15F	TGAGGTGGATGGRGTGACAA	6782-6802
PPRRG-15R	CAGYCCGATTAGACTCAGRA	7461-7482
PPRRG-16F	CAAAGGGARAGRATCAATGC	7335-7354
PPRRG-16R	ACACGTTGTYTTCATCTCG	7955-7974
PPRRG-17F	CTGTCTMGGYAGGRCAGTAA	7886-7905
PPRRG-17R	AGGTCGAGTCTTRCATGCTT	8476-8495
PPRRG-18F	GCCTACCTCYGAYCTCAYGG	8342-8362
PPRRG-18R	GAGAGGATTGCCYYTGAAAT	8998-9018
PPRRG-19F	AGGAGCGAGCATGCAATC	8922-8939
PPRRG-19R	CCTTCTTGAAGCTCMGAGA	9604-9624
PPRRG-20F	GGGTGGBGAAACAGTCAAY	9487-9506
PPRRG-20R	TGCCRCATGCTCYAGTATC	10229-10248
PPRRG-21F	TGTTGCTCTGCTTGAACCRY	10124-10144
PPRRG-21R	TARGAGCCATTCACCACGTA	10834-10852
PPRRG-22F	TGGCTGCACTGAAGAATGAG	10693-10713
PPRRG-22R	ATCYARRTCGGGRGGGCAAT	11419-11438
PPRRG-23F	GRYTRCCATCCTTCTTTCAA	11349-11369
PPRRG-23R	GATCTGTTGGAAAATYTTGAGGA	11965-11988
PPRRG-24F	TTGCAACAACRTCTCGCYAAG	11899-11919
PPRRG-24R	GGAAAGGCGRGCTARKACTC	12580-12599
PPRRG-25F	CTATAGCYGGGATGTTGGAY	12508-12527
PPRRG-25R	ATCTYGCWACCCTCACRAGG	13195-13215
PPRRG-26F	TCTACATCCACCAACCTAGCC	13128-13148
PPRRG-26R	AGGAGYTCCCCATCTGGTA	13809-13828
PPRRG-27F	GMGATGACGACATCAAYAGY	13678-13697
PPRRG-27R	CAAGGGGGAGARAARTCAGTC	14432-14452
PPRRG-28F	GMGGTTCCATYAAACAAATC	14314-14334
PPRRG-28R	TAGAGCCGATTTTYCCYAAA	15005-15024
PPRRG-29F	GAGACACTCCCAACCAAGGA	14934-14954
PPRRG-29R	TYCGCACYAGCAGTTTCTCA	15721-15741
PPRRG-30F	TGTGGTCTCTCGATCAATGG	15735-15748
PPRRG-30R	TCTCATGGTGGAGGAGAAGG	15929-15948

The LINII virus from this study was detected in a town at the border with Benin Republic and it has been reported that lineage II PPRV is circulating in neighbouring Benin Republic (Dundon *et al.*, 2015). LINIV was detected in a town at the border with Niger Republic (Figure 3.1). It is possible that lineage IV PPRV may be in Niger Republic and Chad, as it is circulating in the region, specifically in Cameroon, Morocco, Sudan and Ethiopia (Muniraju *et al.*, 2013). LINIV is likely to have come from any of these African countries, the Middle

East, India or Turkey where lineage IV PPRV strains circulate. The length of the Nigerian borders with Chad (85 km), Niger (1,608 km) and Cameroon (1,975 km) are porous. It is likely that the lineage IV viruses circulating currently in the country originated from these neighbouring countries via transhumance movement and trades.

The diversity/conservation of amino acids (aas) at each site was calculated using quality scores calculated in Clustal W (Larkin *et al.*, 2007). The N, M, F and L genes were well conserved with a range of conserved sites ranging from 83.5 - 88.1 % (Table 3.2; Figure 3.3). The N_{CORE} portion of the N protein (aas 1 - 422) was very well conserved with 93.1 % of the sites conserved and a mean (100 - quality score) of 1.85, with high amino acid (aa) diversity at the 5' end (aas 423 - 525) of the N protein. The greatest diversity could be found in the P protein, with only 61.5 % of the sites conserved and a mean (100 - quality score) of 13.32. The second most diverse protein was the H protein, with only 71.1 % of the sites conserved.

Table 3.2: Comparison of PPRV genome sequences available in public databases to (A) KR828813_NG2013 (LINIV) and (B) KR828814 NG2012 (LINII). MF-GC% - The GC % of the untranslated region between the M and the F genes, Nt. ID% - nucleotide identity (%).

A)

Genome	Lineage	MF-GC%	Nt. ID%	Protein identity (%)							
				N	P	V*	C	M	F	H	L
KR828813 NG2013	IV	67.1	100	100	100	100	100	100	100	100	100
KJ867542 IN1996	IV	68.2	96	96.5	95.6	94.2	90.9	97.9	98.1	95.4	98.2
KR140086 IN1996	IV	68.6	96	97.1	95.8	NA	NA	98.8	98.1	96.0	98.4
KC594074 MA2008	IV	66.2	95	97.1	95.0	93.2	89.8	99.1	97.2	95.2	97.8
KJ867541 ET2010	IV	68.1	95	97.3	94.1	92.6	90.3	98.8	97.2	94.7	97.9
NC006383 TR2000	IV	68.9	95	97.1	94.4	92.9	90.9	97.6	97.4	96.3	98.2
KM091959 CN2013	IV	69.7	95	96.0	94.6	NA	NA	98.8	97.8	95.7	97.8
FJ905304 CN2007	IV	69.3	95	96.7	94.1	72.1	88.1	98.5	97.2	95.5	98.3
JF939201 CN2007	IV	69.5	95	96.9	94.1	72.1	88.1	98.5	97.4	95.5	98.2
KP260624 CN2014	IV	69.6	95	96.5	94.4	92.2	90.9	98.8	97.8	95.7	97.6
JX217850 CN2008	IV	69.4	95	96.5	93.7	91.2	87.0	98.5	97.4	95.8	98.1
KF727981 IN1996	IV	68.9	94	96.0	91.7	NA	NA	95.5	94.8	93.5	95.3
KM089832 CN2014	IV	69.8	94	96.0	94.4	73.1	91.5	98.8	97.6	95.8	97.5
KM089830 CN2014	IV	69.9	94	96.0	94.6	73.1	90.3	98.8	97.6	95.4	97.2
KM089831 CN2014	IV	69.8	94	95.8	45.2	72.5	90.3	98.8	97.8	95.5	97.7

*The V-protein sequence in a number of the GenBank submissions has been incorrectly annotated or is not available (NA).

B)

Genome	Lineage	MF-GC%	Nt. ID%	Protein identity (%)							
				N	P	V	C	M	F	H	L
KR828814 NG2012	II	68.9	100	100	100	100	100	100	100	100	100
KJ466104 GH2010	II	66.2	97	98.8	97.2	97.6	97.7	99.1	98.3	98.1	99.4
KM212177 SN2013	II	67.8	97	99.2	96.4	96.6	97.1	99.4	98.3	97.8	99.3
X74443 NG 1975	II	67.6	96	97.7	94.1	92.6	93.7	98.5	97.6	96.5	98.3
EU267274 NG 1976	II	67.5	96	98.0	94.3	77.8	93.7	99.7	98.1	96.7	98.7

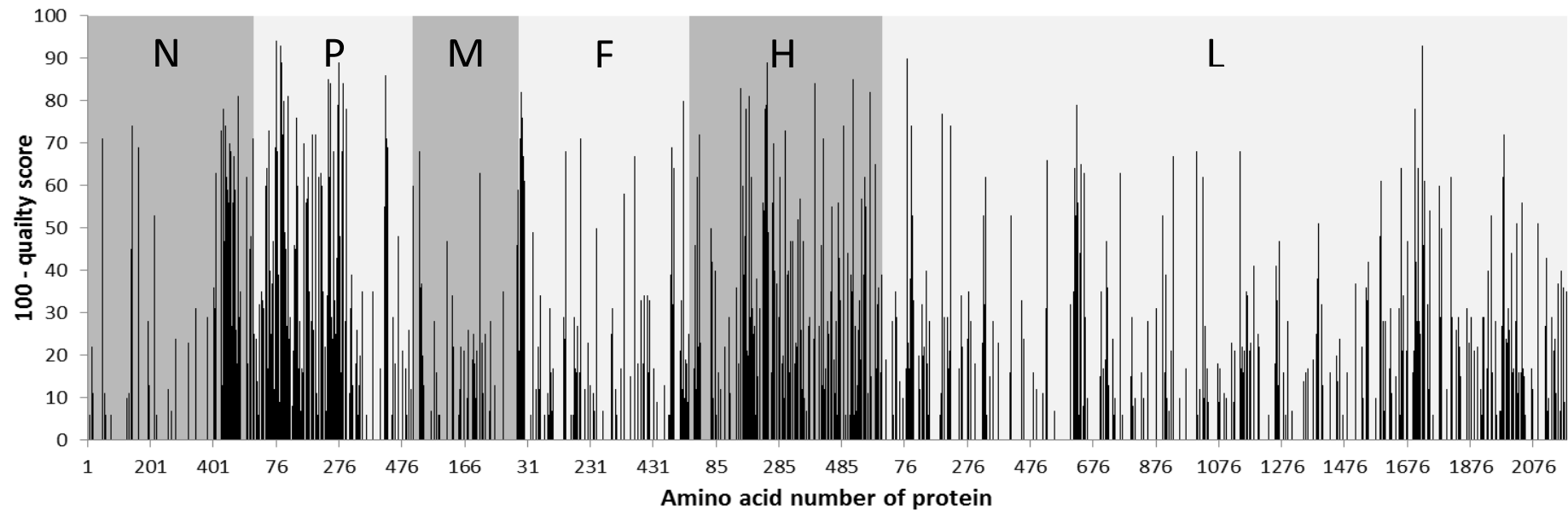


Figure 3.3: Diversity of the amino acid composition at each site of the PPRV proteome, determined using quality scores calculated in Clustal X2 (Larkin *et al.*, 2007). A value of 0 indicates conserved sites and the higher the score, the higher the amino acid diversity at the site.

Positive or purifying selection was examined by performing a codon-based Z-test of selection in MEGA v6 (Tamura *et al.*, 2013). The whole genome was under strong purifying selection ($p < 0.001$), as well as when genes were examined individually ($p < 0.001$). Analysis of the third position of the codons in DAMBE (Xia & Xie 2001) showed saturation of sites (Figure 3.10). As PPRV is a RNA virus with a higher mutation rate than DNA viruses, these results are to be expected.

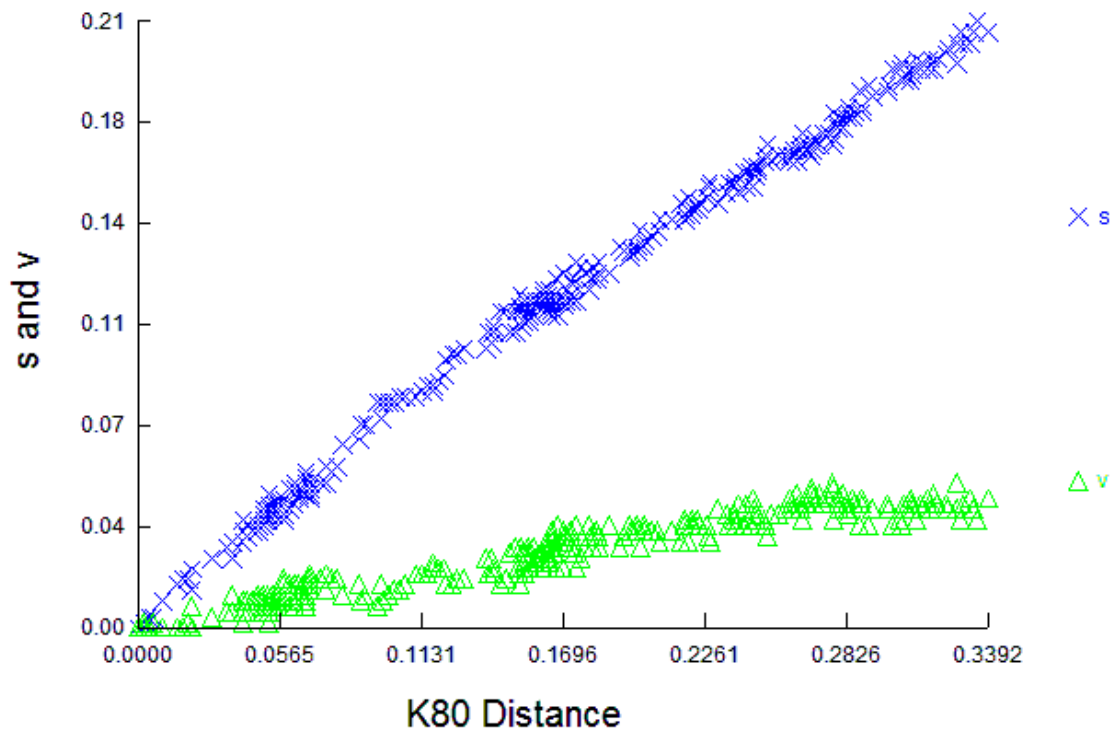


Figure 3.4: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank ($n = 27$) against K80 distance using DAMBE (Xia & Xie, 2001) for N protein.

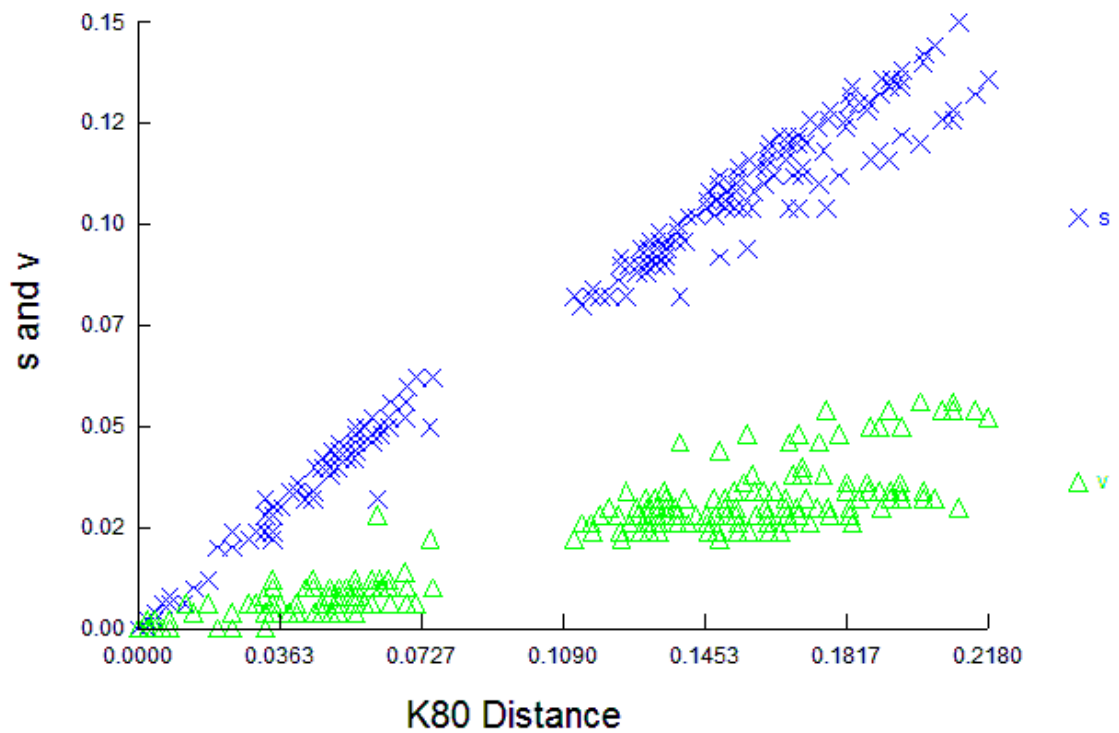


Figure 3.5: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank (n = 27) against K80 distance using DAMBE (Xia & Xie, 2001) for P protein.

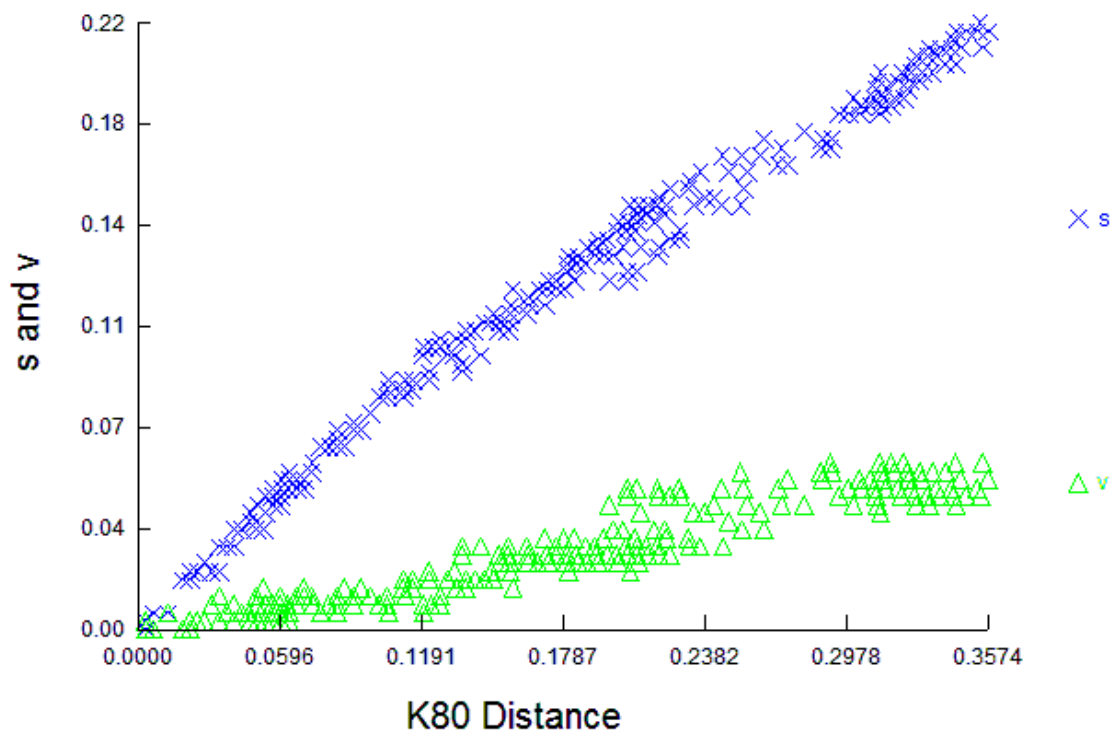


Figure 3.6: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank (n = 27) against K80 distance using DAMBE (Xia & Xie, 2001) for M protein.

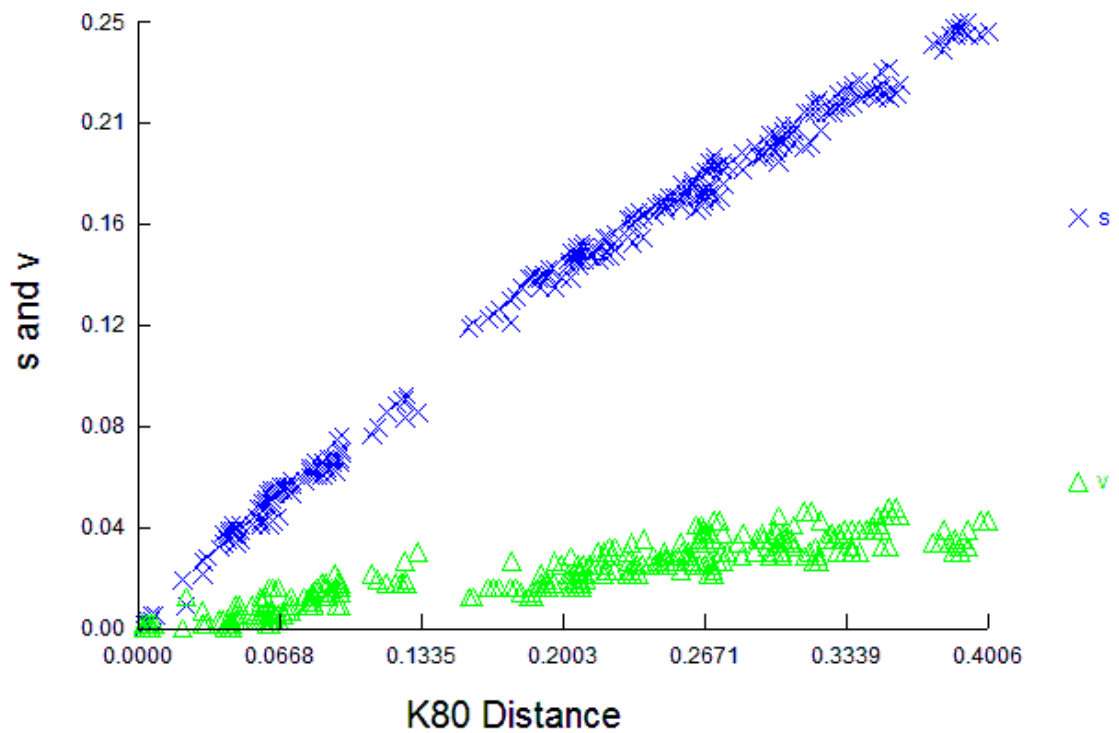


Figure 3.7: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank (n = 27) against K80 distance using DAMBE (Xia & Xie, 2001) for F protein.

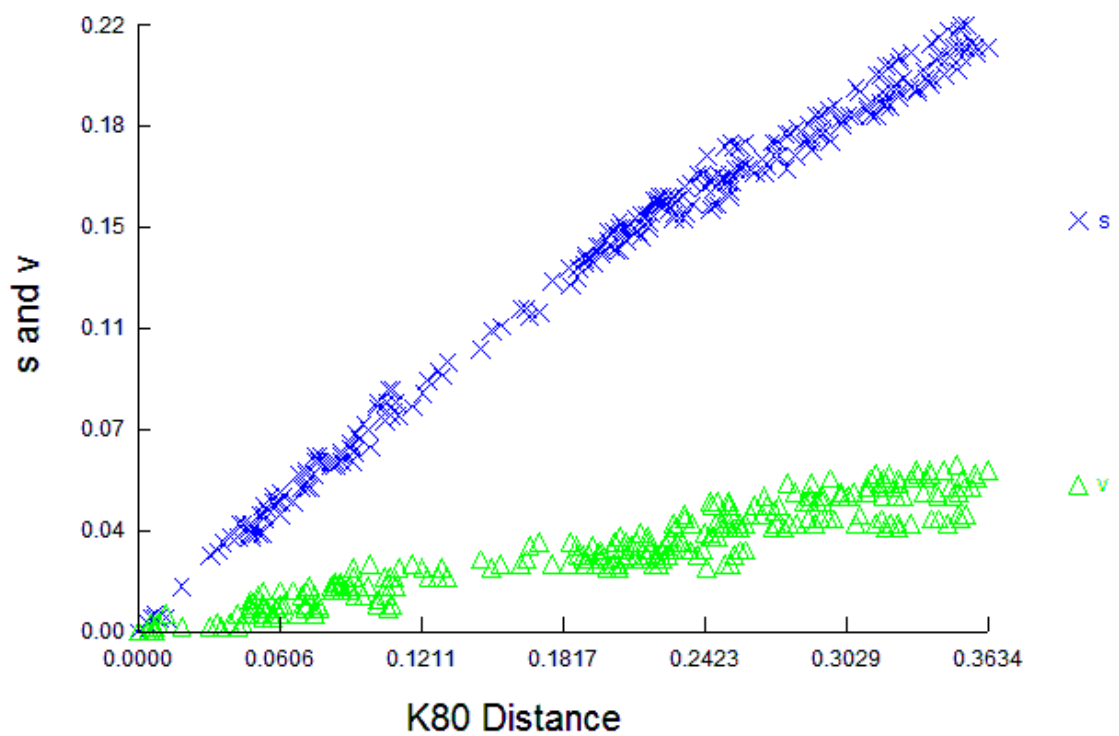


Figure 3.8: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank (n = 27) against K80 distance using DAMBE (Xia & Xie, 2001) for H protein.

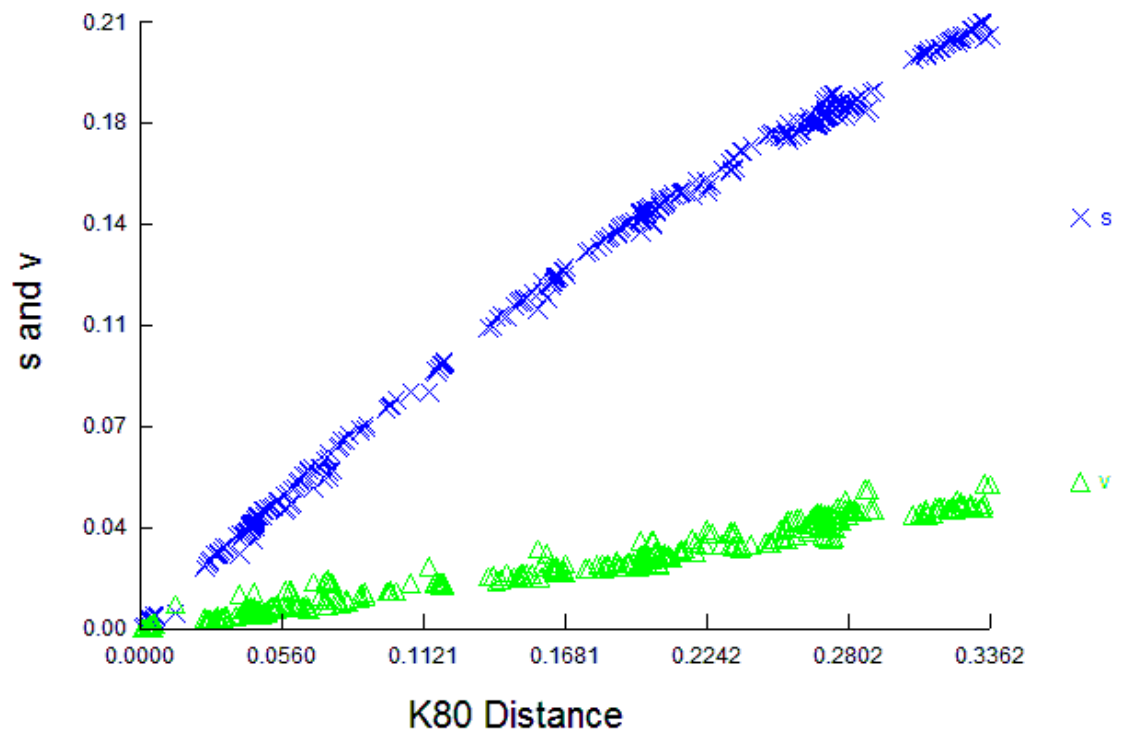


Figure 3.9: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank (n = 27) against K80 distance using DAMBE (Xia & Xie, 2001) for L protein.

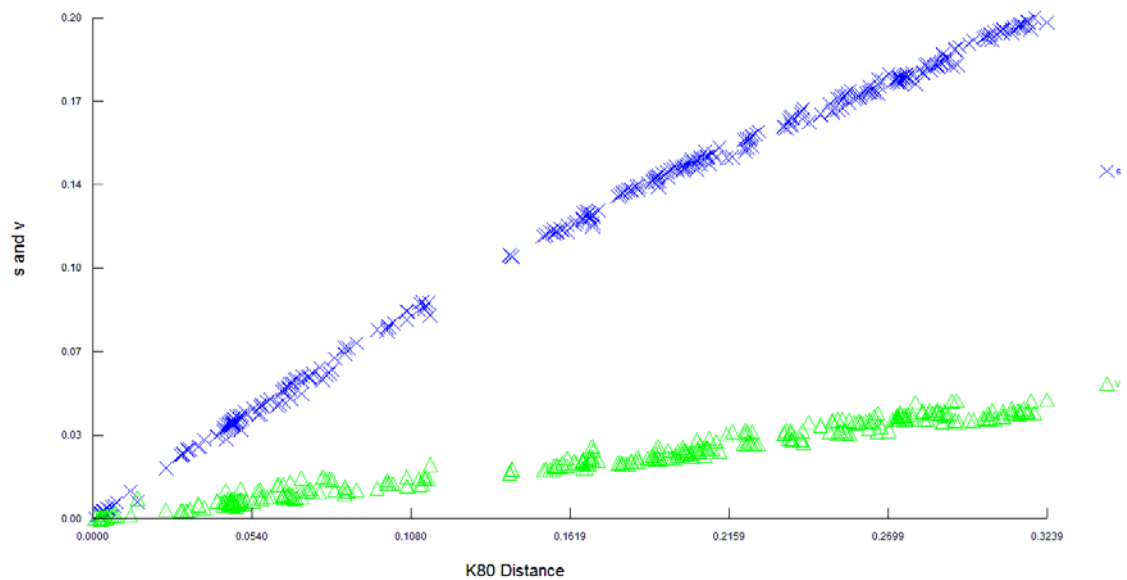


Figure 3.10: Plot of transitions and transversions in whole genome PPRV sequences available on GenBank (n = 27) against K80 distance using DAMBE (Xia & Xie, 2001) for the whole genome

Phylogenetic analyses using neighbor-joining, maximum parsimony and Bayesian methods yielded trees of similar topology. The trees showed four distinct PPRV lineages (Figure 3.11);

similar to what was seen when the partial N or F gene was used for the analysis (Woma *et al.*, 2015). LINII formed a monophyletic group with recent isolates from Ghana (KJ466104_GH2010) and Senegal (KM212177_SN2013), as well as with the Nigerian vaccine strain (X74443_NG1975). X74443_NG1975 was isolated in 1975 and is the oldest lineage II PPRV sequenced. LINIV was most closely related to KR140086_IN1994 and KJ867542_IN1996. The latter is an Indian isolate and is being used as a vaccine (Sungri/96) in that country; it was also the first lineage IV virus to be sequenced, the former isolate was only sequenced two years ago.

The Nigerian vaccine strain (X74443_NG1975) is the oldest PPRV lineage II whole genome sequenced. Likewise, KJ867542_IN1996 is the oldest Indian isolate to be sequenced. We therefore compared the rate of transversions between LINII and X74443_NG1975; and LINIV and KJ867542_IN1996 (Figure 3.12). The amino-terminal part of the N protein (aa 1 – 400) was well conserved, while the carboxyl terminal (aa 404 – 530) varied greatly. Most of the transversions of the lineage II N protein were changes from hydrophobic to hydrophobic and from hydrophobic to hydrophilic aas. Among both the lineage II and IV viruses, the P and H proteins showed the highest number of transversions. All types of transversions (e.g hydrophobic to hydrophilic, hydrophobic to hydrophobic) were present in the P protein between the lineage II viruses. The M protein was the most conserved of all the proteins in PPRV as the least amount of transversions occurred in this protein. A hydrophobic to hydrophilic transversion aa 171 of the M was present in both lineage II and IV. The transversions of the lineage II F protein were from hydrophobic to hydrophilic, hydrophobic to hydrophobic and hydrophilic to hydrophobic. All transversion types were observed in the H protein of lineage II viruses. The L protein of PPRV, although very large, was well conserved and only a low number of transversions were observed. Hydrophobic to hydrophilic changes were not observed in the L protein between LINII and X74443_NG1975.

The N protein was well conserved among the PPRV, with only a few aa changes between LINII and X74443_NG1975 throughout the entire 525 aa length (Figure 3.13). The N protein aa sequence of recent lineage II viruses from Ghana, Nigeria and Senegal differed from the older Nigerian isolates in three positions (Table 3.3). There were 18 aa differences in the N protein between LINIV and the closely related KJ867542_IN1996 from India (Figure 3.13).

The P protein ORF is situated at nt positions 1807 to 3336. The P protein is 509 aas in size and not as conserved as N (Figure 3.3). LINII and LINIV differed by 30 and 22 aas compared with X74443_NG1975 and KJ867542_IN1996 respectively (Figure 3.13). The P protein aa

sequence of recent lineage II viruses from Ghana, Nigeria (LINII) and Senegal differed from the older Nigerian isolates in six positions. Two aa changes in the P protein of Nigerian lineage II viruses differed from other lineage II viruses (Table 3.4). A tyrosine at position 110 (Y110) is present in LINII, LINIV, X74443_NG1975 and KJ867542_IN1996. It has also been reported in PPRV KJ466104 GH2010 (Dundon *et al.*, 2014).

The M protein ORF is situated between nt positions 3438 to 4445 and is 335 aa in size. M protein is the most conserved protein in the PPRV (Figure 3.10). Nigerian lineage II viruses had one distinct aa change compared to other West African lineage II viruses in the M (Table 3.4).

The F-protein cleavage site GRRTRR (aa position 108) as described by Meyer & Diallo (1995) for PPRV X74443_NG1975 was also present in both LINII and LINIV. The F protein ORF is situated between nt positions 5526 to 7166 and is 546 aas in size. It is highly conserved (Figure 3.10). The F protein aa sequence of recent lineage II viruses from Ghana, Nigeria and Senegal (KJ466104 GH2010, LINII and KM212177 SN2013) differed from the older Nigerian (X74443_NG1975 and EU267274_NG1976) isolates in two positions. The F gene of all lineage II viruses from Nigeria differed from other lineage II West African viruses in one aa position (Table 3.4).

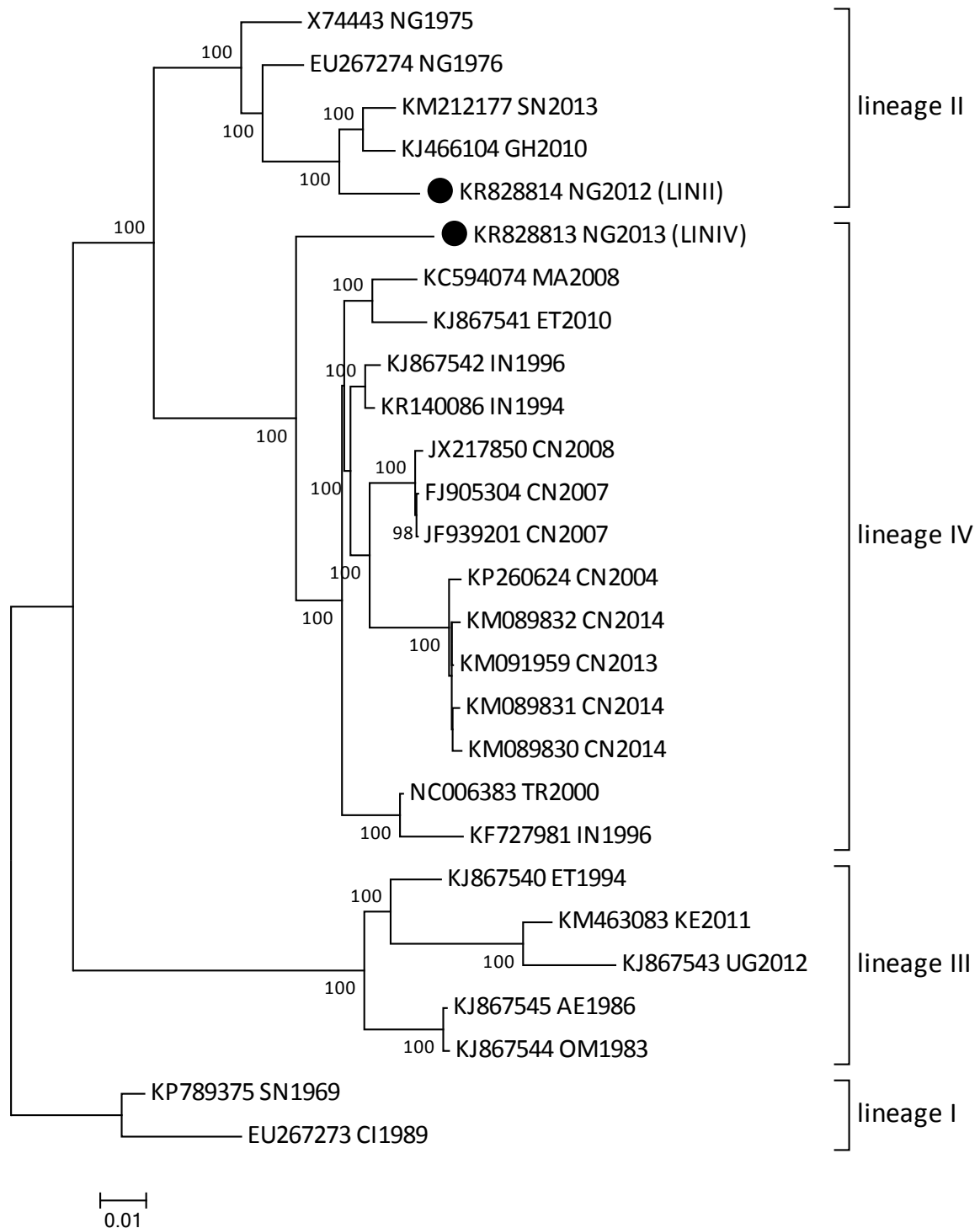


Figure 3.11: PPRV full genome phylogenetic analysis. Unrooted phylogram calculated using Bayesian inference, GTR+I+Γ substitution model and 1 × 10⁶ iterations, showing the relationship between the full genome sequences from this study (indicated by black circles, ISO 3166 country code and year of sample collection) with other published full genome sequences obtained from GenBank (indicated by accession number, ISO 3166 country code and year of isolation). The posterior probabilities are indicated at the nodes of the tree.

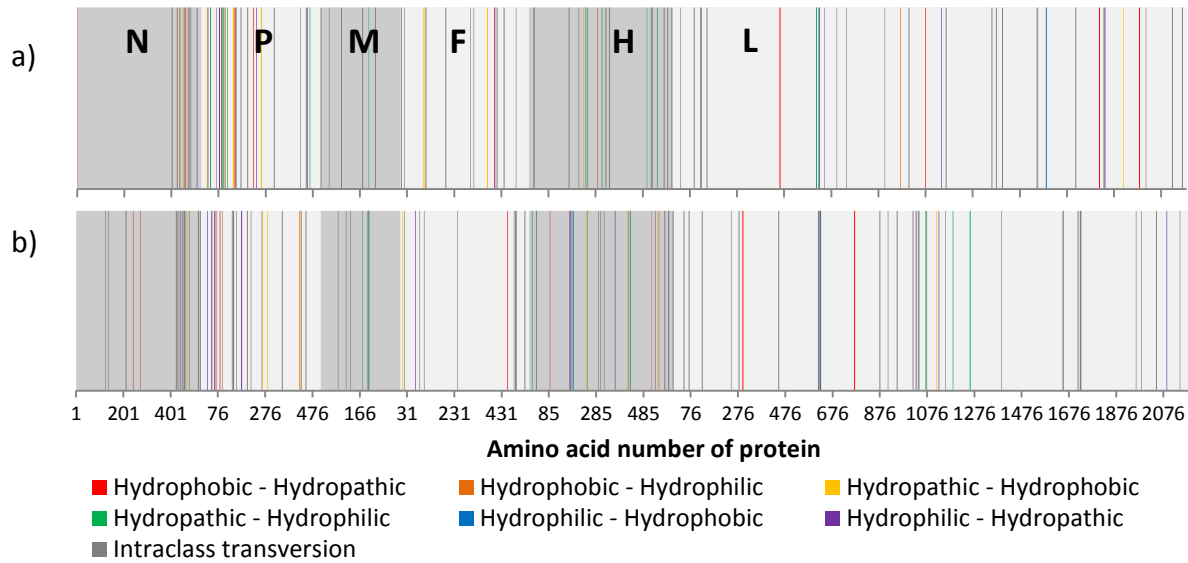


Figure 3.12: Transversions in a pairwise comparison between a) LINII and X74443_NG1975, both PPRV lineage II viruses, and b) LINIV and KJ867542_IN1996, both PPRV lineage IV viruses. The colour of the bar indicates the type of transversion (e.g. hydrophobic to hydrophilic indicated in green). Alternating grey blocks indicate the different PPRV proteins.

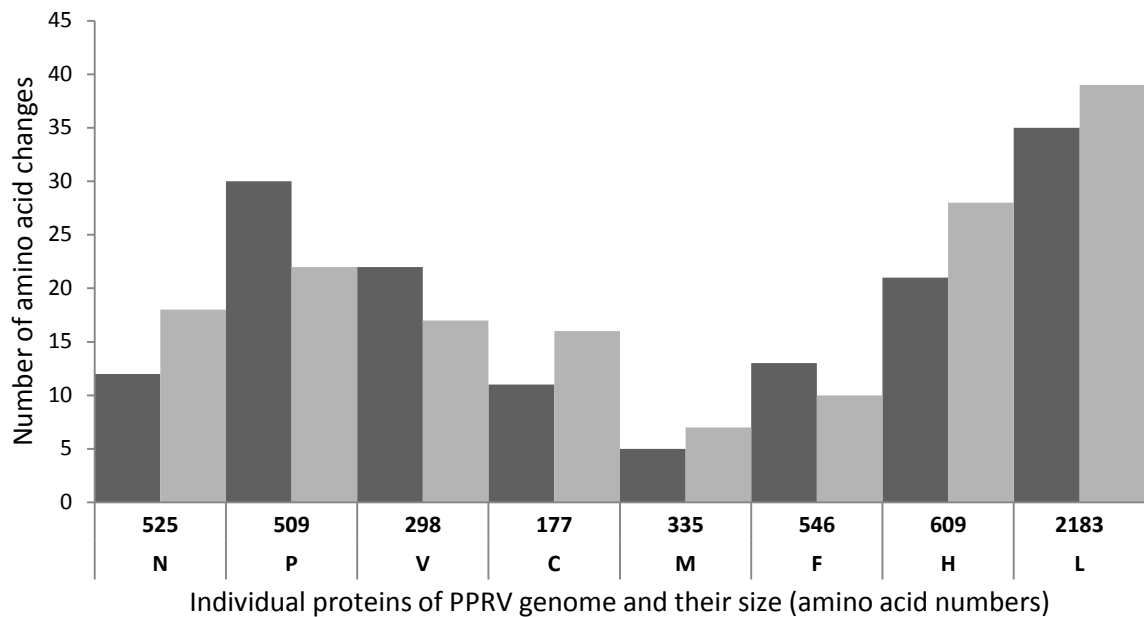


Figure 3.13: Comparison of the different number of amino acids changes between various closely related isolates of PPRV. Dark grey columns = differences between LINII and X74443 NG1975, light grey columns = differences between LINIV and KJ867542 IN1996.

Table 3.3: Diversity/conservation of amino acids of the different proteins of PPRV, using quality scores calculated in Clustal W: the lower the quality score, the greater the degree of conservation, * - conserved 3' region of the N protein (Larkin *et al.*, 2007), aa - amino acids.

Protein	No. of aa	No. of conserved aa	% of conserved sites	Mean (100 - quality score)
N	525	444	84.6	5.48
N _{CORE} *	422	393	93.1	1.85
P	509	313	61.5	13.32
M	335	295	88.1	2.78
F	546	456	83.5	4.53
H	609	433	71.1	9.19
L	2183	1867	85.5	3.84

Table 3.4: Unique amino acid residues of different groups of PPRV lineage II isolates. Isolates were grouped into RI = Recent Isolates [KJ466104_GH2010, KR828814_NG2012 (LINII) and KM212177_SN2013], OI = Old Isolates (X74443_NG 1975 and EU267274_NG1976), NI = Nigerian Isolates [KR828814_NG2012 (LINII), EU267274_NG1976 and X74443_NG1975) and Others = KJ466104_GH2010 and KM212177_SN2013. AP = letter indicating the protein and followed by the amino acid positions on the protein. Amino acids are indicated by the IUPAC-IUB one letter notation.

AP	N456	N469	N482	P30	P40	P50	P66	P79	P89	P91	P241	F94	F194	M32
RI	P	L	G	Q	F		S	G	D	N		A	I	
OI	D	T	S	K	L		P	D	N	S		T	V	
NI						P					T			I
Others						H					M			V

AP	H171	H233	H246	H247	H250	H327	H350	H488	H520	H560	H574	H589
RI	F	I		D	Q	V			R		S	M
OI	L	T		G	H	A			K		Y	I
NI			P				Y	R		R		
Others			L				H	K		K		

AP	L35	L93	L120	L142	L147	L612	L698	L902	L1004	L1401	L1547	L1551	L1780	L2010
RI	N	Q	V		D	N	K	V	P	K	I	V		V
OI	D	D	I		E	T	R	I	T	R	V	I		I
NI				T									V	
Others				N									A	

The H protein ORF is situated between nt positions 7326 to 9155 and is 609 aas in size. The H proteins of LINII differed by 21 aa when compared to X74443_NG1975, while LINIV differed by 28 aa when compared to KJ867542_IN1996. The H protein aa sequence of recent lineage II viruses from Ghana, Nigeria and Senegal (KJ466104 GH2010, LINII and KM212177

SN2013) differed from the older Nigerian (X74443_NG1975 and EU267274_NG1976) isolates in eight positions, while all lineage II viruses from Nigeria differed from other lineage II West African viruses in four positions.

The L protein ORF is situated at nt positions 9288 to 15839 and is 2183 aa in size. The L protein is an RNA-dependent RNA polymerase. It is the largest of the PPRV proteins and is highly conserved. The L proteins of LINII differed by 35 aa compared to X74443_NG1975, while LINIV differed by 39 aa compared to KJ867542_IN1996 (Figure 3.13). The L protein aa sequence of recent lineage II viruses from Ghana, Nigeria and Senegal (KJ466104 GH2010, LINII and KM212177 SN2013) differed from the older Nigerian (X74443_NG1975 and EU267274_NG1976) isolates in 13 positions. The recently isolated lineage II viruses had 13 distinct aa changes from the older isolates. Lineage II viruses from Nigeria differed from other lineage II West African viruses in two positions (Table 3.4).

The antigenome promoter (trailer) of LINII and LINIV are represented by nt 15912–15948 as reported by other studies. The identity between PPRV strains in these regions is high (Chard *et al.*, 2008).

3.4. Discussion

The genome lengths of both LINII and LINIV were 15,948 nucleotides (nt), which agreed with sequence data of other strains of PPRV (Bailey *et al.*, 2005; Chard *et al.*, 2008; Bao *et al.*, 2012; Muniraju *et al.*, 2013; 2014; Salami *et al.*, 2014; Dundon *et al.*, 2014; 2015), and also conformed to the “rule of six” proposed by Calain & Roux (1993). According to this rule, to form a proper template for efficient replication, the virus RNA must contain a total number of nucleotides which must be a multiple of six.

The organization of the genomes of LINII and LINIV were the same as those described previously with a 107 nt genome promoter region at the 3’ end followed by the transcription units for the N, P (V, C), M, F, H and L proteins and the antigenome promoter at the 5’ end (Bailey *et al.*, 2005). Each transcription unit was separated by an untranslated region of 120 – 160 nt that contained the gene end signal to direct polyadenylation of the transcript, the intergenic region and the gene start signal. Of the eight PPRV proteins, only three (N, P and L) are absolutely required for replication and transcription of the viral RNA (Chard *et al.*, 2008).

The N protein can be divided into two regions. The first 420 aas are highly conserved (Figure 3.12) across the genus and across PPRV strains, forming a region known as N_{CORE}. The N_{CORE} has been suggested to play a role in N-N self-assembly and N-RNA interactions. The terminal 105 aas of N starting at position 421 to 525, referred to as N_{TAIL}, are less conserved and is considered an intrinsically disordered domain within the N protein. The N_{TAIL} is likely to be involved in interactions between N and other proteins such as P and the P-L polymerase complex (Chard *et al.*, 2008).

The N protein open reading frames (ORF) of LINII and LINIV are situated between nt positions 108 to 1685 and they translate to 525 aas, similar to other PPRV isolates. The N protein has the nuclear export signal (LLKSLALF) at aa positions 4 to 11, and a nuclear localization signal (TGVMISML) at aa positions 70 to 77 (Chard *et al.*, 2008; Dundon *et al.*, 2014). The reasons for the nuclear export and localization signals in N are unclear, as these viruses replicate in the cytoplasm of cells, although aggregates of N have been identified within the nucleus at late stages of infection and nuclear inclusion bodies are characteristic of infections by morbilliviruses (Huber *et al.*, 1991; Chard *et al.*, 2008).

There will be need to further characterize the phosphorylation sites within the PPRV P protein since we observe Ser 49 and Ser 151 phosphorylation sites in PPRV LINIV but not in LINII.

There is an unusually long (1,080 bp) and highly GC-rich untranslated region (UTR) between the M and F open-reading frames in all PPRV sequenced. The UTR is not conserved between PPR viruses and its function is unclear. The GC content of the UTR for LINII and LINIV was 68.9 % and 67.1 % respectively, and was similar to the UTR of X74443_NG1975 which had a GC content of 67.6 % (Table 3.2). It has been suggested that the long 3' UTR of the MeV M mRNA had a role in up-regulation of M protein production and the long 5' UTR of the F mRNA causes a decrease in F protein production. Together these effects may alter cytopathogenicity of the virus, and increase viral survival in nature (Takeda *et al.*, 2005; Chard *et al.*, 2008).

At aa position 291 of the LINII and LINIV PPRV L protein, a leucine zipper motif is evident as reported in other PPRV. This protein region is involved in nucleic acid binding and is formed when leucine residues, from adjacent alpha-helices, interdigitate to stabilize the L protein (Bailey *et al.*, 2005). The L protein of LINII and LINIV had the conserved pentapeptide QGDNQ motif (aa position 771–775) as documented for other PPRV (Bailey *et al.*, 2005,

Dundon *et al.*, 2015). The conservation of the D and N in this motif is known to be an absolute requirement for polymerase activity; and the GD residues of this motif constitute part of a structure that is predicted to be the core polymerase motif (Bailey *et al.*, 2005).

In conclusion, we have sequenced two virulent strains of PPRV (LINII and LINII) currently co-circulating in Nigeria and found the genome organization of these viruses to conform to those of other morbilliviruses. We have also highlighted the unique aa residues in the individual proteins of the various lineages of PPRV.

CHAPTER

4. DEVELOPMENT OF A LINEAGE-SPECIFIC REAL TIME PCR ASSAY FOR PPRV

4.1. Introduction

Conventional reverse transcription polymerase chain reaction (RT-PCR) tests are available for the detection of PPRV nucleic acids (Forsyth & Barrett, 1995; Couacy-Hymann *et al.*, 2002; Balamurugan *et al.*, 2006). RT-PCR can detect PPRV but lineage differentiations require sequencing and phylogenetic analysis.

Real time RT-PCR is preferred over conventional RT-PCR because it is more rapid to perform, has higher sensitivity and much lower chance of contamination. Five group-specific real time RT-PCR assays have so far been described for PPRV. Three of these assays target the N gene using TaqMan® chemistry (Bao *et al.*, 2008; Kwiatek *et al.*, 2010; Batten *et al.*, 2011), while two target the M gene using either TaqMan® (Balamurugan *et al.*, 2010) or SYBR Green (Abera *et al.*, 2014) chemistry.

This report describes the development of a lineage-specific TaqMan® real time RT-PCR based on the H gene. Knowledge of the virus lineage can help pinpoint the origin of an outbreak. In view of the fact that not all laboratories are equipped to carry out sequence analysis, this assay will provide an alternative to the conventional RT-PCR and other group-specific real-time RT-PCR diagnostic assays that already exist, thereby helping in rapid lineage specific clinical diagnosis of PPRV.

4.2. Materials and methods

4.2.1. Primer and probe design

The whole genome sequences of all (27) currently available PPRV strains of different origins (in GenBank) including two whole genome isolates from this study were aligned using Clustal W. A manual scan of the whole PPRV transcriptome was performed to identify lineage specific aa residues. A lineage-specific aa residue was identified in the H gene aa position 399. The region was back-translated into nucleotide sequences. The oligonucleotide primers and the lineage-specific probes for real time RT-PCR amplification of

PPRV were designed using the Primer Express software version 3.0.1 (Applied Biosystems, Carlsbad, USA) and the consensus sequence of the PPRV H gene.

A Blastn search was done on the PCR product of Genbank reference number EU267273_C11989 to identify somewhat similar sequences and to check for specificity of the assay.

4.3. Results

Lineage-specific aa residues were identified (Table 4.1). A lineage-specific residue at aa 399 of the H gene was identified (Figure 4.1). TaqMan® MGB probes (Table 4.2) were designed to span this residue. The aa residues were back translated into nucleotides (Figure 4.2) to enable the primer and probe design.

The P protein was not as conserved as the N protein. Lineage I and III sequences each contained fourteen unique aa residues, lineage II contained seven and lineage IV six unique aa on the P protein. The M protein of PPRV was the most conserved in the entire genome and only lineage III sequences contained any unique aa residues (three) on this protein.

Lineage I sequences contained two unique residues on the F protein, lineage II one, lineage III seven and lineage IV two. However, aa position F524 (aa position 1893 on the whole genome) showed unique aa T for lineage I and III, A for lineage II and I for lineage IV; lineage III also had a unique aa residue P at F525. This aa position 1893 was also considered as a possible target for the development of the assay but there was more variation around the primer sites, so it would have been more difficult.

The H protein was highly diverse, with seven unique aa residues each in both lineage II and IV sequences, lineage III sequences contained the most (sixteen) aa residues in the H protein, while ten unique aa residues were present in lineage I sequences. The L protein was well conserved, but due to its large size (2183 aa), lineage I contained 18, lineage II four, lineage III 34 and lineage IV ten unique aa residues.

Blastn revealed additional H gene sequences (partial coding sequences) not originally aligned in the development of the assay since only full genome sequences were used. The additional sequences (accession number AY602954 – AY602974) all belonged to lineage IV PPRV and were identical to the designed primers and probes.

The entire genome of PPRV was aligned to identify areas of lineage specific aa residues. The N protein of PPRV was highly conserved. Lineage I, II and IV sequences each contained only two unique aa residues. Lineage III sequences contained the highest amount of unique aa residues (ten) on the N protein.

The primer and probe sequences (Table 4.2). developed for this assay were determined using the least conserved part of the hemagglutinin (H) protein gene in order to achieve high lineage-specificity for PPRV detection.

Table 4.1: Lineage-specific amino acid residues (AA ID - lineage) of PPRV and the residues of the other lineages (AA ID - others). The positions of the lineage-specific residue on the protein (AA position - protein), genome (AA position - genome) and nucleotide position (Nt position - genome) are indicated in the table. Amino acids are indicated by single letter IUPAC code. The assay was developed from H399, highlighted in red.

Lineage	Protein	AA position - protein	AA position - genome	Nt position - genome	AA ID - lineage	AA ID - others
I	N	54	54	269	K	R
I	N	467	467	1508	P	L/M
I	P	21	546	1869	T	A
I	P	59	584	1983	D	S/N
I	P	68	593	2010	V	I
I	P	98	623	2100	I	V
I	P	168	693	2310	W	R
I	P	172	697	2322	T	A
I	P	174	699	2328	H	N
I	P	192	717	2382	A	E
I	P	199	724	2403	N	S/G
I	P	242	767	2532	A	V/T/G
I	P	310	835	2736	G	E/D
I	P	337	862	2817	E	D/H
I	P	453	978	3165	I	V
I	P	503	1028	3315	T	A
I	F	95	1464	5810	K	R
I	F	180	1549	6065	S	N
I	H	90	2005	7595	N	D
I	H	98	2013	7619	V	I
I	H	187	2102	7886	S	G
I	H	275	2190	8153	H	Y
I	H	283	2198	8174	G	D
I	H	399	2314	8522	V	I/L/S
I	H	450	2365	8675	S	R/K
I	H	472	2387	8741	L	F
I	H	596	2511	9113	K	T/M/A
I	H	600	2515	9125	I	T

Lineage	Protein	AA position - protein	AA position - genome	Nt position - genome	AA ID - lineage	AA ID - others
I	L	129	2653	9674	T	R/K
I	L	194	2718	9869	V	T/I
I	L	211	2735	9920	A	V
I	L	650	3174	11237	S	G
I	L	720	3244	11447	V	I/G
I	L	1406	3930	13505	D	E
I	L	1551	4075	13940	T	V/I
I	L	1618	4142	14141	L	V
I	L	1621	4145	14150	T	S
I	L	1639	4163	14204	V	M/I
I	L	1660	4184	14267	G	D/E
I	L	1817	4341	14738	D	G
I	L	1877	4401	14918	L	F
I	L	1887	4411	14948	N	T
I	L	1931	4455	15080	G	S/A
I	L	2026	4550	15365	R	K
I	L	2115	4639	15632	R	A
I	L	2118	4642	15641	G	K/R
II	N	448	448	1451	G	E
II	N	452	452	1463	R	K
II	P	32	557	1902	A	T
II	P	134	659	2208	N	D
II	P	171	696	2319	A	V
II	P	177	702	2337	S	A/V
II	P	209	734	2433	I	T
II	P	220	745	2466	N	H
II	P	339	864	2823	L	I
II	F	524	1893	7097	A	T/I
II	H	163	2078	7814	V	I
II	H	239	2154	8042	T	A/V
II	H	241	2156	8048	K	D/N
II	H	264	2179	8117	E	G
II	H	269	2184	8132	I	V
II	H	399	2314	8522	I	V/L/S
II	H	452	2367	8681	A	V/I
II	L	256	2780	10055	N	S
II	L	645	3169	11222	T	I/V
II	L	1655	4179	14252	I	T/A
II	L	2118	4642	15641	K	G/R
III	N	13	13	146	K	R
III	N	320	320	1067	V	I
III	N	430	430	1397	H	P/Y/S
III	N	432	432	1403	S	P
III	N	442	442	1433	T	I
III	N	449	449	1454	K	R
III	N	454	454	1469	A	T
III	N	462	462	1493	G	E
III	N	465	465	1502	D	G/S
III	N	480	480	1547	G	E

Lineage	Protein	AA position - protein	AA position - genome	Nt position - genome	AA ID - lineage	AA ID - others
III	P	41	566	1929	D	S/N/G
III	P	77	602	2037	T	P/S
III	P	83	608	2055	Y	F
III	P	87	612	2067	N	T
III	P	137	662	2217	L	V/F/I
III	P	177	702	2337	V	A/S
III	P	201	726	2409	R	G
III	P	215	740	2451	T	V/I
III	P	221	746	2469	P	S/F
III	P	268	793	2610	F	S
III	P	295	820	2691	P	L
III	P	380	905	2946	E	D
III	P	381	906	2949	V	T/I
III	P	419	944	3063	P	Q
III	M	127	1161	3818	R	K
III	M	161	1195	3920	T	S
III	M	219	1253	4094	I	V
III	F	9	1378	5552	S	L/F
III	F	47	1416	5666	Q	R/H
III	F	148	1517	5969	A	T
III	F	295	1664	6410	M	I
III	F	298	1667	6419	V	I
III	F	399	1768	6722	I	V
III	F	525	1894	7100	P	S
III	H	15	1930	7370	T	N
III	H	24	1939	7397	T	I
III	H	27	1942	7406	K	R
III	H	110	2025	7655	R	K
III	H	189	2104	7892	P	L/Q
III	H	211	2126	7958	N	E
III	H	222	2137	7991	I	V
III	H	263	2178	8114	F	L
III	H	266	2181	8123	S	G
III	H	338	2253	8339	I	V
III	H	399	2314	8522	L	I/V/S
III	H	419	2334	8582	V	L/S
III	H	471	2386	8738	A	G
III	H	490	2405	8795	L	P
III	H	535	2450	8930	D	E
III	H	553	2468	8984	F	Y
III	L	85	2609	9542	V	I
III	L	100	2624	9587	G	S
III	L	129	2653	9674	R	T/K
III	L	219	2743	9944	C	Y
III	L	259	2783	10064	K	R
III	L	275	2799	10112	A	T
III	L	321	2845	10250	V	I
III	L	325	2849	10262	S	G
III	L	328	2852	10271	D	N

Lineage	Protein	AA position - protein	AA position - genome	Nt position - genome	AA ID - lineage	AA ID - others
III	L	331	2855	10280	I	T
III	L	412	2936	10523	V	A
III	L	524	3048	10859	E	K
III	L	526	3050	10865	H	P
III	L	615	3139	11132	Q	R
III	L	616	3140	11135	K	G/E/D
III	L	620	3144	11147	D	S/N
III	L	621	3145	11150	R	Q
III	L	624	3148	11159	T	K/N/S
III	L	645	3169	11222	V	I/T
III	L	760	3284	11567	H	Y
III	L	897	3421	11978	L	F
III	L	1023	3547	12356	T	I
III	L	1176	3700	12815	A	S
III	L	1203	3727	12896	K	R
III	L	1460	3984	13667	T	A
III	L	1707	4231	14408	N	K
III	L	1708	4232	14411	T	L
III	L	1730	4254	14477	R	G
III	L	1814	4338	14729	I	T
III	L	1840	4364	14807	L	I/V
III	L	1864	4388	14879	E	K
III	L	1931	4455	15080	S	G/A
III	L	1943	4467	15116	V	A
III	L	2004	4528	15299	Y	F/L
IV	N	453	453	1466	Q	R
IV	N	517	517	1658	V	T/I
IV	P	33	558	1905	I	L
IV	P	71	596	2019	N	G
IV	P	74	599	2028	Y	D
IV	P	164	689	2298	T	P
IV	P	188	713	2370	A	D
IV	P	265	790	2601	Q	R
IV	F	70	1439	5735	A	S
IV	F	199	1568	6122	G	S
IV	F	524	1893	7097	I	T/A
IV	H	173	2088	7844	K	R
IV	H	267	2182	8126	P	A
IV	H	286	2201	8183	R	S/N
IV	H	361	2276	8408	D	N
IV	H	399	2314	8522	S	I/L/V
IV	H	504	2419	8837	V	T/I
IV	H	516	2431	8873	I	V
IV	L	46	2570	9425	V	I
IV	L	81	2605	9530	H	R
IV	L	83	2607	9536	R	C
IV	L	104	2628	9599	K	R
IV	L	624	3148	11159	K	T/N/S
IV	L	699	3223	11384	I	V

Lineage	Protein	AA position - protein	AA position - genome	Nt position - genome	AA ID - lineage	AA ID - others
IV	L	718	3242	11441	N	D
IV	L	1268	3792	13091	N	D
IV	L	1698	4222	14381	V	I
IV	L	2181	4705	15830	I	V

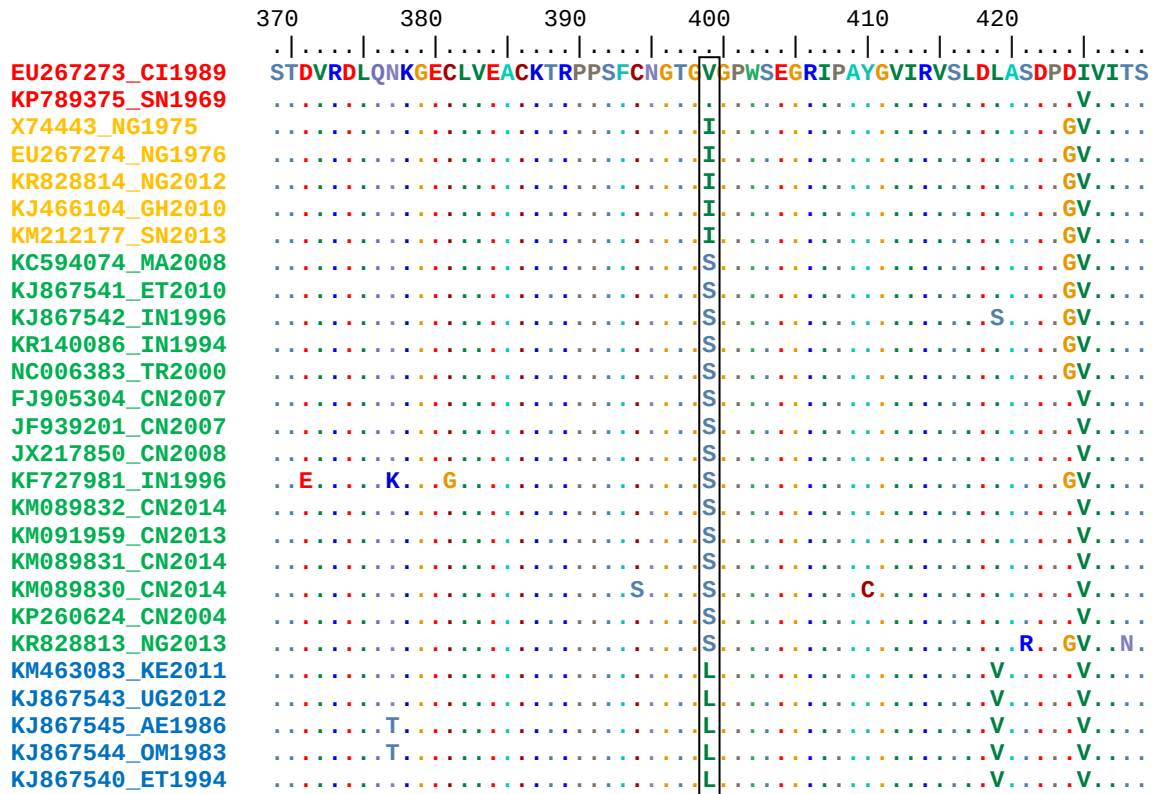


Figure 4.1: Lineage-specific amino acid residue indicated by black box. Sequence names are coloured according to lineage (red - lineage I, orange - lineage II, green - lineage IV and blue - lineage III). The amino acids of the H protein are numbered according to the Genbank reference sequence (NC_006383).

Table 4.2: Primers and TaqMan® MGB probes for the lineage-specific diagnosis of PPRV.

Primer/Probe	Sequence (5' – 3')	T _M (°C)	% GC	Length (bp)
Forward primer	CGACCTCCYTCATTTWGCAATG	59.1	48	22
Reverse primer	CCGYARGCRGGGATTCTC	59.7	64	18
Lineage I probe	CACRGGAGTGGGCCCAT	69.0	68	17
Lineage II probe	CACAGGAATAGGCCCATG	69.0	56	18
Lineage III probe	TACAGGGTTAGGCCCATG	68.0	56	18
Lineage IV probe	CACAGGATCWGGCCCGTG	69.0	67	18

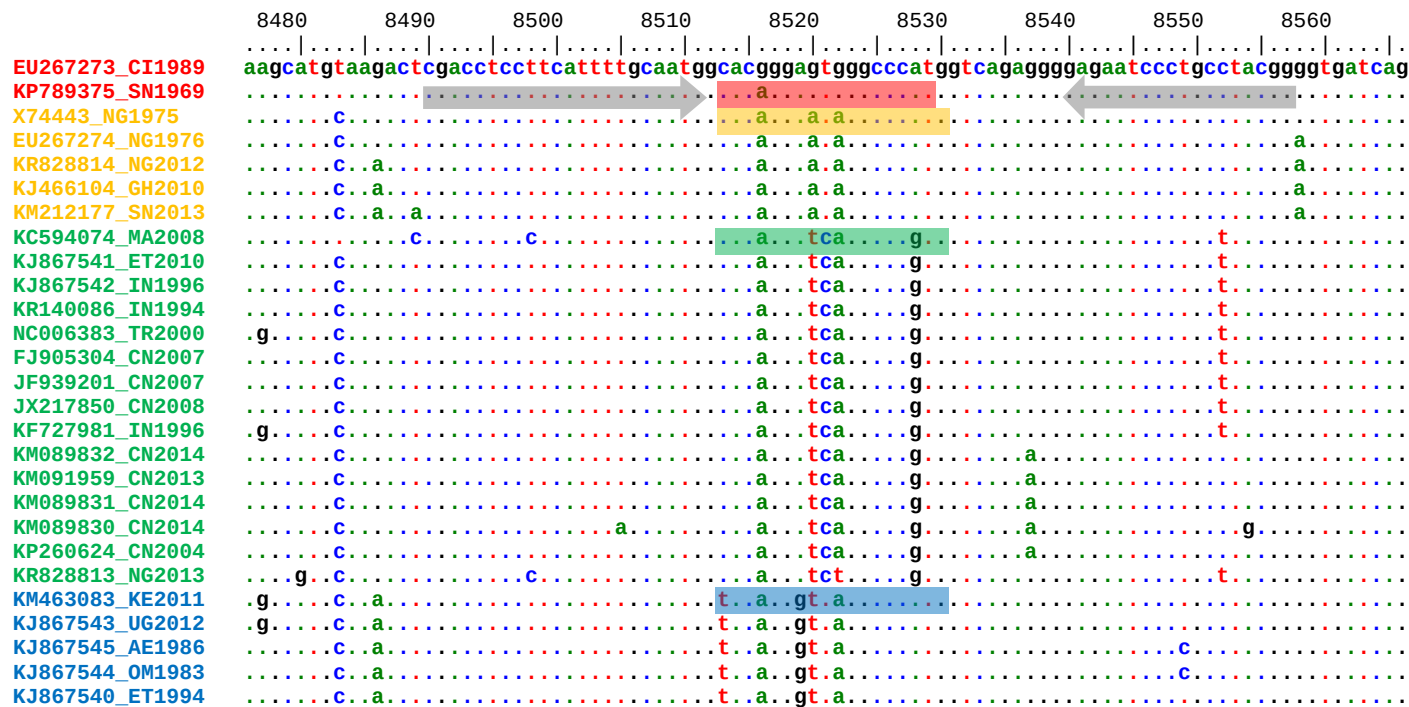


Figure 4.2: Location of a lineage-specific real-time RT-PCR assay on the H gene of PPRV. Primers are indicated by grey arrows, lineage-specific TaqMan® MGB probes indicated by coloured rectangles. Sequence names are coloured according to lineage (red - lineage I, orange - lineage II, green - lineage IV and blue - lineage III). Nucleotides are numbered according to the Genbank reference sequence (NC_006383).

4.4. Discussion

The recent outbreaks of PPRV in new areas of the world necessitated the need to develop an efficient protocol for lineage-specific surveillance of the disease.

The aa residues in the H protein were used to develop a single test that can differentiate various PPRV lineages without the need to sequence or the need to perform a phylogenetic analysis. However, the design of primers and probes in this variable region to discriminate individual lineages of PPRV from each other and from other morbilliviruses is challenging since the accumulation of mismatches in the primer/probe-binding within the PPRV group may result in detection failures.

The test was developed using the TaqMan® probes because of their higher sensitivity and specificity (Mackay, 2004). They are replacing gel-based conventional PCR in diagnostic and research laboratories (McGoldrick *et al.*, 1998; Leutenegger *et al.*, 2001; Rodriguez-Sanchez *et al.*, 2008).

It is hoped that this new RRT-PCR assay will be able to identify each lineage of PPRV in sheep, goat, and camel samples. The test need to be evaluated to ensure there is no cross reactivity between lineages of PPRV and with range of viruses which have genetic or structural similarities to PPRV. Assay evaluation is required to ensure that it does not detect phylogenetically or symptomatically related viruses of ruminants (such as rinderpest, bluetongue, and bovine viral diarrhea viruses). Once validated, this assay could be included in the panel of assays recommended by the OIE.

This study was unable to test the assay because of lack of access to all the different lineages of PPRV. However, the one-step TaqMan® real-time RT-PCR assay is a simple, sensitive, specific and rapid method for the lineage-specific diagnosis of PPRV.

CHAPTER

5. SEROPREVALENCE OF PPRV IN NIGERIAN SMALL RUMINANTS³

5.1. Introduction

There are many documented reports of the endemic nature of PPRV in Nigeria (Isoun & Mann 1972; Taylor & Abegunde 1979; Obi *et al.*, 1983; Shamaki 2002; Okoli 2003; Odo 2003; Ogunsanmi *et al.*, 2003; Obidike *et al.*, 2006; Ezeibe *et al.*, 2008; Ibu *et al.*, 2008; El-Yuguda *et al.*, 2010; Emikpe & Akpavie 2010; Ularamu *et al.*, 2012). Outbreaks have continued to occur in the country during the last 40 years, despite the introduction of the tissue culture rinderpest vaccine (TCRV) against PPRV in the 1980s and the use of the homologous PPRV vaccine (Diallo *et al.*, 1989; Diallo, 2003) since 1998. The National Veterinary Research Institute (NVRI) Vom, Nigeria, produces a 50-dose PPRV vaccine vial using the Nigeria 75/1 strain, with the recommendations that small ruminants must be vaccinated from three months of age and thereafter every 12 months.

Small ruminants play an important role in agricultural food production and in sustainable employment in Nigeria and the control and eradication of PPR is therefore a priority, in order to ease poverty and improve the health and husbandry of animals kept by resource-poor people in this developing country. This study was designed to determine the seroprevalence and distribution of PPRV antibodies in small ruminants in rural households, farms, live animal markets and slaughter slabs across different states in all the agro-ecological zones of Nigeria. This information will be helpful to develop a progressive control programme with the aim to eradicate PPR from Nigeria.

5.2. Materials and Methods

5.2.1. Study area

Each agro-ecological zone consists of administrative structures called States subdivided into Local Governments. Two states were selected at random from each agro-ecological zone:

³ The results presented in this chapter has been accepted for publication in the Onderstepoort Journal of Veterinary Research (Ms. Ref. No. 1035). Authours contributions: T.Y .W (NVRI Vom) was *responsible for experimental and project design and wrote the draft manuscript*. P.S.E (NVRI Vom) performed the statistical analysis, D.G.B (NVRI Vom), J.O.I (NVRI Vom), L.T (NVRI Vom), D.Y.D (NVRI Vom) and L.S (NVRI Vom) performed some of the experiments. H.M.K (Ahmadu Bello University), D.J.U.K (Abubakar Tafawa Balewa University), D.B (University of Birmingham) and D.S (NVRI Vom) made conceptual contributions. M.Q (University of Pretoria) was the project leader and contributed in writing the manuscript.

the north eastern agro-ecological zone is montane savannah and samples were collected from Adamawa and Taraba States; the south eastern agro-ecological zone is tropical rain forest and samples were collected from Anambra and Imo States; the south southern agro-ecological zone is mangrove/swamp and samples were collected from Akwa Ibom and Cross River States; the north central agro-ecological zone is Guinea/derived savannah and samples were collected from Kwara and Plateau States; the north western agro-ecological zone is Sudan and Sahel savannah and samples were collected from Kano and Sokoto States; the south western agro-ecological zone is tropical rain forest and samples were collected from Ogun and Ondo States (Iloeje, 2001).

5.2.2. Sample size and sample collection

The OpenEpi v2 open source calculator, SSPropor (Kevin *et al.*, 2009) was used to calculate the total number of samples to be collected per state using the equation: Sample size (n) = $DEFF \times Np(1-p) / [d^2/Z_{1-\alpha/2}^2 \times (N-1) + p(1-p)]$, where DEFF = design effect for cluster surveys, N = population size, p = hypothesized % frequency of outcome factor in a population and d = confidence limits as $\pm \%$. The calculated sample size per state was 379 (Table 5.1).

The combined population of domestic small ruminants in Nigeria in 2007 was estimated at 90 million (FAOSTAT 2007). Nigeria has a 36 state structure and a figure of 2,000,000 small ruminants per state was estimated for sample size calculations (in effect an infinite population). A percentage frequency of outcome factor in the population was hypothesized to be 44 % $\pm$ 5 based on a study by Shamaki (2002). A confidence limit of 5 % and design effect of one was used.

Multistage sampling was performed with four hierarchical stages. The first level of selection was the state. Two states were selected randomly from each agro-ecological zone of the country. Within each state, local governments, districts and villages were selected randomly. Within each selected village, animals were sampled from different homes, from the live animal market and from the slaughter slab. These were selected purposely because of logistical constraints.

About five ml of blood was collected by venipuncture. Sera were separated within 24 h after centrifugation at a relative centrifugal force of 1000 for 10 min. All samples were then stored at -20 °C until used.

Table 5.1: Apparent and true prevalence of PPRV in small ruminants in Nigeria, 2010 - 2013.

State	No. of samples analysed	No. positive	Apparent prevalence (%)	True prevalence (%)	X ²	p-value
Adamawa	379	76	20.05	20.52	30.9	0.001
Taraba	379	106	27.97	29.51		
Anambra	379	74	19.53	19.92		
Imo	379	102	26.91	28.31		
Akwa Ibom	379	94	24.80	25.91		
Cross River	379	56	14.76	14.52		
Plateau	379	79	20.84	21.41		
Kwara	379	101	26.65	28.01		
Kano	379	108	28.49	30.11		
Sokoto	379	84	22.16	22.91		
Ogun	379	67	17.68	17.82		
Ondo	379	71	18.73	19.02		
Sex						
Male	1,925	421	21.87	22.58		0.321 0.571
Female	2,623	597	22.76	23.59		
Species						
Sheep	1,059	244	23.04	23.91	0.220	0.639
Goat	3,489	774	22.18	22.93		
Age						
Adult	3,040	664	21.38	22.55	0.977	0.323
Young	1,508	354	23.48	24.41		
TOTAL	4,548	1,018	22.38	23.16		

5.2.3. Competitive enzyme linked immunosorbent assay (c-ELISA)

The commercially available c-ELISA kit (IDVET, France) for PPRV was used for this study. This diagnostic kit detects antibodies directed against the nucleoprotein of PPRV and was developed by a FAO reference laboratory (CIRAD-EMVT, Montpellier, France). The microplates were supplied in strips, already pre-coated with PPRV recombinant nucleoprotein. The kit contained anti-NP-HRP concentrated conjugate (10×), positive and negative controls, dilution buffers, wash concentrate (20×), substrate solution and stop solution (0.5 M H₂SO₄). The test was performed according to the manufacturer's instructions (Libeau *et al.*, 1995). The ELISA micro plates were read with an immunoskan reader (Flow laboratories, UK) with a filter of 450 nm. The optical density (OD) was recorded and the test was validated when the mean value of the negative control OD (ODNC) was greater than 0.7 and the mean value of the positive control OD (ODPC) was less than 0.3 of the ODNC.

The OD values were converted to competition percentage (CP) using the following formula: $CP = OD \text{ sample} / ODNC \times 100$. The samples with $CP \leq 35\%$ (cut-off) were considered positive for PPRV infection.

5.2.4. Statistical Analysis

The overall and group apparent prevalence (total positive/total sample analysed) and the exact binomial 95% confidence intervals were computed. Pearson's chi-squared test was used to determine if there were significant differences in the number of positive and negative tests results within each variable. A $p < 0.05$ was considered significant for all tests. The true prevalence together with 95 % confidence intervals for all categories was calculated using the Rogan-Gladen adjusted estimator of “true” prevalence (Rogan & Gladen, 1978). True prevalence (TP) was calculated using the following equation: $TP = (AP + Sp - 1) / (Se + Sp - 1)$ where AP = apparent prevalence, Sp = specificity and Se = sensitivity. The sensitivity of the c-ELISA test has been reported to be 90 % and specificity 98 % (Saliki *et al.*, 1993). All analyses were performed in R statistical software version 3.0.1 (R 2015).

5.3. Results

At a 95 % confidence level, a sample size of 379 animals per state was calculated. A total of 4,548 field serum samples were collected from 12 states across the agro-ecological zones of the country. No animal was known to have been vaccinated against PPRV before or at the time of sampling. Some animals appeared to have clinical signs compatible with PPR during sampling (Figure 5.1). Small ruminants were transported in vehicles that were often overcrowded, over long distances from the north of the country to the live animal markets in the south (Figure 5.2). On arrival at the markets, the animals were tied together as they awaited buyers (Figure 5.3).







Figure 5.1: Clinical signs (compatible with PPR) observed during sampling (2010 – 2013) in various locations in Nigeria (A - frothy salivation and muco-purulent nasal discharge, B - soiled anal region due to profuse diarrhoea, C - matted eyelids, salivation and nasal discharges, D - raised hair coat, depression, increased rectal temperature, E - frothy salivation, death).



Figure 5.2: Small ruminants were transported in an overcrowded vehicle from the north eastern part of the country to the south. Note the exposure to weather elements (sun, rain) in addition to the stress of transportation over long distances without feed or water.

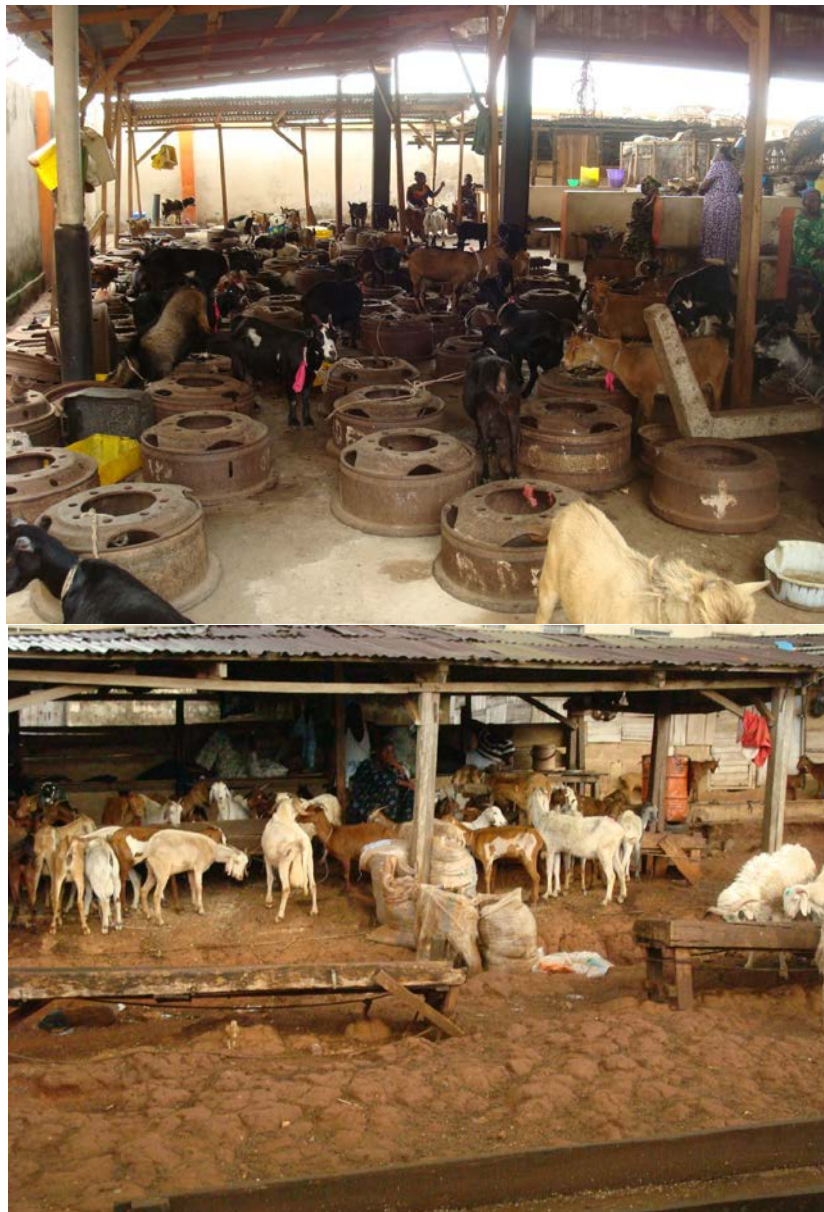


Figure 5.3: Live animal market in a south western Nigerian city. The animals were tied together as they awaited buyers. At the end of the day, the animals not sold were returned to the flock until the next market day.

The overall apparent prevalence estimate of sera positive for PPRV antibodies was 22.38 % ($n = 1,018/4,548$, 95 % confidence interval [CI]: 21.79 - 24.57), with a range from 14.5 – 30.1 %. The overall true prevalence estimate was 23.16 % (Table 5.1).

There were significant differences in the distribution of PPRV antibodies in small ruminants between states ($p = 0.001$). The highest seroprevalence was in Taraba State (29.51 %), while the lowest seroprevalence was observed in Cross River State (14.52 %) (Figure 5.4). The highest seroprevalence of PPRV in sheep where $n > 6$ was in Kano State (33.12 %, $n = 157$) and in goats it was in Taraba state (27.97 %, $n = 236$). The seroprevalence varied between localities, with a range of 12.64 % (Ikot-Omin, Cross River State) to 33.33 % (Kassa, Taraba

State). No spatial pattern was evident in the distribution of PPRV antibodies in small ruminant - the seroprevalence did not appear to be higher in states neighbouring other countries, nor higher in one region of the country.

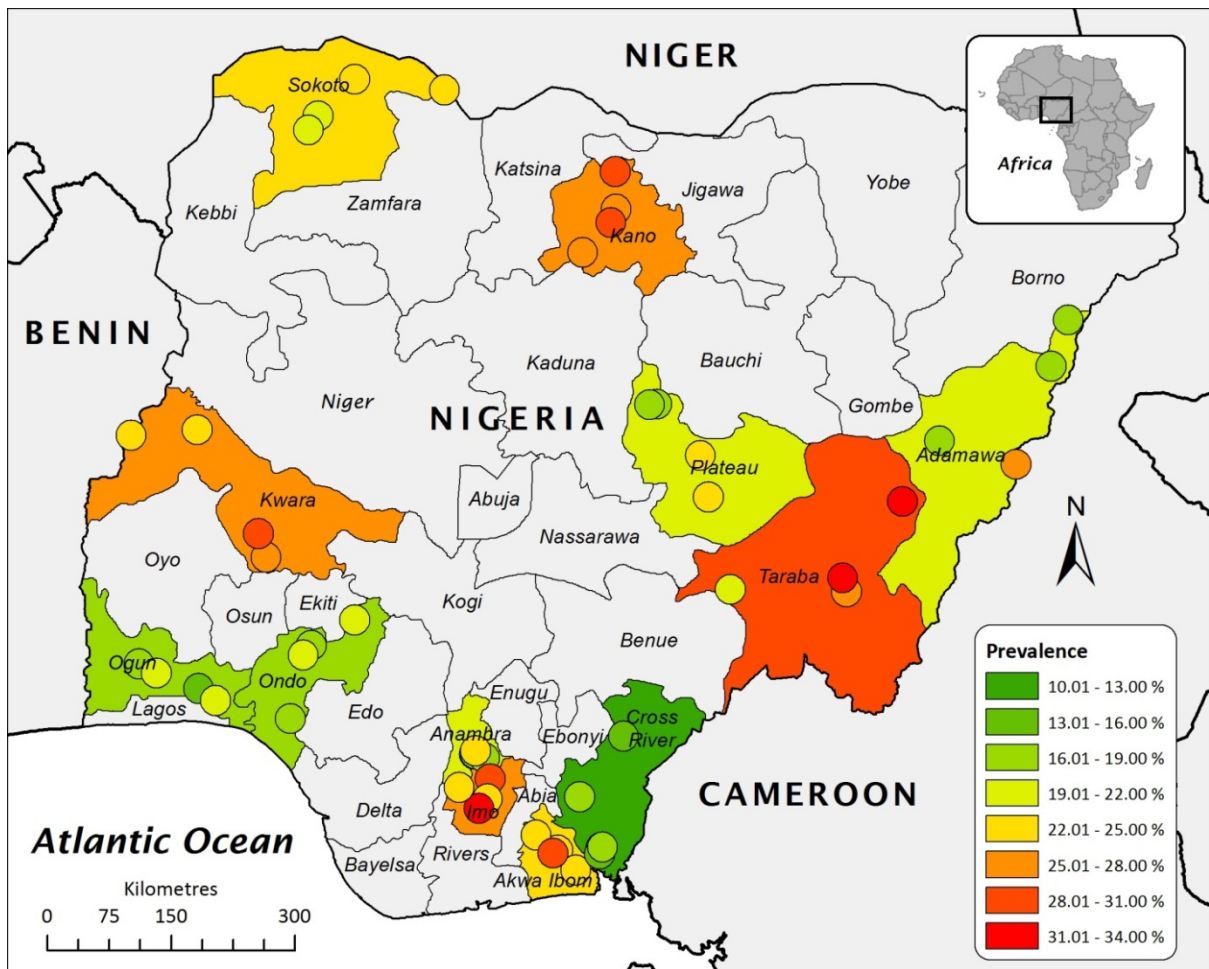


Figure 5.4: Prevalence of PPRV antibodies in each locality indicated by coloured circles. The colour of each state indicates the overall prevalence in that state.

From the 4,548 small ruminants that were sampled, 42.3 % were males and 57.7 % females. True prevalence was 22.58 % for males and 23.59 % for females. There was no significant difference in the PPRV seroprevalence between male and female small ruminants ($p = 0.571$).

The animals that were sampled ranged in age from three months to three years. The true prevalence of PPRV in animals above one year of age was 22.55 % and 24.40 % in animals between three and 12 months of age. There was no significant difference between the seroprevalence of PPRV in small ruminants over one year of age and those between three and 12 months of age ($p = 0.323$).

A total of 3,489 serum samples were collected from goats and 1,059 serum samples from sheep. The true prevalence in goats was 22.93 % and 23.91 % in sheep. There was no significant difference between the seroprevalence of PPRV in sheep and goats ($p = 0.639$).

The sample size in each locality varied from 43 (Ikot Eneobong, Cross River State) to 111 (Kassa, Taraba State) with a median of 95 (Figure 5.5). In most localities in the south of Nigeria, sheep were seronegative for PPRV. In this region the sample sizes in sheep were either small (Akwa Ibom, Anambra and Imo States) or no sheep were available for sampling (Cross River State), as most farmers breed with goats and sheep are scarce. However in Idanre and Fajaw, both in Ondo State, all 14 and 17 sheep respectively, were seronegative for PPRV. The highest seroprevalence of PPRV in sheep in a locality where the sample size was greater than two was in Gaya, Kano State (38.24 %, $n = 34$). The most sheep sampled in a locality ($n = 44$) was in Bafarawa (Sokoto State), where the seroprevalence of antibodies to PPRV in sheep was 22.73 % (Table 5.1).

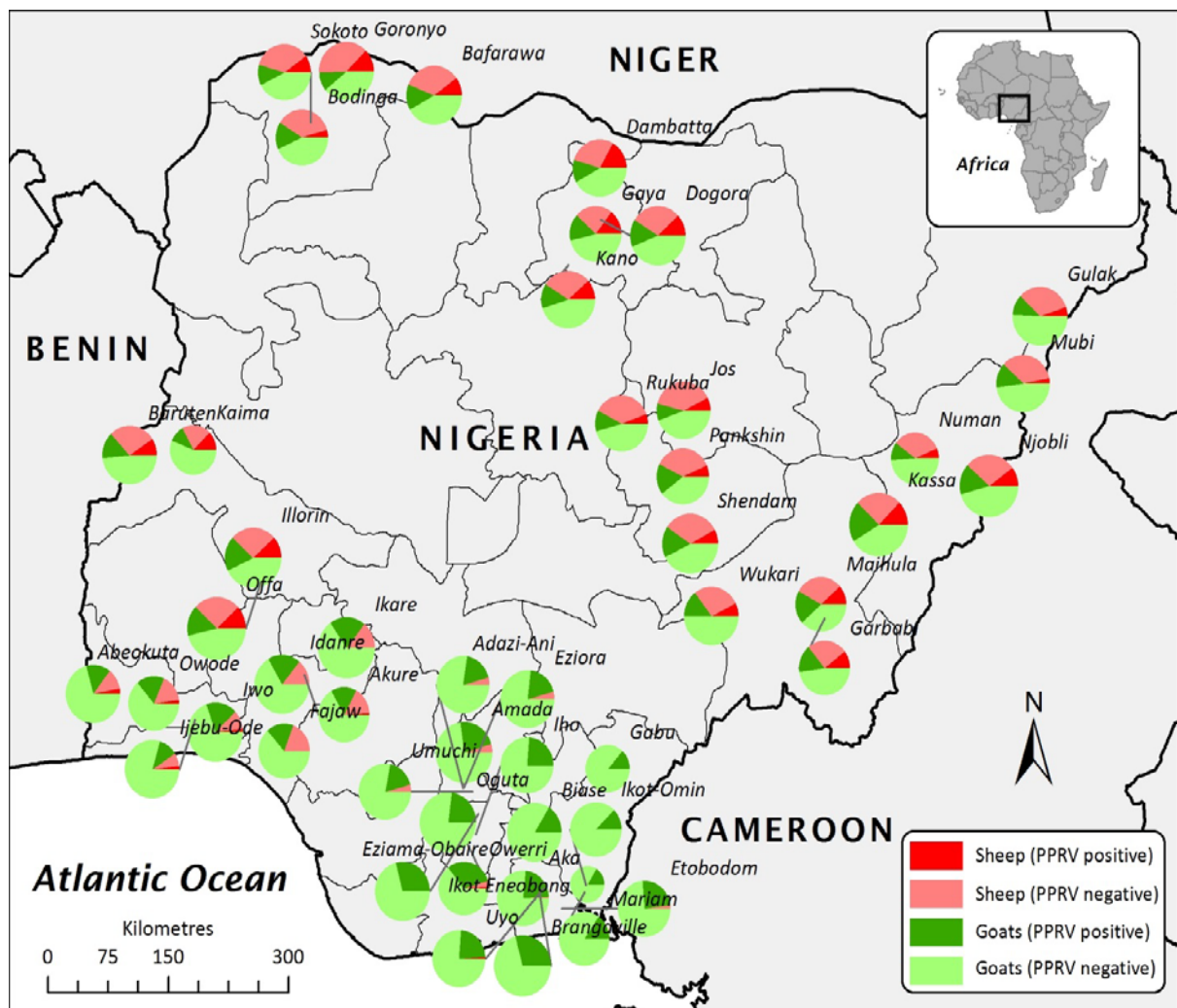


Figure 5.5: The proportion of sheep and goats seropositive to PPRV in Nigeria. The size of the pie symbol is relative to the number of samples collected.

Goats were present in all localities sampled, with a range of 43 (Ikot Eneobong, Cross River State) to 104 (Brangaville, Akwa Ibom State). The prevalence in goats ranged from 12.36 % (Ijebu-Ode, Ogun State) to 34.00 % (Maihula, Taraba State) (Figure 5.5).

The seroprevalence of PPRV antibodies in each animal category was very similar (20.44 % in male adult sheep, 22.29 % in female adult sheep, 21.64 % in young male sheep, 23.78 % in young female sheep, 23.89 % in adult male goats, 22.09% in adult female goats, 26.21 % in young male goats and 24.02 % in young female goats). The highest seroprevalence was 66.67 % in young female sheep from Kwara State (n = 18).

5.4. Discussion

Analysis of 4,548 samples from sheep and goats confirmed the endemic nature and wide distribution of PPRV throughout Nigeria. This study found an overall current seroprevalence of PPRV antibodies in small ruminants in Nigeria to be 23.16 %. Taylor & Abegunde (1979) in the 1970s reported a seroprevalence of 57 % and 44 % in sheep and goats respectively. Obi *et al.*, (1983) reported 52.2 % and 53.7 % in sheep and goats in the 1980s while Shamaki (2002) reported a seroprevalence of 44 % from samples analysed between 1995 and 1999. These differences may be due to the sampling locations, sample frames, seasons of the year, different sampling techniques and laboratory tests used. It is also possible that the higher seroprevalence obtained previously were due to sampling during outbreaks. The world-wide eradication of rinderpest virus, which cross-reacts with PPRV in serological tests, may also have been responsible for the lower seroprevalence we found in this study.

These results are similar to a study in Ethiopia which reported a seroprevalence of 33 % in sheep and 67 % in goats in the 1990s, but another study a decade later found the seroprevalence to be 9 % in goats and 13 % in sheep (Roeder *et al.*, 1994; Abraham *et al.*, 2005). The difference was less in Sudan (51.9 % compared to 67.2 %) in studies also conducted ten years apart (Haroun *et al.*, 2002; Saeed *et al.*, 2010).

Taraba State in the north-east agro-ecological zone had the highest seroprevalence rate of 29.51 % while Cross River State in the south-south agro-ecological zone has the lowest seroprevalence rate of 14.52 %. This observation may be due to the prevailing management practices in the various states and rainfall, which affects the growth of grasses. Most of the small ruminants in Taraba state are on a free range (agro-pastoralism) system since the

farmlands for crops are a distance from most villages and the zone is a savannah with a longer dry season period. Most animals in Cross River State are tied at home and grass is provided since crops are planted in the backyard. Cross River State is a rain forest zone with abundant rains that enables grass to grow almost all the year round. The sheep breeds observed in Taraba State were of the Uda, Balami and Yankassa breeds while those seen in Cross River State were predominantly of the West African dwarf (WAD) stock. The WAD breeds of goats were also seen in Cross River State while those in Taraba State were a mix of both the WAD and the Sokoto Red. It is not clear whether these breed differences in small ruminants had any effect on the difference in seroprevalence rate observed in the two states.

Transportation, management practices and marketing play major roles in the epidemiology of PPRV. We observed that animals from different households and localities are usually transported together in close proximity (Figure 5.2) to the market. At the market, the animals are tied together (Figure 5.3) in stalls as they awaited buyers. Any animal not sold is returned to the flock and new animals bought also end up in new flocks. These practices are conducive to outbreaks of diseases. New infections leading to outbreaks normally occur when animals are transported together or when they are tied together (transportation stress, overcrowding, malnutrition) in the live animal markets or kept for safety reasons with a relative from another locality.

In this study, there was an over-representation of female animals (2,623 females and 1,925 males). This may be due to the practice of sacrificing males when the need to sell or slaughter animals arises.

The c-ELISA is an ideal test to carry out a serological survey of PPR virus antibodies in small ruminants. The c-ELISA was used in this study because of the merits it has over other serological techniques, which are more laborious and time consuming.

The results of this study may have been biased by the sampling technique employed. Due to logistical constraints and the complexity of the pastoral husbandry practices, it was not possible to systematically select the different villages, farms, households and the animals to include in this study. Live sheep and goat markets and slaughter slabs were also sampled randomly in each locality visited. A more accurate seroprevalence may emerge if randomized sampling is done where the actual small ruminant population in each state (data such as this is currently not available in Nigeria) is taken into account.

An important observation during the course of sampling is the apparent lack of awareness among the small ruminant owners of the vaccination status of their flocks. The control and eradication of PPR is a priority in order to ease poverty and improve the health and husbandry of animals kept by people in developing countries. It is encouraging to note that between 18 - 23 November 2013, heads of veterinary laboratories and directors of veterinary services from the ECOWAS sub-region gathered in Lagos, to develop a framework for the progressive control of PPR in the region. This type of sub-regional approach also took place in southern Africa and a PanAfrican strategy for the progressive control of PPRV has also been developed (Elsawalhy *et al.*, 2010).

This study provides an overview of the current antibody seroprevalence to PPRV in the small ruminant population in Nigeria and also confirms a low level of awareness about vaccination among small holder famers in rural settings.

CHAPTER

6. SEROPREVALENCE OF PPRV IN NIGERIAN CAMELS⁴

6.1. Introduction

Camel production is important in northern Nigeria because of the adaptation of this animal species to areas where there is shortage of forage and water and adverse climatic conditions. Despite this important role of camels for pastoralists, the species has not received enough attention with regard to improvements in its health status and productivity (Tefera & Gebreah 2001; Swai *et al.*, 2011; Touil *et al.*, 2012). Although camels are comparatively hardy animals and only few viruses are known to cause diseases in this species, they are susceptible to many infectious diseases (Bekele 1999; Tefera & Gebreah 2001; Swai *et al.*, 2011; Touil *et al.*, 2012) and may serve as reservoirs of infection to other in-contact animals during grazing. Camels may be a potential pathogen carrier in the transmission of several vector-borne infections such as bluetongue virus, African horse sickness virus, West Nile virus and Rift Valley fever virus (Touil *et al.*, 2012). In Nigeria, the camel population is located in the Northern States and camels represent an important economic resource for the pastoral population in these States. Evidence of camels infection by PPRV has been recorded in many reports (Shamaki 2002; Haroun *et al.*, 2002; Abraham *et al.*, 2005; Khan *et al.*, 2008; Chauhan *et al.*, 2009; Albayrak & Gür, 2010; Khalafalla *et al.*, 2010; Kinne *et al.*, 2010; Balamurugan *et al.*, 2012). Seroprevalence in camels varied considerably depending on the countries or regions investigated and the type of assay employed: it can be as high as 38 % (Daneji *et al.*, 1997; Roger *et al.*, 2001; Abraham *et al.*, 2005; Abubakar *et al.*, 2008; Ibu *et al.*, 2008).

In Nigeria camel breeding is concentrated mainly in the northern region with an estimated population of about of 20 000 heads (Blench, 1993; FAO, 2012). There are no large scale camel farms in Nigeria. Most camel owners own between two to six camels. Camels are sent to slaughter either because of age, ill health, or when the owner needs money.

The aim of this study was to determine the seroprevalence and extent of PPRV antibodies in camels in Northern Nigeria. The results of this study will enhance the knowledge of the

⁴ The results presented in this chapter has been published in Tropical Animal Health and Production (2015) 47: 603 – 606. Authours contributions: T.Y .W (NVRI Vom) was *responsible for experimental and project design and wrote the draft manuscript*. P.S.E (NVRI Vom) performed the statistical analysis, H.G.U (NVRI Vom), S.C.C (NVRI Vom), I.I.L (NVRI Vom), D.B.B (NVRI Vom), N.D.T (NVRI Vom) and G.B.A (NVRI Vom) performed some of the experiments. A.D. (IAEA), D.J.U.K (Abubakar Tafawa Balewa University), D.B (University of Birmingham) and D.S (NVRI Vom) made conceptual contributions. M.Q (University of Pretoria) was the project leader and contributed in writing the manuscript.

epidemiology of PPR in the country as camels share grazing land and drinking points with sheep, goats and cattle.

6.2. Materials and Methods

Abattoirs in Maiduguri (Borno State), Kano (Kano State), Katsina (Katsina State) and Sokoto (Sokoto State), northern Nigeria were selected as sites for sample collection. These sites were selected, as they are the only major camel abattoirs in their States. The majority of the camels in Nigeria (an estimated 80 %) occur in these four states and they cover a combined area of about 70,714 km² and are the desert gateways with important camel trade. The other 20 % of the camel population occur in the states of Yobe, Jigawa, Kebbi and Zamfara (Figure 6.1).

Our aim was to collect a maximum of 384 samples per state based on calculations using a prevalence of 50 %, a confidence of 95 % and a margin of error of 5 % (Dohoo *et al.*, 2009). Convenience sampling was used: samples were collected from every fifth slaughtered camel every other day (excluding Sundays) between July 2011 and April 2013.

Approximately 10 ml of blood was collected from the jugular vein of each slaughtered animal in a serum vacutainer tube (Becton Dickson, UK). Each sample was labelled using codes to describe the state, sex, age, and body condition score of the camel. The serum was obtained after allowing the blood to clot overnight, and centrifugation at 2500 *g* for 15 min. Samples were transported to the laboratory on ice and stored at –20 °C until analyzed.

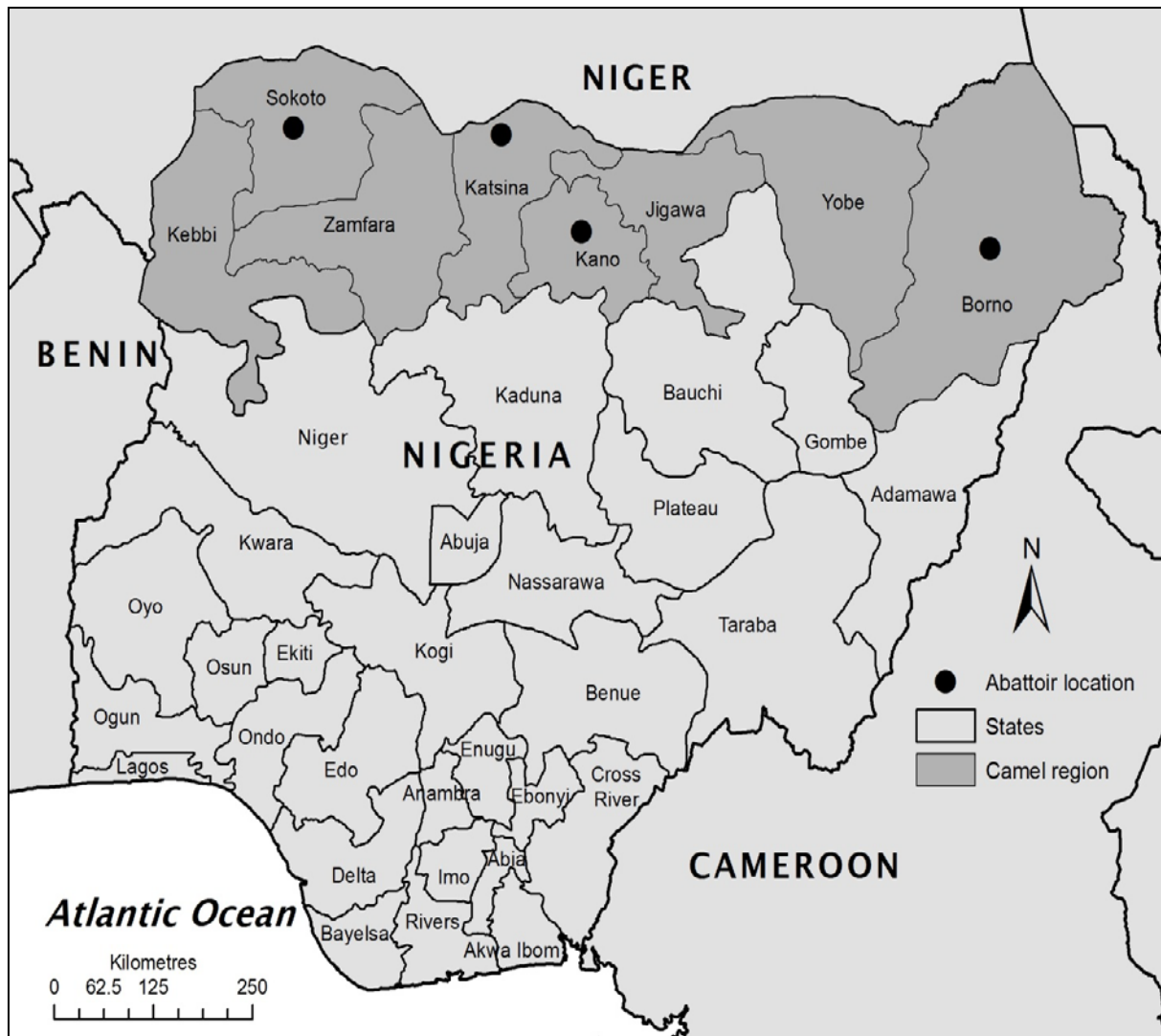


Figure 6.1: Map of Nigeria showing the States where camels are predominantly found and where samples were collected.

6.2.1. Competitive enzyme linked immunosorbent assay (c-ELISA)

A PPRV H-protein-based monoclonal antibody (Mab) competitive enzyme linked immunosorbent assay (c-ELISA) kit (BDSL, UK) was used for the detection of antibodies to PPRV according to the manufacturer's instruction (Anderson *et al.*, 1991). All serum samples tested in the present study were processed in duplicates and a cut-off value of 50 % was used. The ELISA micro plates were read with an immunoskan reader (Flow laboratories, UK) with an inference filter of 492 nm. The reader was connected to a computer loaded with ELISA data information (EDI) software which was used to automate the reading and calculate the percentage inhibition (PI) values. The OD (optical density) values were converted to percentage inhibition using the following formula:

$$PI = 100 - (OD \text{ control or test serum} / OD \text{ MAb control}) \times 100$$

The samples with $PI \geq 50\%$ (cut-off) were considered positives for PPRV infection.

6.2.2. Statistical analysis

The overall and group apparent prevalence (total positive/total sample analysed) and the exact binomial 95 % confidence intervals were computed. Pearson's chi-squared test was used to test if there were significant differences in the number of positive and negative tests results within each variable. A $p < 0.05$ was considered significant for all tests. The true prevalence together with 95 % confidence intervals for all categories was calculated using the Clopper-Pearson exact method (Clopper & Pearson, 1934). True prevalence = $(AP + Sp - 1) / (Se + Sp - 1)$ where AP is apparent prevalence, Sp is specificity and Se is sensitivity. The sensitivity of the cELISA test has been suggested to be 90 % and specificity as 98 % (Saliki *et al.*, 1993). All analyses were performed in R statistical software version 3.0.1 (R, 2015).

6.3. Results

A total of 1,517 sera samples were collected in abattoirs from camels submitted for slaughter. The study subjects were all ages (ranging between 1 – 10 years) and sexes. Overall prevalence estimate of serum positive results for PPRV was 3.36 % ($n = 51/1517$, 95 % CI: 2.51 – 4.39) (Table 6.1).

Kastina State had the highest seroprevalence rate of 4.05 % while the lowest rate of 3.0 % was observed in Borno State, however, there were no significant differences in prevalence between the states ($p = 0.892$). Of the 1,517 camels sampled, 984 (64.9 %) were males and 533 (35.1 %) females. There was no significant difference in the PPRV prevalence between male and female camels ($p = 0.742$) (Table.6.1).

The prevalence differed significantly ($p < 0.001$) by body score. The prevalence was highest in camels with poor body condition score (16.67 %). There was a statistically significant difference between camel aged ≤ 5 years and those > 5 years ($p = 0.004$) (Table 6.1).

Table 6.1: The proportion of camels in each category and mean serum antibody prevalence profiles of each variable investigated during the study ($n = 1,517$).

	No. of samples analyzed	No. positive	% positive	χ^2	p-value
States					
Borno	433	13	3.00	0.6189	0.892
Kano	517	17	3.29		
Katsina	296	12	4.05		
Sokoto	271	9	3.32		

Sex					
Males	984	32	3.25	0.1084	0.742
Females	532	19	3.57		
Body Score					
Poor	42	7	16.67	24.7109	< 0.001
Fair	846	29	3.43		
Good	628	15	2.39		
Age Category					
Adult (> 5 years)	1241	34	2.74	8.2043	0.004
Young (≤5 years)	275	17	6.18		

Using a cut-off level of 50 % and, sensitivity of 90 % and specificity of 98 %, the overall true prevalence estimate of serum positive results for PPRV in the studied camel population was calculated to be 1.55 %. The true prevalence per state was calculated to be 1.14 %, 1.46 %, 2.33 % and 1.50 % for Borno, Kano, Kastina and Sokoto states respectively. True prevalence was 1.43 % for males and 1.79 % for females. True prevalence for camels aged 5 years and above was 1.62 % and for those below 5 years it was 4.75 %. In the body condition score category, true prevalence was calculated to be 16.67 %, 1.62 % and 0.44 % for the poor, fair and good status respectively (Table 6.2).

Table 6.2: Apparent (AP) and true prevalence (TP), with 95 % confidence interval (CI) of PPRV in camels in Nigeria.

Variables	AP (%)	95 % CI	TP (%)	95 % CI
States				
Borno	3.00	1.61 – 5.08	1.14	0.00 – 3.49
Kano	3.29	1.93 – 5.21	1.46	0.00 – 3.65
Katsina	4.05	2.11 – 6.97	2.33	0.13 – 5.65
Sokoto	3.32	1.53 – 6.21	1.50	0.00 – 4.78
Sex				
Males	3.25	2.24 – 4.56	1.43	0.27 – 2.91
Females	3.57	2.16 – 5.52	1.79	0.19 – 4.00
Body Score				
Poor	16.67	6.97 – 31.36	16.67	5.65 – 33.37
Fair	3.43	2.31 – 4.89	1.62	0.35 – 3.28
Good	2.39	1.34 – 3.91	0.44	0.00 – 2.17
Age Category				
Adult (> 5 years)	2.74	1.91 – 3.81	1.62	0.84 – 2.05
Young (≤5 years)	6.18	3.64 – 9.71	4.75	1.87 – 8.77
Overall	3.36	2.51 – 4.39	1.55	0.58 – 2.72

6.4. Discussion

Camel production is important in northern parts of Nigeria. It is commonly used as source of draught power and it is also a ready source of animal protein for the human population. PPR is endemic in Nigeria and in many parts of Africa and Asia. The FAO and the OIE has initiated a global PPR control programme like the one which has been successfully implemented for the eradication of rinderpest. Since camels are among potential susceptible animal species, we therefore decided to sample Nigerian camels between 2011 and 2013 to determine the seroprevalence of PPRV in this species.

In the study area, majority of the rural household economy is based on the pastoralism and different animal species co-mingling at grazing and drinking points, and the live animal markets. In those conditions, pathogens may circulate from one species to another whatever the sensitivity of the animal. This situation highlights the importance of including camels among the group of animals to be monitored for PPRV infection and to define their role in the epidemiology of the disease in Nigeria and elsewhere.

In this study, the overall apparent prevalence for all states studied was 3.36 %. The PPRV antibodies that were detected result from natural infection of the camels, as there is no documented evidence that camels are vaccinated against PPRV in and around Nigeria. This agrees with earlier reports of 4 % (out of 250 samples) from camels in Sokoto (Daneji *et al.*, 1997), Kano and Maiduguri (Ibu *et al.*, 2008). The seroprevalence rate detected in this survey is also comparable to studies done in other African countries. PPRV seroprevalence rate in camels has been reported to be 3 % in Ethiopia (Abraham *et al.*, 2005), 2.6 % in Tanzania (Swai *et al.*, 2011), 4.2 % (using the serum neutralization test) and 14 % a decade later in Egypt (Ismael *et al.*, 1992; Haroun *et al.*, 2002). A lower seroprevalence rate of 0.3 % was recorded in camels in Sudan (Saeed *et al.*, 2010) even though there was an outbreak of PPR in camels in the country with devastating clinical signs (Khalafalla *et al.*, 2010).

The variation in seroprevalence of PPRV in camels observed in different countries might be attributed partly to differences in diagnostic techniques, sample size and method, seasons of the year, management system, health care practices and presence of other concurrent disease infections (Bekele 1999; Roger *et al.*, 2001; Khalafalla *et al.*, 2010). Although the detection of PPR antibodies in camels in this study may not indicate clinical disease, it remains to be investigated whether the existence of the virus in camels has any epidemiological significance of virus persistence and transmission to other animal species.

The higher seroprevalence of PPRV among camels with poor body condition score (16.67 %, $p < 0.001$) and the statistically significant difference ($p = 0.004$) observed between aged (≤ 5

years) and younger (> 5 years) camels in this study may be due to the ability of PPRV, like all other morbilliviruses to cause severe immunosuppression in its host.

The results of this study may have been biased by the convenience sampling employed. Currently, there is no census data of camels on a state level in Nigeria. Some of the camels are brought into the country through the porous borders with Niger and Chad to village live animal markets for sale. The sampled abattoirs are the only ones in the sampled states. The others in the sampled states are slaughter slabs and these do not slaughter camels but cattle, sheep and goats. A more accurate prevalence will emerge if a randomized sampling is done and the camel population in each state is taken into account. This however was not done due to logistic constraints.

In conclusion, the findings of this study show that PPRV is circulating in Nigerian camels and there is a need for further investigations into the genome characterization and epidemiology of PPRV, to determine the role camels play in the endemicity of PPRV in the Nigerian sheep and goat populations.

CHAPTER

7. GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

7.1. General discussion

The fragility of PPRV means that efficient virus transmission occurs only through close contacts between infected and susceptible animals. Such conditions are often met in and around shared watering holes and grazing sites. As was observed during this study, transportation of animals from different villages in one vehicle to the market, and the live animal markets are all ideal areas for virus transmission. In some parts of Nigeria with a high percentage of Muslims, peaks of PPR outbreaks are recorded just after the Eid festival when there is increased trade in sheep. Another period of high frequency in PPR outbreaks is the dry hammattan period (December – March), when sheep and goats are allowed to graze in the fallows after crop harvest, and there is a better preservation of the virus under “cold” conditions and/or the tendency of the animals to huddle closer to each other for warm (Ezeibe *et al.*, 2008; Abubakar *et al.*, 2009). The effect of cold weather and its associated stress also reduce immunity and increase the risk to PPR in sheep and goats. PPR outbreaks in the same area occur in cycles of three years. This is due to the fact that animals that recover from PPR are protected for life, so PPRV can only be maintained in large populations if there is a turnover of susceptible hosts (newborns, transhumance, and newly purchased animals). This knowledge is essential when planning a nationwide vaccination campaign.

The most common outcome of PPR is death with case fatality rates that may exceed 90 % in naïve populations (Diallo, 2006). Camels, buffaloes, cattle and small ruminant wildlife species are susceptible. Ruminant wildlife, by sharing grazing areas and water points with domestic animals, may play a role in the epidemiology of the disease; however, their role remains undefined (Banyard *et al.*, 2010). This study found antibodies to PPRV in camels while an earlier study detected PPRV nucleic acid in a captive warthog (Shamaki, 2002). The warthog may be an accidental host.

The FAO (2009) estimated that 63 % of small ruminants globally, are at risk of PPR, especially those from southern Africa, central Asia, South East Asia, China, Turkey, and southern Europe. Apart from being a major disease burden, the economic impact of PPR is considerable, with significant effects on food security and animal production. It is a listed animal disease, which if identified, has to be notified to the OIE.

The number of sheep and goats in countries which reported PPR to the OIE numbered more than 750 million in 2002 (Diallo, 2003). Currently, about one billion domestic small ruminants are at risk of PPR infection. Most countries affected by PPR cannot afford stringent but drastic sanitary control measures, such as a stamping out policy, hence the only effective means to control PPR is by vaccination. This approach has advantage because immunity from morbillivirus infection or vaccination is life-long. Although PPR vaccines are available from many manufacturers, studies have shown that the vaccination coverage of small ruminants is so low, about 5 %, that effective control of the disease is not possible (Diallo, 2003). In the case of rinderpest, it was estimated that a minimum herd immunity of 75 – 80 % was needed for its control (Rossiter & James, 1989) and this is probably the case for PPR as well. Since the turnover of small ruminants is high, at an annual rate of about 33 %, it means that every three years all animals of a flock will be susceptible to PPR in the absence of vaccination. Unfortunately, it seems that in most cases, there are no actual effective programmes for the control of PPR in place and vaccinations are implemented mainly as precautions in the face of PPR outbreaks, to avoid the spread of the disease (Libeau *et al.*, 2014).

The impact of PPR in wildlife populations (some endangered) in Asia has been severe, with high fatalities recorded in bharal in Tibet (Bao *et al.*, 2011, 2012), ibex in Pakistan (Abubakar *et al.*, 2011) and wild goats in Kurdistan (Hoffmann *et al.*, 2012). All these were related to PPRV-infected livestock. The huge impact on small ruminant production has resulted in PPR emergence as an essential global animal health concern and has been proposed as the next animal disease to be eradicated after rinderpest (FAO, 2013).

There is the need for more studies on PPRV genetic diversity in order to unravel its evolutionary path, particularly since data from some geographic regions and individual countries are under-represented. All phylogenetic analyses undertaken to date revealed four distinct lineages of PPRV with strong bootstrap support for the critical nodes using adequate small variable sequences. Branching into four lineages was observed with analysis of the complete open reading frames of major structural protein genes, notably the N, M, F, H (Balamurugan *et al.*, 2010a) and L genes (Minet *et al.*, 2009). This study used the complete genomes for phylogenetic analysis and it showed that the mutation rate differs between genes, thus there is no constant evolutionary rate. However, we obtained the same differentiation of lineages, suggesting that analysis of individual genes is sufficient to determine the PPRV lineage of an isolate.

Lineage identification shows the likely origin of the virus causing a new outbreak. PPRV lineage identification showed that the outbreak in Morocco in 2008, the first time the virus had been seen in north Africa, was a lineage IV virus, and therefore had not come from west Africa, where all the viruses have been lineages I or II, but was likely to have come from the Middle East, Turkey or Sudan, where lineage IV PPRV strains circulate. PPRV isolates in Yemen and Oman are mostly lineage III, reflecting the major movements of sheep and goats from the Horn of Africa to these countries. Interestingly, older outbreaks of PPRV in Sudan were lineage III, while recent outbreaks have been lineage IV, suggesting a change in animal movement patterns in that region. Virus transmission is through direct contact with infected animals, or through contact with fresh secretions or faeces from infected animals. Transfer of the virus across any distance is through the movement of livestock, usually through migration or trade; introduction of disease into new areas is almost always associated with the movement of livestock and the first level of control is therefore the restriction of movement of animals from endemic areas, with rigorous quarantine and surveillance procedures if a total ban is not practicable (Libeau *et al.*, 2014).

Currently, PPRV suspected samples need to be amplified, sequenced and phylogenetically analysed to determine the lineage to which they belong. Since most laboratories may not have the facilities for sequencing, this study developed a lineage specific real time TaqMan®-based PCR for the diagnosis of PPRV. Once this test is evaluated and approved, it will contribute as an important test for tracing the possible origin of outbreaks in new geographical areas.

Although a highly effective vaccine has existed since 1989 (Diallo *et al.*, 1989), PPRV remains an emerging problem in other regions and animal species. Libeau *et al.*, (2014) have given the following reasons:

- negligence to the small ruminant health sector in all the countries now enzootic for the disease, leading to a lack of performing diagnostic tests,
- size and more rapid mobility of small ruminant populations,
- Cessation of rinderpest circulation, which is generally mild in small ruminants and which cross reactive antibodies allowed for an additional gain in protection.

All the above reasons hold true for the Nigerian situation. This study confirmed that most people in rural areas don't vaccinate against PPR even though there is an effective, government subsidized homologous vaccine produced by the National Veterinary Research Institute, Vom. The majority of small ruminants are owned by poor farmers who have little

contact with field veterinarians and cannot afford the cost of vaccination on a routine basis. A high number of these farmers are also nomadic in nature, moving their animals to other parts of the country, as dictated by the rainfall and grass/water availability. Politicians and governments at all levels conduct sporadic vaccination campaigns from time to time, this uncoordinated campaigns may play a role in the epidemiology of PPRV.

The treatment of animals affected by PPRV involving the administration of anti-PPRV serum or antibodies in association with antidiarrhoeal medicines has been reported in Nigeria (Anene *et al.*, 1987; Akpan *et al.*, 1999). However, this approach may not be of practical use in the field, given the low price of individual sheep and goats.

Although sub-Saharan Africa, the Middle East, and Asia experienced severe epidemics, the genetic variation of PPR viruses suggested that outbreaks observed, at least until late 1990, were seeded locally within each of the four specific geographic basins where PPR is endemic. An exception was noted in the Middle East where a virus belonging to lineage III was isolated within wide circulation of lineage IV viruses, most likely as a result of the importation of a PPRV affected animal from East Africa into this region, where this lineage was widespread. Co-circulation of these two PPRV lineages in a region that imports animals from both East Africa, (lineage III), and many Asian countries (lineage IV), is expected. This study confirmed that both lineage II and IV viruses are co-circulating currently in Nigeria and that lineage IV seems to be replacing lineage II. The lineage IV virus seems to be the predominant lineage in circulation globally. Constant increase of disease incidence has been associated with this lineage, particularly that which occurred in North and East Africa and in Asia, from 2004 to 2013, thus suggesting an increase in virulence (Kwiatiek *et al.*, 2011).

The availability of bivalent or polyvalent PPR vaccines will be very useful for control in endemic countries. Because PPR and capripox share almost the same geographical distributions, the F and H protein genes of PPRV have been inserted into the capripox virus genome to produce a dual thermostable recombinant vaccine to protect small ruminants against these two economically important diseases (Berhe *et al.*, 2003; Chen *et al.*, 2010). A similar attempt had been made in Cameroon to protect goats from PPR and capripox by single immunisation with combined vaccine containing live attenuated viruses (Matrencher *et al.*, 1999). A combination of PPR, orf, and sheep pox vaccines has been developed in India (Hosamani *et al.*, 2006; Chaudhary *et al.*, 2009). Single vaccination against two or more diseases can significantly bring down the cost of vaccination and reduce the stress to the animal.

Transhumance and trade, as a factor in the spread of PPRV is an area that deserves special consideration and further investigation. Host susceptibility is considered of great importance in the manifestation of the disease. An example is the PPR outbreak reported by Diop *et al.* (2005), who described very severe symptoms in animals of West African Dwarf breeds, while those of the West African Long-Legged breed showed mild symptoms. However, the idea that different breeds/species may show different clinical signs requires further examination.

In addition to the 'emergence' of PPR due to improved recognition, there is also the possibility that the eradication of rinderpest may have facilitated the spread of PPR, since sheep/goats exposed to RPV are immune to PPRV (Taylor, 1979). Circulating rinderpest in cattle may have suppressed PPR spread by partially immunising in-contact sheep/goat flocks. However, PPR has spread dramatically in the past ten years, appearing in countries that have been free of rinderpest for many decades (example China, Morocco), and whose sheep/goat populations, therefore, could not have been protected by the RP antibodies (Libeau *et al.*, 2014).

It is a generally held view that the factors that favoured the eradication of rinderpest were: a safe and reliable vaccine covering all strains of the disease; the availability of simple and effective diagnostic tests; a short infectious period (no persistence or carrier state); transmission only by close contact; an economic incentive for local and national participation and compliance with the programme (Rweyemamu *et al.*, 2006). Reports from the experience during the rinderpest eradication programme have indicated that the importance of epidemiologically targeted vaccination, extensive and continued surveillance to ensure the continued absence of disease and the preparation of proper contingency plans against re-emergence or re-introduction of the virus were important (Baron *et al.*, 2011). It was also considered of critical importance that the programme was time-limited, which put pressure on countries to proof and confirm that they were free of disease by a certain date (2010). In the situation of PPRV, it is clear that many of the requirements for the eradication of this disease are already well established as for RPV. Like RPV, there is only one serotype of PPRV. Diagnostic tests are available for PPR including a similar safe attenuated homologous vaccine (Baron *et al.*, 2011).

It is essential to fully understand the role of wildlife in the transmission and potential maintenance of PPRV in the environment in order to be able to initiate successful control strategies (Baron *et al.*, 2011). A major concern in the past has centered on the economic incentive to control PPR; sheep and goats are of much less individual value than cattle, and

have a shorter working life, which means a faster turnover of animals, and a consequent need for more vaccine and people to administer it in the field. Accurate assessment of benefit-cost ratios are difficult but possible, and the need to take into account the socioeconomic benefits in both the agricultural sector and other areas dependent on it (example trade, food distribution/retail) (Baron *et al.*, 2011). Such analysis has only recently been carried out for rinderpest (Roeder & Rich, 2009), where the results indicated that the real benefits were much higher than had been originally estimated. The ever-advancing spread of PPR has made the economic impact of the disease, and consequently the benefits of its eradication, much greater. The imperative for coordinated action is therefore much stronger (Baron *et al.*, 2011).

7.2. Conclusions

The specific research aims of this work were: to serosurvey PPRV in camels, sheep and goats in Nigeria; to isolate PPRV from naturally infected animals; to characterize the isolates using molecular techniques and to sequence the full genome of representative current viral isolates from Nigeria and compare them with historical isolates used worldwide as vaccine strain (Nigeria75/1). In line with these objectives, the major contribution to knowledge of this work was findings that more than one lineage of PPRV is circulating currently in Nigeria, the “emerging” Asian lineage IV may be gradually replacing the “traditional” lineage II in the country; the isolation in cell cultures and full genome sequencing of current field viruses which was last done in 1976 and the current seroprevalence report of PPR in camels, sheep and goats from all the agro-ecological zones of the country. This work has also increased the sequences of PPRV available globally in the GenBank.

Nigeria is home to approximately 90 million small ruminants (FAO, 2007). Sheep and goats play a significant socio-economic role in the life of rural people. PPR is considered the most important single impediment to the health of small ruminants in Nigeria and most countries where it is endemic. Considering the economic importance of the effect of PPR on domestic small ruminants, there is the need for constant review of PPR protocols in terms of proper recognition, management and control. Much more needs to be done to understand the epidemiology of PPR, for example, the role of cattle and camels in the epidemiology of the disease, variation in the virulence of virus strains, and maintenance of the disease in an enzootic status.

This work was conceived with the main aim of increasing small ruminant production in Nigeria through improved serosurveillance, isolation and molecular characterization of

currently circulating PPRV isolates in camels, sheep and goats. This work serves as a tool for effective planning for a nationwide vaccination campaign for improved control of PPR in Nigeria. Improved control of PPR will be beneficial to farmers, as it will boost their potentials to produce small ruminants, increase their economic power and provide meat and milk products to meet the minimum human requirement for protein of animal origin. Controlling PPRV would have positive market and non-market benefits, such as healthier animals (example increased fertility, increased meat quality and quantity) additional income through renting of animals, increased milk production and eventually improved market access. This should lead to a reduction in poverty – in terms of hunger eradication, child mortality reduction and improved human health.

7.3. Limitations in this study

The results of the serosurvey in small ruminants study may have been biased by the sampling technique employed. Due to logistic constraints and the complexity of the pastoral husbandry practices, it was not possible to systemically select the different villages, farms, households and the animals to include in the study. Meanwhile, live sheep and goats markets and slaughter slabs were also sampled randomly in each locality visited. A different values of prevalence may have emerge if a randomized sampling is done in each of the actual small ruminant populations in each state (data such as this is currently not available in Nigeria).

The serosurvey in camels may have been biased by the convenience sampling employed. Currently, there is no nationwide or state-level census data for camels in Nigeria. Some of the camels are brought into the country through the porous borders with Niger and Chad to village live animal markets for sale. The sampled abattoirs are the only ones in the sampled states. The others in the sampled states are slaughter slabs and these do not slaughter camels but cattle, sheep and goats. A more definitive prevalence may emerge if a randomized sampling is done and the actual camel population in each state is taken into account. This however was not done due to logistic constraints.

The real time assay developed in this study has not been evaluated due to financial and other constraints. This evaluation is necessary to ensure that the assay differentiates PPRV from other morbilliviruses and between lineages of PPRV.

Other limitations were: lack of adequate funding to sample all the states; insecurity and consequent inaccessibility in parts of the country which decreases the options of states

available for sample collection. Lack of a biotechnology company producing and/or retailing reagents in Nigeria sometimes meant a long wait between the order/purchase and material arrival/delivery in Nigeria which delayed certain aspects of project implementation. In Nigeria, like many low income countries, science is a minor component of the cultural environment and to most people and many politicians, science is a curiosity performed in high-income countries. This type of thinking coupled with political instability (appointment of a new minister of Agriculture for example) contributed immensely to the stoppage of research funding by the Competitive Agricultural Research Grant Scheme of the Agricultural Research Council of Nigeria.

7.4. Recommendations

There should be nationwide campaigns to vaccinate small ruminants at least once every year either around February/March (weeks before the rains) or November/December (just before the cold harmattan). The vaccination programmes should aim at whole village flocks instead of individual or household flocks. Accurate and reliable livestock census for each village, district, local and state government will be of great help in infectious disease control, this is currently lacking in Nigeria.

Thermostability of the available live vaccines is a major concern under tropical conditions. Endemic countries like Nigeria should adopt the Xerovacc (or other more relevant) technology to produce an indigenous thermostable PPR vaccine. The current efforts to control PPR in the country will be boosted with an indigenous thermostable vaccine since electricity supply to maintain the cold chain requirement of the present vaccine is deficient.

There should be a sub-regional programme within various regions (ECOWAS, SADC) to control and possibly eradicate PPR, similar to what was done for rinderpest eradication. There is need to reinvigorate the extension services by evaluating vaccination service delivery systems and training villagers to establish sustainable vaccination service models that make reliable and affordable control services available to farmers, especially women and children.

There should be an effective surveillance and reporting system which is currently lacking, as most small ruminants small holder farmers handle the outbreaks by either self medicating with antibiotics or slaughter before disease notification to the veterinary authorities. Religious, political and intra-tribal crises prevent effective surveillance even when reports of outbreaks are received from certain parts of the country. There is also the added problems

of logistics such as vehicles for implementation of field activities. Political stability is a key component of PPR control and/or eradication.

Some aspects of the history of PPR are still missing and require further data analysis, for example when PPRV separated from ancestral viruses and when PPRV first appeared in small ruminant populations.

The success of PPR control should rely on all the innovations made in the fields of virus identification and epidemiology, investigation and disease modelling. The integration of molecular epidemiology and quantitative knowledge is a key element to understand the bio-ecological processes and enable dynamic mapping of health risks. It is essential to elucidate the role of evolutionary factors critical on the emergence, virulence, and adaptation to novel hosts. Epidemiologically, the role of camels and wildlife in PPR spread and maintenance needs to be investigated.

Studies on the pathogenesis of PPR are limited and the contribution of secondary infections to the pathology of PPR remains to be determined.

To carry out a PPR control programme to a successful conclusion (PPR eradication), there is need for vaccines that differentiate infected from vaccinated flocks (DIVA), along with compatible diagnostic tests.

More work is needed in the area of biological antivirals. There is a need for efficient therapeutics for virus control to be used under emergency situations. The development of curative medicines in addition to vaccines as an improvement in PPR control will be useful. A promising approach is the possibility of blocking the expression of virus genes using RNA interference (RNAi). Preliminary studies carried out on this new technology to inhibit PPRV replication showed promising results (de Almeida *et al.*, 2007).

There is need for further studies on the socio-economic impact of PPR on the local economy and the cost to benefit ratio of PPR control or eradication in countries where it is endemic. Multi-disciplinary (one health approach) research involving sociologists, psychologists, biostatisticians and other professionals to help elucidate the effects of PPR on individual households and communities is imperative.

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Appendix 2: Protocol modification

Ref: V082/11

11 March 2014



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Dear Dr Quan

**PROTOCOL V082/11: DEVELOPMENT OF A THERMOSTABLE VACCINE AND MOLECULAR
PHYLOGENY OF PESTE DES TEPITS RUMINANTS (PPR) IN NIGERIA – *Dr YT Woma***

I am pleased to inform you that the additions to the above protocol were approved by the Research Committee.

As no change in ethical procedures are involved, it is not necessary for the AEC to approve the protocol again.

I trust that you will find this in order.

Kind regards

A handwritten signature in black ink, appearing to read 'Niesje Tromp'.

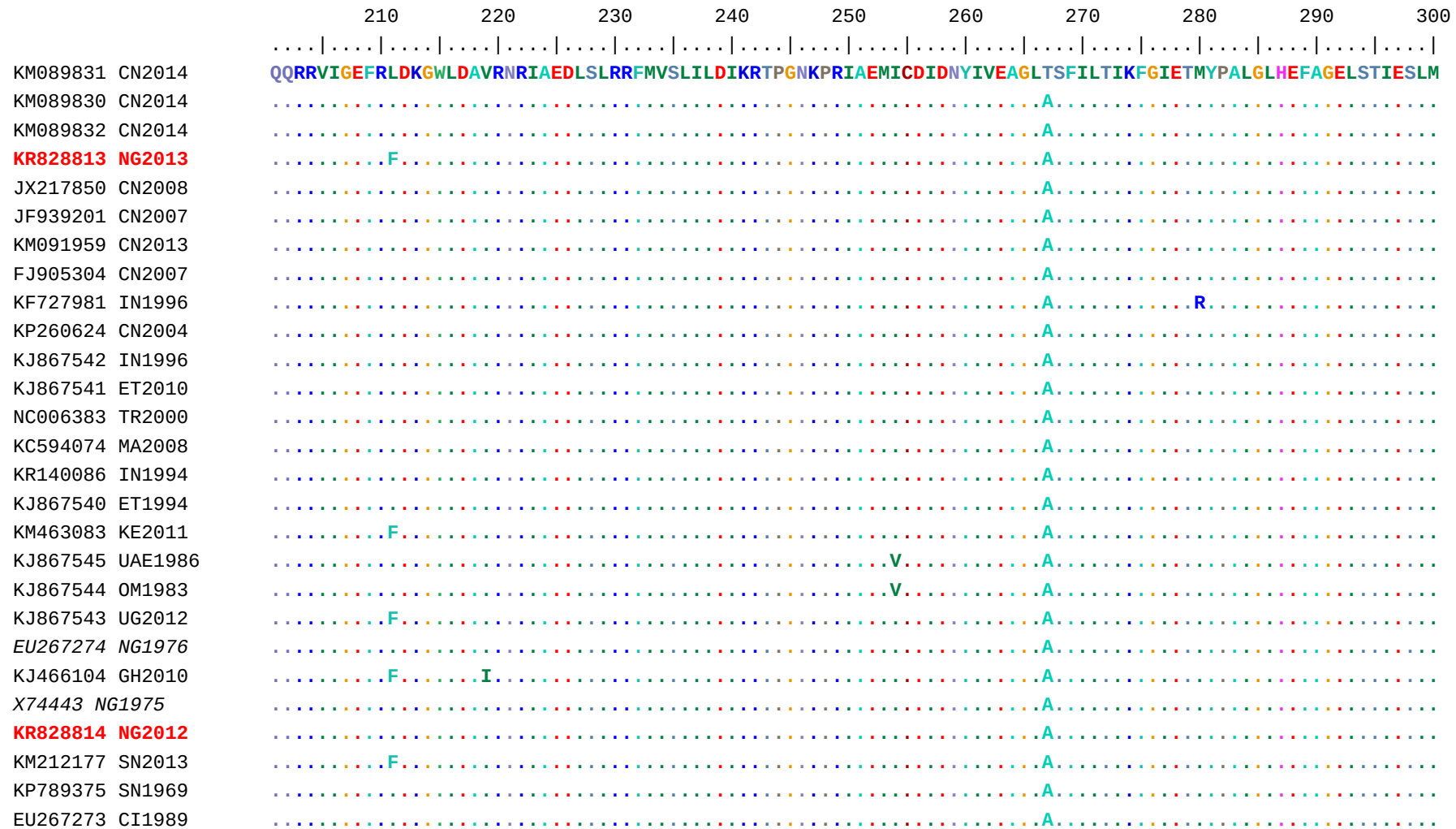
NIESJE TROMP
SECRETARY: RESEARCH COMMITTEE

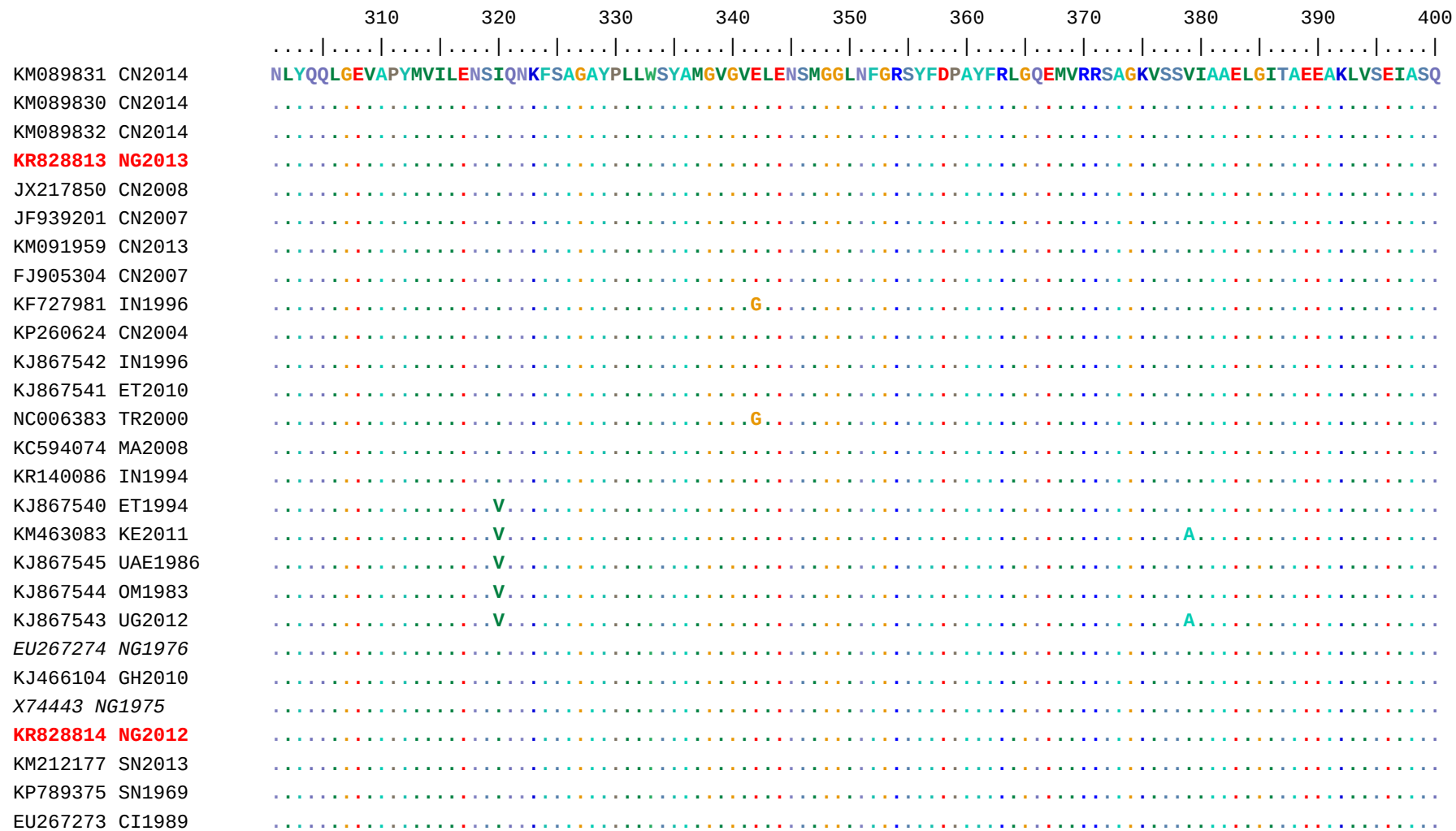
Copy: Dr YT Woma, Researcher (s27313736@tuks.co.za)

Appendix 3: N protein sequence alignments of the PPRV from this study and reference strains

	10	20	30	40	50	60	70	80	90	100
	...	...	...	...	...	...	...	...	...	...
KM089831 CN2014	MATLLKSLALFKRNKDKAPTASGSGGAIRGIKNVVIIVPIPGDSSITTRSRLLDRLVRLAGDPDINGSKLTGVMISMLSLFVE	SPGQLIQRITDDPDVSIR								
KM089830 CN2014										
KM089832 CN2014										
KR828813 NG2013					I					
JX217850 CN2008										
JF939201 CN2007										
KM091959 CN2013										
FJ905304 CN2007										
KF727981 IN1996	I				I					
KP260624 CN2004					I					
KJ867542 IN1996					I					
KJ867541 ET2010					I					
NC006383 TR2000					I					
KC594074 MA2008					I					
KR140086 IN1994					I					
KJ867540 ET1994					I					
KM463083 KE2011					I					
KJ867545 UAE1986					I					
KJ867544 OM1983					I		K			
KJ867543 UG2012					I					
EU267274 NG1976					I					
KJ466104 GH2010					I					
X74443 NG1975					I					
KR828814 NG2012					I					
KM212177 SN2013					I					
KP789375 SN1969					I		K			
EU267273 CI1989					I		K		L	

		110	120	130	140	150	160	170	180	190	200
...		...	...	...	...	...	...	...	...	...	...
KM089831	CN2014	LVEVVQSTRSQSGLTFFASRGADLDNEADMYFSTEGPSSGGKKRINWFENREIIDIEVQDPPEEFNMLLASILAQVWILLAKAVTAPDTAADSELRRWKYT									
KM089830	CN2014										
KM089832	CN2014										
KR828813	NG2013	S.....S.....									
JX217850	CN2008										
JF939201	CN2007										
KM091959	CN2013										
FJ905304	CN2007										
KF727981	IN1996	S									
KP260624	CN2004										
KJ867542	IN1996										
KJ867541	ET2010										
NC006383	TR2000										
KC594074	MA2008	S.....									
KR140086	IN1994										
KJ867540	ET1994	S.....S.....A.....									
KM463083	KE2011	A.....SR.....A.....									
KJ867545	UAE1986	SP.....S.....A.....									
KJ867544	OM1983	SP.....S.....A.....									
KJ867543	UG2012	A.....SR.....A.....									
EU267274	NG1976	C.....A.....P.....									
KJ466104	GH2010	S.....A.....									
X74443	NG1975	S.....A.....									
KR828814	NG2012	S.....									
KM212177	SN2013	S.....A.....									
KP789375	SN1969	S.....A.....									
EU267273	CI1989	S.....A.....									



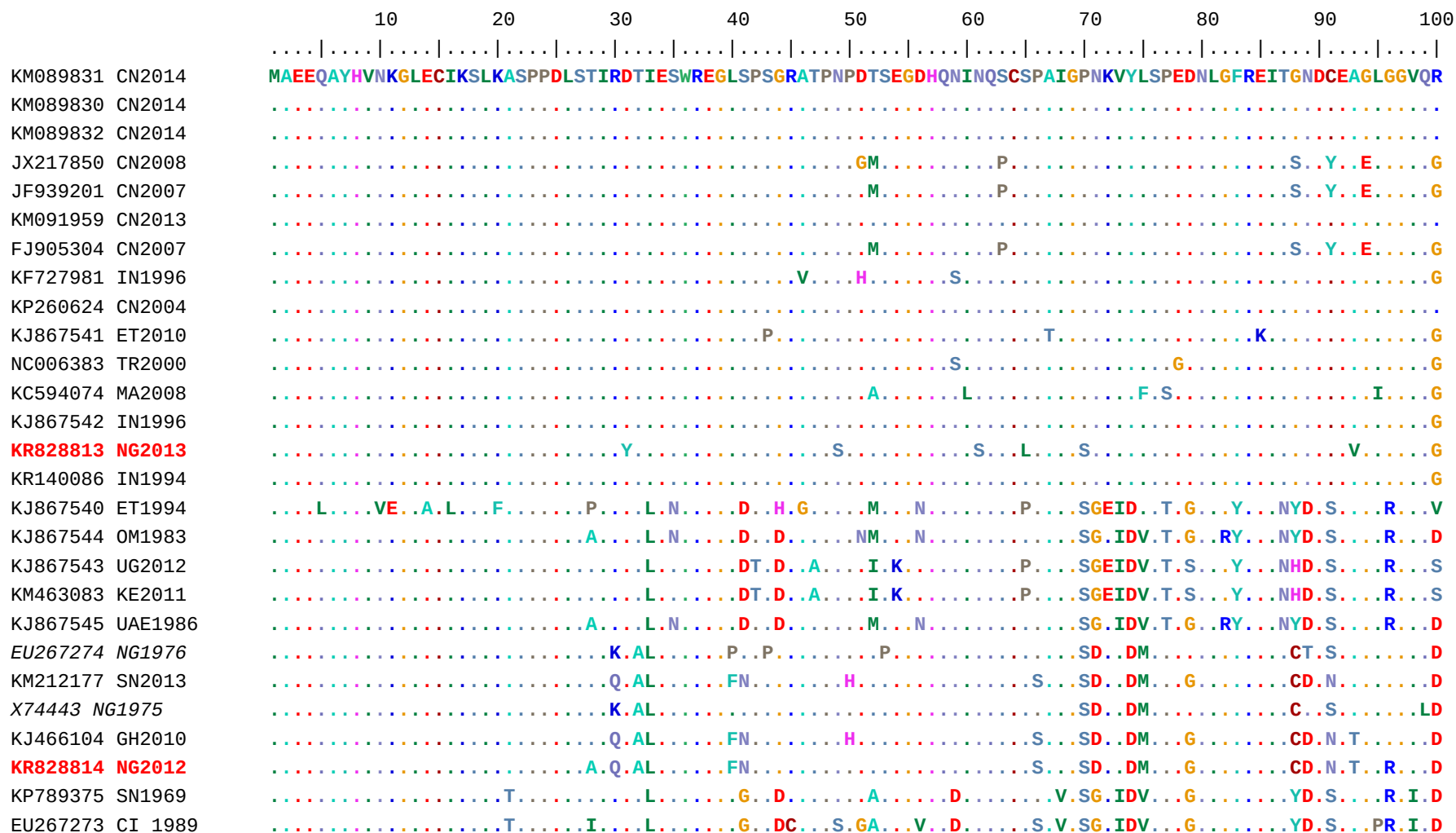


	410	420	430	440	450	460	470	480	490	500
										
KM089831 CN2014	AGDERTARGTGPRQAQVSFLQHTGGGESSAPATREGVKAATPNGAEERDRKQTRPGRPRGETSGQLLLDIMPEDILRESGQNPREAQRSALFRLQA									
KM089830 CN2014										
KM089832 CN2014										
KR828813 NG2013	T.....									
JX217850 CN2008										
JF939201 CN2007										
KM091959 CN2013										
FJ905304 CN2007										
KF727981 IN1996										
KP260624 CN2004										
KJ867542 IN1996										
KJ867541 ET2010	T...I.....									
NC006383 TR2000										
KC594074 MA2008										
KR140086 IN1994										
KJ867540 ET1994	T.....									
KM463083 KE2011	T.....									
KJ867545 UAE1986	T..G..T.....									
KJ867544 OM1983	T..G..T.....									
KJ867543 UG2012	T.....									
EU267274 NG1976	T.E..V.....									
KJ466104 GH2010	T.E..V.....									
X74443 NG1975	T.....									
KR828814 NG2012	T.E..V.....									
KM212177 SN2013	T.E..V.....									
KP789375 SN1969	T.....									
EU267273 CI1989	T.....									

	510	520
		
KM089831 CN2014	MAKILEDQEEGEDNSQVYNDKDLLG	
KM089830 CN2014		
KM089832 CN2014		
KR828813 NG2013	IH.....S	
JX217850 CN2008	N.....	
JF939201 CN2007	N.H.....	
KM091959 CN2013		
FJ905304 CN2007	N.....	
KF727981 IN1996		
KP260624 CN2004		
KJ867542 IN1996		
KJ867541 ET2010		
NC006383 TR2000		
KC594074 MA2008		
KR140086 IN1994		
KJ867540 ET1994	G.....I.....S	
KM463083 KE2011	G.....T.....S	
KJ867545 UAE1986	G.....I.....S	
KJ867544 OM1983	G.....I.....S	
KJ867543 UG2012	G.....T.....S	
<i>EU267274 NG1976</i>	IH.....S	
KJ466104 GH2010	I.....S	
<i>X74443 NG1975</i>	I.....S	
KR828814 NG2012	G.....I.....S	
KM212177 SN2013	I.....S	
KP789375 SN1969	G.....I.....S	
EU267273 CI1989	G.G.....N.I.....S	

Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities.

Appendix 4: P protein sequence alignments of the PPRV from this study and reference strains



		110	120	130	140	150	160	170	180	190	200					
																
KM089831	CN2014	EGSNSQVQRYHVVYSHGGEEIEGLEDA	DSL	VVQADPPFANVFN	GGEDGSDSDVDSGPDDPGRG	TL	YDRGSVAGNGIAGST	DVEKLE	GADI	QEV	LNSQKGK					
KM089830	CN2014				V			D		R						
KM089832	CN2014				V			D		R						
JX217850	CN2008	R..... Y.....			V	I		D		S	DV	R				
JF939201	CN2007	K..... Y.....			V	I		D		S	DV	R				
KM091959	CN2013				V			D			R					
FJ905304	CN2007	K..... Y.....			V	I		D		S	DV	R				
KF727981	IN1996	K..... Y..... V..... DH..... V..... SS..... S..... D.....									DV	R		V		R
KP260624	CN2004				V			D			R					
KJ867541	ET2010	K...P... CY.....			V	I		D			DV	R				
NC006383	TR2000	KR..... Y.....			V			S	D		DV	R				
KC594074	MA2008	K..... Y.....			V	I		D			DV	R				S
KJ867542	IN1996	K..... Y..... S..... V..... I.....						D			DV	R				
KR828813	NG2013	K..... Y..... V..... DIL..... E..... D.....									V	R				S
KR140086	IN1994	K..... Y..... S..... V..... I.....						D			DV	R				
KJ867540	ET1994	Y...HP... I..... R..... Q..... MG..... L...T... ST..... D...HP..... D...DDVK..... D..... S.....														
KJ867544	OM1983	R...D..... L...T... SR..... D...DP..... D...DVVK..... DN..... S.....														
KJ867543	UG2012	R...P...P... I..... M..... L...T... SR..... D...DP..... D...DVVK..... DN..... S.....														
KM463083	KE2011	R...P...P... I..... M..... L...T... SR..... G..... D...DP..... D...DVVK..... DN..... S.....														
KJ867545	UAE1986	R...D..... L...T... SR..... D...DP..... D...DVVK..... DN..... S.....														
EU267274	NG1976	KR...D..... Y..... N...V...T... N..... DP..... PA..... DVSR..... D..... S.....														
KM212177	SN2013	KR...D..... Y..... N..... N...I...T... D..... N..... DP..... PA..... DVSR..... D..... S.....														
X74443	NG1975	K..... Y..... N...V...T... D..... N..... DP..... A..... DVSR..... D..... S.....														
KJ466104	GH2010	K...D..... Y..... N..... N...V...T... D..... N..... T...DP..... PA..... DVSR..... D..... S.....														
KR828814	NG2012	K...D..... Y..... N..... N...V...T... D..... N..... DP..... PA..... DVSR..... D..... RS.....														
KP789375	SN1969	K..... V...T... S..... S...DP..... W...T...H...V...K..... D...A..... N.....														
EU267273	CI 1989	K...D...I..... K..... V...T... S..... S...DP..... W...T...H...DV...R..... D...A..... N.....														

	210	220	230	240	250	260	270	280	290	300
										
KM089831 CN2014#	GGRFQGGKTLRVPEIPDVKH	SRPSAQSIKKGGTEGNSVSSGTVIECL	SISGATQTVPE	SRWESSE	QNASVGSVLKSARS	AKTIQG	STQES	GTIAS	LTQP	KE
KM089830 CN2014		TDGNSVS	GTVIECL	SISGATQTVPE	SRWES	EQNASVGSVLKSARS	AKTIQG	STQES	GTIAS	LTQPKE
KM089832 CN2014		TDGNSVS	GTVIECL	SISGATQTVPE	SRWES	EQNVSVGSVLKSARS	AKTIQG	STQES	GTIAS	LTQPKE
JX217850 CN2008		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKSAGS	AKTIQG	STQES	GTIAS	LTQPKE
JF939201 CN2007		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKSAGS	AKTIQG	STQES	GTIAS	LTQPKE
KM091959 CN2013		TDGNSVS	GTVIECL	SISGATQTVPE	SRWES	EQNASVGSVLKSARS	AKTIQG	STQES	GTIAS	LTQPKE
FJ905304 CN2007		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKSAGS	AKTIQG	STQES	GTIAS	LTQPKE
KF727981 IN1996	V.....	TDGNSVS	GTVTECL	SISGATQAVLE	SRWES	EQNASVG	VLKSARSAETIQ	GLTQES	GTIAS	LTQPKE
KP260624 CN2004	K.....	TDGNSVS	GTVIECL	SISGATQTVPE	SRWES	EQNASVGSVLKSARS	AKTIQG	STQES	GTIAS	LTQPKE
KJ867541 ET2010		TDGSSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKS	AKSAKTIQ	GLTQES	GTIAS	LTQPKE
NC006383 TR2000		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKSARSA	ETIQ	GLTQES	GTIAS	LTQPKE
KC594074 MA2008		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKS	AKSAKTIQ	GLTQES	GTIAS	LTQPKE
KJ867542 IN1996		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKSARS	AKTIQ	ESTQES	GTIAS	LTQPKE
KR828813 NG2013	V.....	TDGNSVS	GTGTECL	SISGATQAVPE	SRWEL	EQNASVGSVLKSARS	AKTIQ	ELTQES	GTIAS	LTQPKE
KR140086 IN1994		TDGNSVS	GTVTECL	SISGATQAVPE	SRWES	EQNASVGSVLKSARS	AKTIQ	ESTQES	GTIAS	LTQPKE
KJ867540 ET1994	R.....T.....P.....	TDGNSVL	GTVTEC	SSISGATQAVPE	SRWES	ERNAFVESVPKSARS	AKTIQ	ELTQES	GTIAS	PPTQPKE
KJ867544 OM1983	R.....T.....N.P.....	TDGNSVL	GTVTEC	SSISGATQAVPE	SRWES	ERNAFVESVPKSARS	AKT	QGLTQES	GTIAPPT	QPKE
KJ867543 UG2012	R.K.....T.....P.....	TDGNSVL	GTVTEC	SSINGATQAVPE	SRWES	ERNAFVESAPKSARS	AKTIQ	ELPQES	GTIAS	PPTQPKE
KM463083 KE2011	R.K.....T.....P.....	TDGNSVL	GTVTEC	SSINGATQAVPE	SRWES	ERNAFVESAPKSARS	AKTIQ	ELPQES	GTIAS	PPTQPKE
KJ867545 UAE1986	R.....T.....N.P.....	TDGNSVL	GTVTEC	SSISGATQAVPE	SRWES	ERNAFVESVPKSARS	AKT	QGLTQES	GTITPPT	QPKE
<i>EU267274 NG1976</i>	I.....N.....	TDGSSAL	GTVTEC	SSISGATQAVPE	SRWES	ERNASVGSVLKSARS	AKTIQ	GLTQES	GTIAS	LTQPKE
KM212177 SN2013	I.....N.....	TDGSSVL	GMVTEC	SSISGATQAVLE	SRWES	ERNASVGSVPKSVRS	AKTIQ	GLTQES	GTIAS	LTQPKE
<i>X74443 NG1975</i>	I.....N.....	TDGNSVL	GTVTEC	SSISGATQAVPE	SRWES	ERNASVGSVPKSARS	AKTIQ	GLTQES	GTIAS	LTQPKE
KJ466104 GH2010	I.....N.....	TDGSSVL	GMVTEC	SSISGATQAVLE	SRWES	ERNASVGSVPKSARS	AKTIQ	GLTQES	GTIAS	LTQPKE
KR828814 NG2012	I.....N.....	TDGSSVL	GTVTEY	SSISGATQAVLE	SRWES	ERNASVGSVPKSARS	AKTIQ	GLTQES	GTIAS	LTQPKE
KP789375 SN1969	G.....	TDGNSVL	GTATECL	INGATRAVPE	SRWES	ERNASVESVPKSARS	AKTIQ	ELTQES	GTIAS	LTQPKE
EU267273 CI 1989	FG.....	TDGNSVS	GTATECL	INGATQAVPE	SRWES	ERNASVESVPKSARS	AKTIQ	ELTPES	GTIAS	LTQPKE

	310	320	330	340	350	360	370	380	390	400
										
KM089831 CN2014	ENDSEY	EYEDDL	LFTEIQ	DVRSIA	KIHD	DDNKT	ILSKLD	SILLK	GEVD	TIKKQ
KM089830 CN2014	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KM089832 CN2014	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
JX217850 CN2008	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
JF939201 CN2007	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KM091959 CN2013	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
FJ905304 CN2007	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KF727981 IN1996	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKPIL	SKLDSIL	.	KGEVD	TIK.
KP260624 CN2004	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KJ867541 ET2010	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
NC006383 TR2000	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KC594074 MA2008	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KJ867542 IN1996	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KR828813 NG2013	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEID	TIK.
KR140086 IN1994	NDSEY	EYED.	LFTEIQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEVD	TIK.
KJ867540 ET1994	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHE.	NKTIL	SKLDSIL	.	KGEID	TIK.
KJ867544 OM1983	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEID	TIK.
KJ867543 UG2012	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEID	TIK.
KM463083 KE2011	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEID	TIK.
KJ867545 UAE1986	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTIL	SKLDSIL	.	KGEID	TIK.
<i>EU267274 NG1976</i>	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTIL	SRLDSLL	.	KGEID	TIK.
KM212177 SN2013	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTLL	SKLDSLL	.	KGEID	TIK.
<i>X74443 NG1975</i>	NDSEF	EYED.	LFTEMQ	EIRASIA	KIHD.	NKTIL	SKLDSLL	.	KGEID	TIK.
KJ466104 GH2010	NDSEY	EYED.	LFTEMQ	DIRASIA	KIHD.	NKTIL	SKLDSLL	.	KGEID	TIK.
KR828814 NG2012	NDSEY	EYED	LFTEMQ	EIRASIA	KIHD.	NKTIL	SKLDSLL	.	KGEID	TIK.
KP789375 SN1969	NDSEY	EYED	GLFTEI	QDIRASIA	KIHE.	NKTIL	SKLESIL	.	KGEID	TIK.
EU267273 CI 1989	NDSEY	EYED	GLFTEI	QDIRASIA	KIHE.	NKTIL	SKLESIL	.	KGEID	TIK.

	410	420	430	440	450	460	470	480	490	500																																																																																								
																																																																																																		
KM089831 CN2014	RD	SG	R	A	E	V	L	K	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	S	S	K	G	Q	L	L	K	D	L	Q	L	K	P	V	D	K	Q	A	S	A	I	G	F	V	P	S	D	H	E	S	R	N	V	I	R	S	I	K	S	S	K	L	N	I	D	H	K	D	Y	L	L	D	L	N	D	V	K	G	S	K	D	L	K	E	F	H	K								
KM089830 CN2014	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KM089832 CN2014	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
JX217850 CN2008	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
JF939201 CN2007	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KM091959 CN2013	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
FJ905304 CN2007	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KF727981 IN1996	DS	.	R	A	L	A	E	V	L	K	.	P	A	V	D	R	F	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	I	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	V	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KP260624 CN2004	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KJ867541 ET2010	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	T	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	S	I	D	H	K	D	Y	L	.	D	L	.	T	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
NC006383 TR2000	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	T	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KC594074 MA2008	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KJ867542 IN1996	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KR828813 NG2013	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	K	N	G	I	K	V	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	V	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KR140086 IN1994	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	I	G	T	K	A	N	S	.	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	N	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M	
KJ867540 ET1994	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	P	K	T	G	F	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	K	S	V	I	R	S	I	.	K	S	.	K	L	N	I	E	H	K	D	Y	L	.	D	L	V	N	D	V	K	G	S	K	D	R	K	E	F	H	K	M
KJ867544 OM1983	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	P	K	T	G	I	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KJ867543 UG2012	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	P	K	T	G	F	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KM463083 KE2011	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	P	K	T	G	F	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KJ867545 UAE1986	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	P	K	T	G	I	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
<i>EU267274 NG1976</i>	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	K	S	G	I	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KM212177 SN2013	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	K	S	G	I	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
<i>X74443 NG1975</i>	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	K	S	G	I	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	E	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N	D	V	K	G	S	K	D	L	K	E	F	H	K	M
KJ466104 GH2010	DS	GR	A	L	A	E	V	L	K	.	P	A	V	D	R	S	Q	K	S	G	I	K	V	N	S	G	.	K	G	Q	L	.	K	D	L	Q	L	K	P	V	D	K	Q	A	S	.	A	I	G	F	V	P	S	D	H	E	S	.	R	S	V	I	R	S	I	.	K	S	.	K	L	N	I	D	H	K	D	Y	L	.	D	L	.	N														

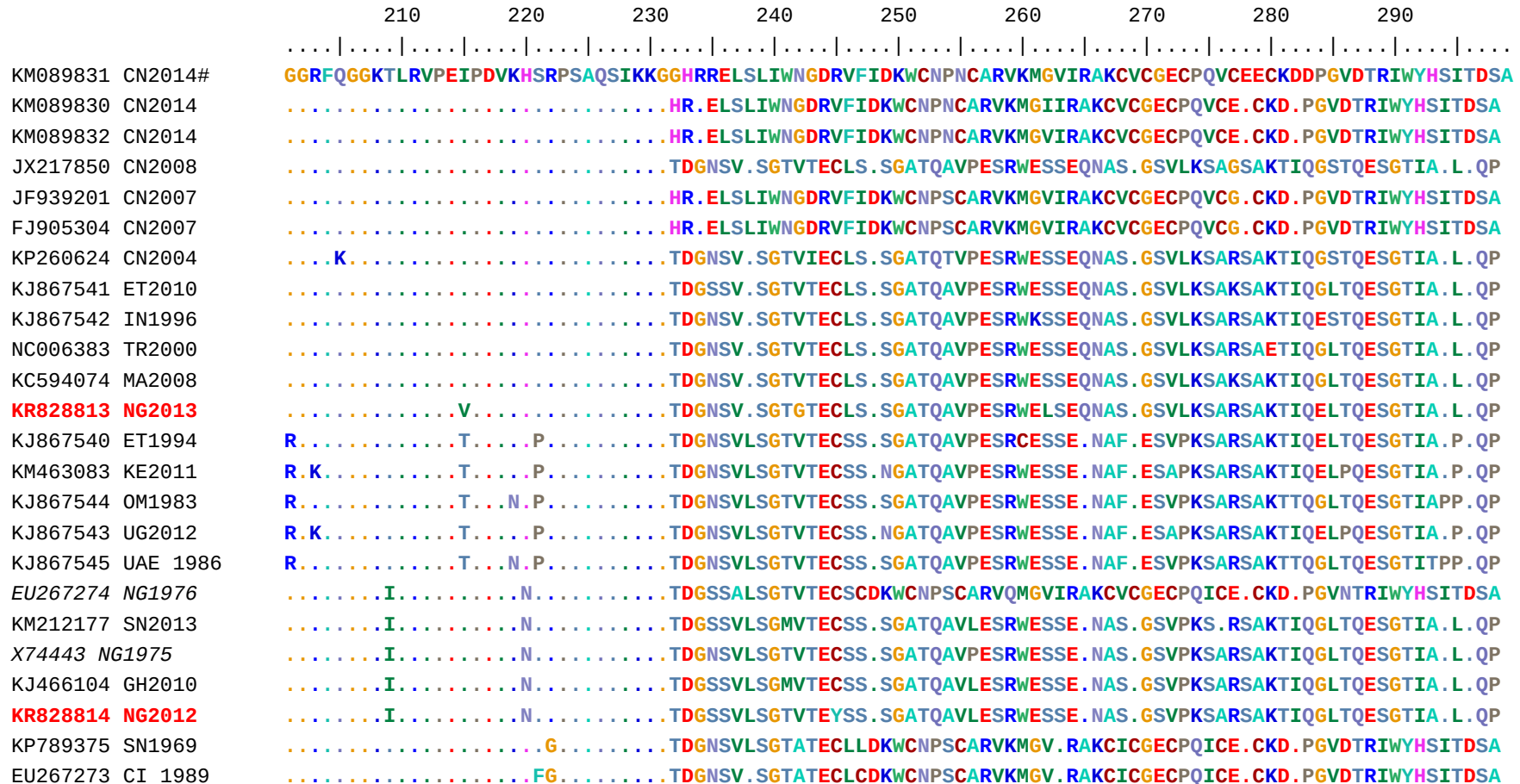
510

....|....|

KM089831 CN2014	MLTAILAKHP
KM089830 CN2014	LTAILAKHP
KM089832 CN2014	LTAILAKHP
JX217850 CN2008	LTAILAKHP
JF939201 CN2007	LTAILAKHP
KM091959 CN2013	LTAILAKHP
FJ905304 CN2007	LTAILAKHP
KF727981 IN1996	LTAILAKHP
KP260624 CN2004	LTAILAKHP
KJ867541 ET2010	LTAILAKHP
NC006383 TR2000	LTAILAKHP
KC594074 MA2008	LTAILAKHP
KJ867542 IN1996	LTAILAKHP
KR828813 NG2013	LTAILAKHP
KR140086 IN1994	LTAILAKHP
KJ867540 ET1994	LTAILAKQP
KJ867544 OM1983	LTAILAKQP
KJ867543 UG2012	LTAILAKQP
KM463083 KE2011	LTAILAKQP
KJ867545 UAE1986	LTAILAKQP
<i>EU267274 NG1976</i>	LTAILAKQP
KM212177 SN2013	LTAILAKQP
<i>X74443 NG1975</i>	LTAILAKQP
KJ466104 GH2010	LTAILAKQP
KR828814 NG2012	LTAILAKHP
KP789375 SN1969	LT . ILAKQP
EU267273 CI 1989	LT . ILAKQP

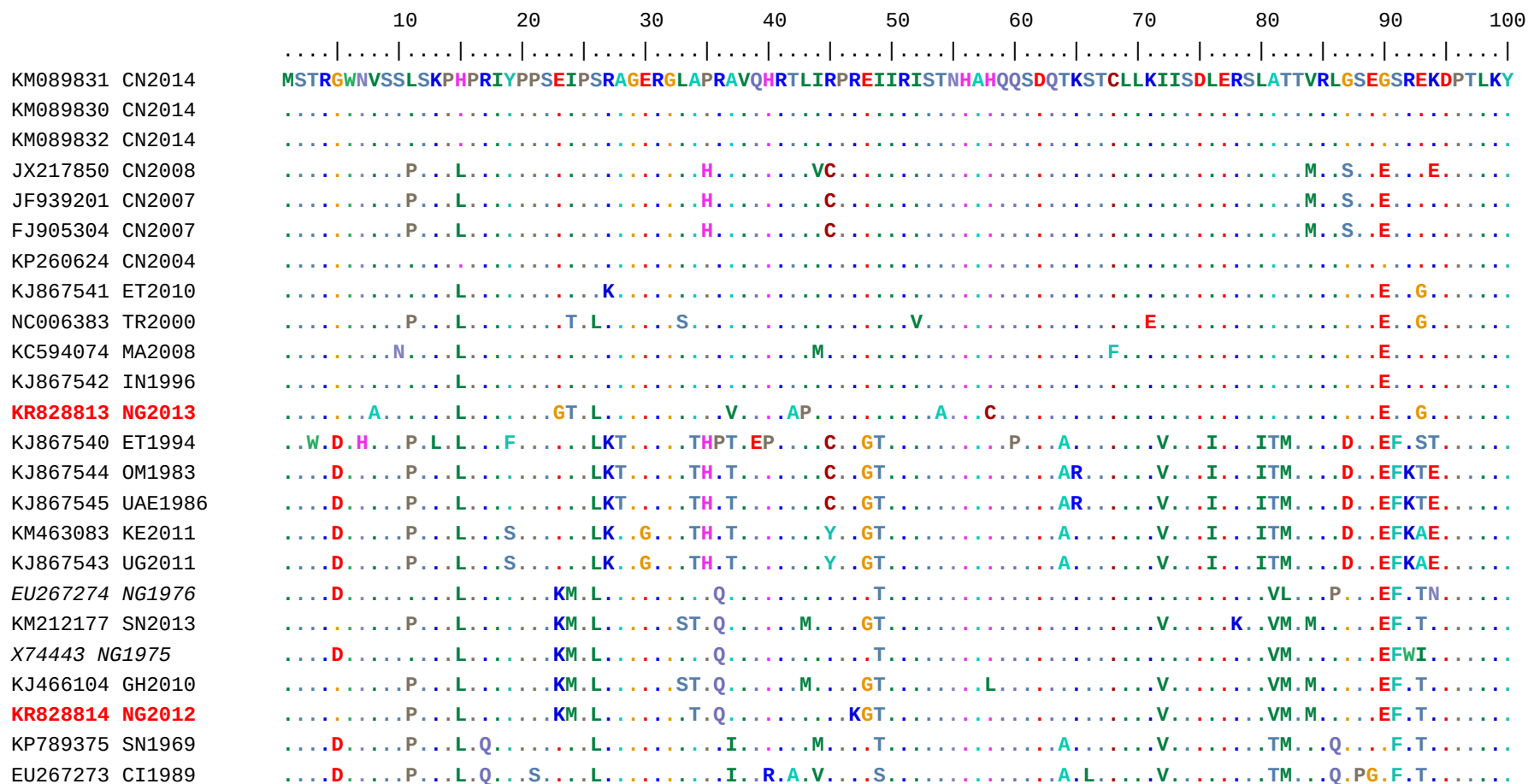
Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities. # = The P-protein of KM089831 CN2014 has two extra GG aas at position 231 and 232. These may be due to incorrect annotation and thus responsible for the aligned picture seen in appendix 4.

		110	120	130	140	150	160	170	180	190	200
											



Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities. # = The V-protein of KM089831 CN2014 has an extra G aa at position 232. This may be due to incorrect annotation and thus responsible for the aligned picture seen in appendix 5.

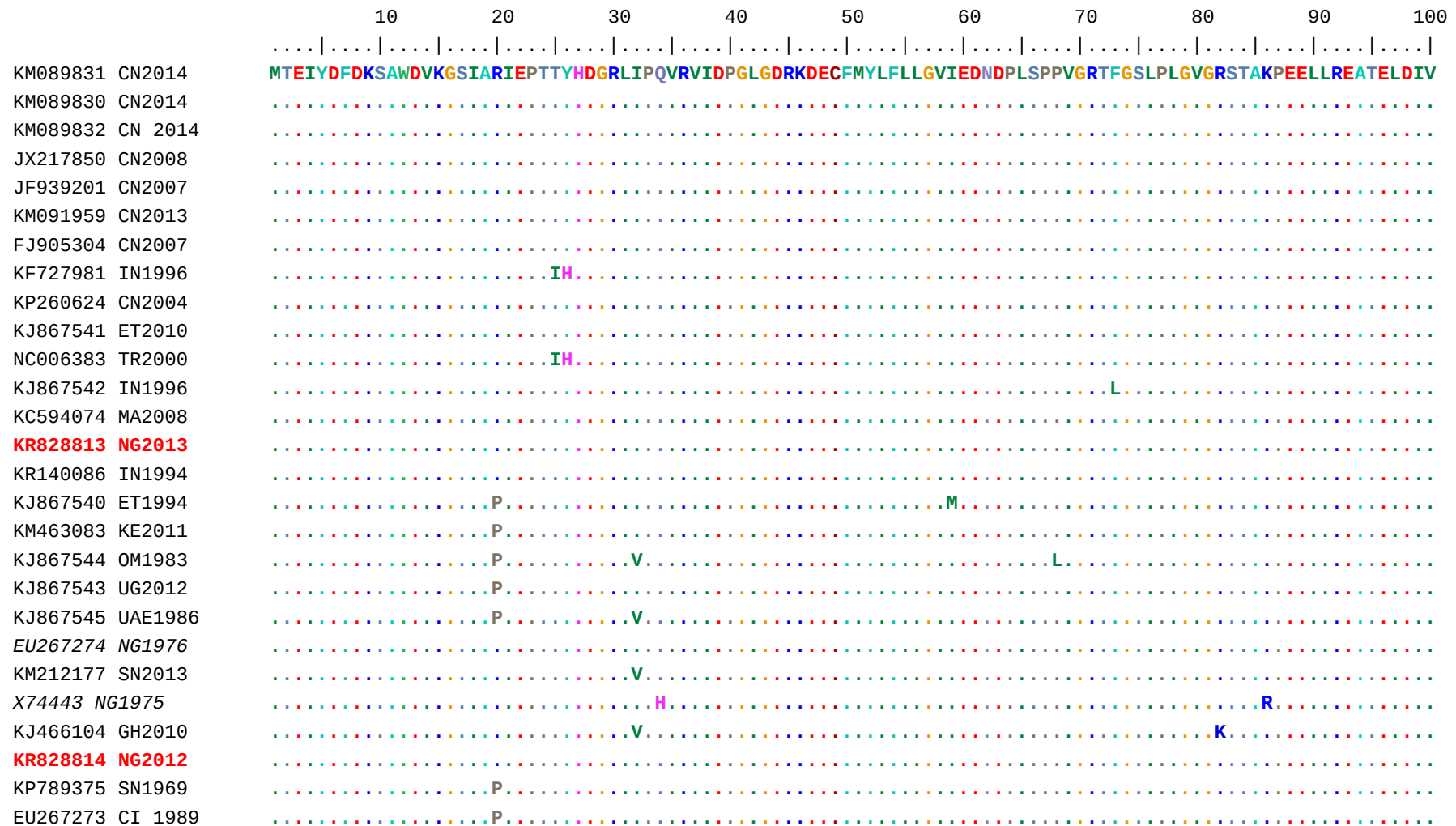
Appendix 6: C protein sequence alignments of the PPRV from this study and reference strains



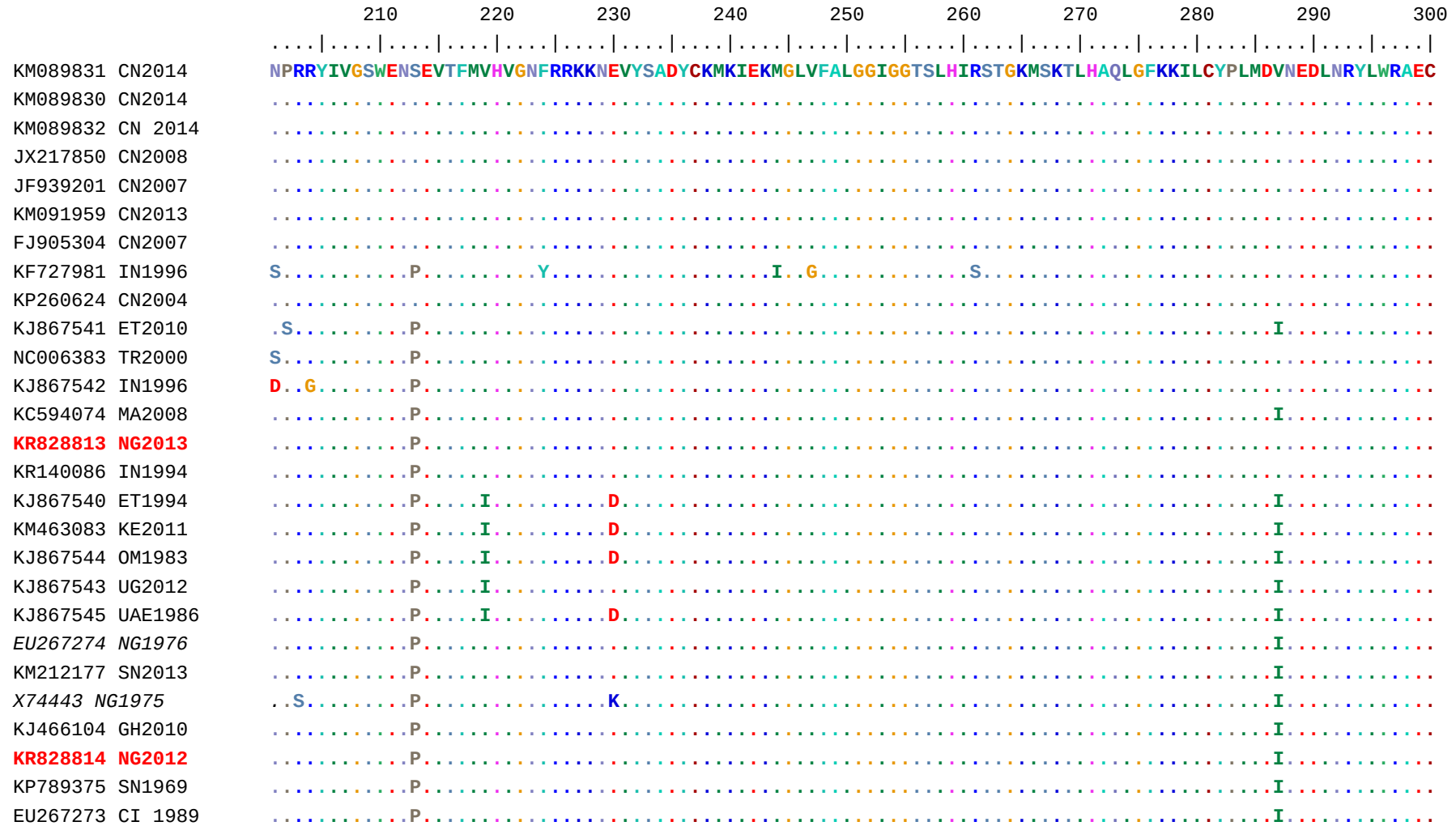
	110	120	130	140	150	160	170
							
KM089831 CN2014	SVTMFIVTGVKRLKDSRMLTSLWFKQILHLLTSSMEERMDLTAMWTLAQMI	PAEVL	YMTG	DLLP	AMVSL	GPQMSKN	
KM089830 CN2014			R.....	T.....		I.....	
KM089832 CN2014			Q.....			I.....	
JX217850 CN2008		A.....	Q.....	Y.....		I.....	M.....
JF939201 CN2007		A.....	Q.....	Y.....		I.....	M.....
FJ905304 CN2007		A.....	Q.....	Y.....		I.....	M.....
KP260624 CN2004			Q.....			I.....	A.....
KJ867541 ET2010	N.A.....	A.....	Q.....			I.....	M.....
NC006383 TR2000		A.....	Q.....			I.....	M.....
KC594074 MA2008		A.....	Q.....			I.....	M.....
KJ867542 IN1996		A.....	Q.....			I.....	M.....R.....
KR828813 NG2013			R.....	Q.....		N.....	I.....D.....
KJ867540 ET1994		A.....		V.....	H.....	P.VQ.....	TDI.....T.MT.S.....S
KJ867544 OM1983		A.....		P.V.....		T.I.....	M.S.P.....
KJ867545 UAE1986		A.....		P.V.....		T.I.....	M.S.P.....
KM463083 KE2011		A.....		P.V.....	V.....	T.I.....	T.M.S.....R.....
KJ867543 UG2011		A.....		P.V.....		T.I.....	T.M.S.....R.....
<i>EU267274 NG1976</i>		A.....		R.....	P.....		I.....M.....Q.....
KM212177 SN2013		I.....	T.....		R.....	P.....	I.....M.....Q.....
<i>X74443 NG1975</i>		A.....		Q.....	P.....		I.....M.....Q.....
KJ466104 GH2010		T.....		R.....	P.....		I.....M.....Q.....
KR828814 NG2012		T.....		R.....	P.....		I.....M.....Q.....
KP789375 SN1969		A.....		Q.....	P.V.....	K.....	I.....S.....
EU267273 CI 1989		A.....		Q.....	P.V.....	K.....	I.....M.....

Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities

Appendix 7: M protein sequence alignments of the PPRV from this study and reference strains



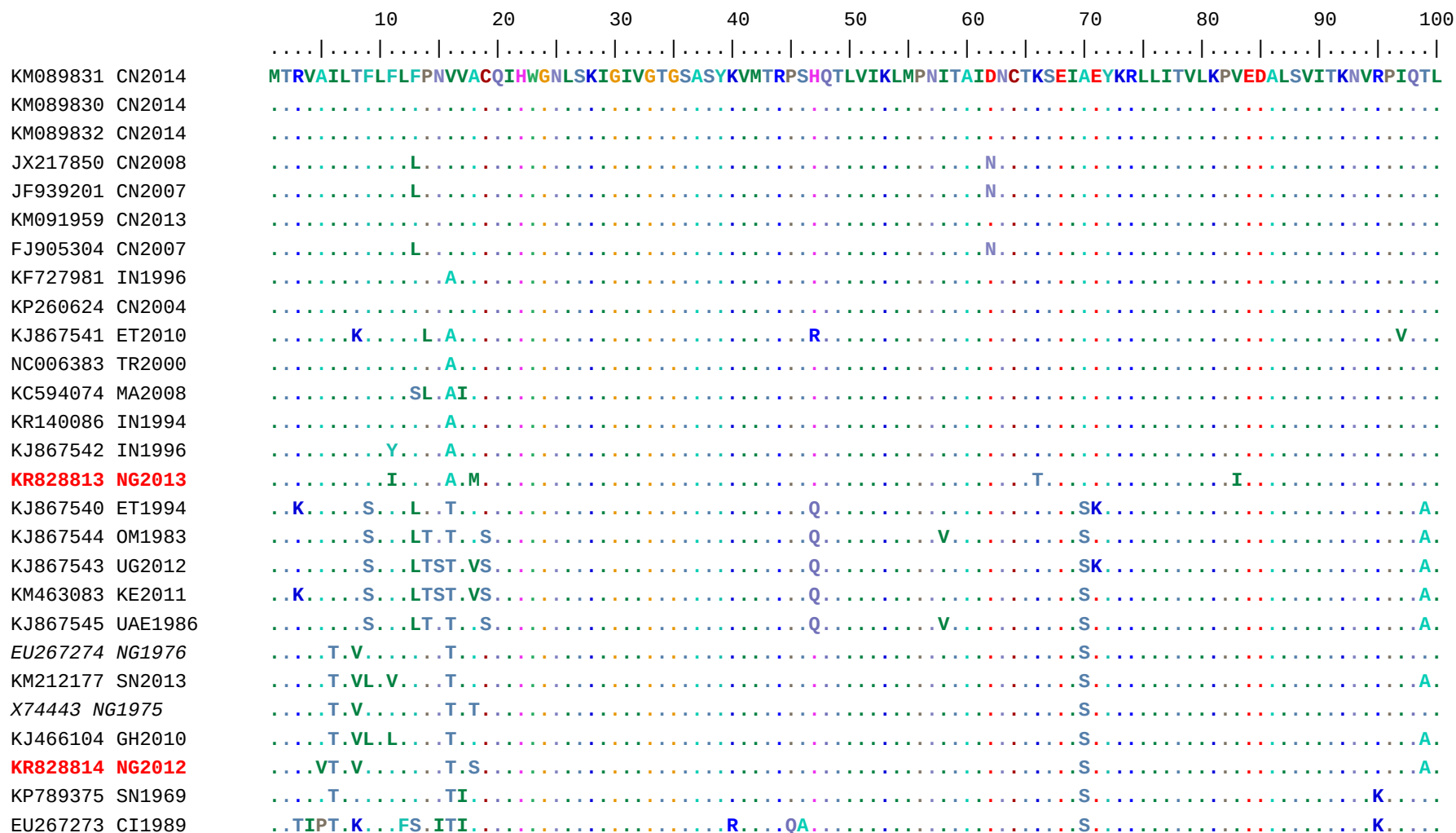
		110	120	130	140	150	160	170	180	190	200
											



	310	320	330
			
KM089831 CN2014	RIVKIQAVLQPSVPQEFRVYDDVIINDDQGLFKIL		
KM089830 CN2014			
KM089832 CN 2014			
JX217850 CN2008			
JF939201 CN2007			
KM091959 CN2013			
FJ905304 CN2007			
KF727981 IN1996	P..P		
KP260624 CN2004			
KJ867541 ET2010			
NC006383 TR2000	P..P		
KJ867542 IN1996			
KC594074 MA2008			
KR828813 NG2013			
KR140086 IN1994	P		
KJ867540 ET1994			
KM463083 KE2011			
KJ867544 OM1983			
KJ867543 UG2012			
KJ867545 UAE1986			
<i>EU267274 NG1976</i>			
KM212177 SN2013			
<i>X74443 NG1975</i>			
KJ466104 GH2010			
KR828814 NG2012			
KP789375 SN1969			
EU267273 CI 1989			

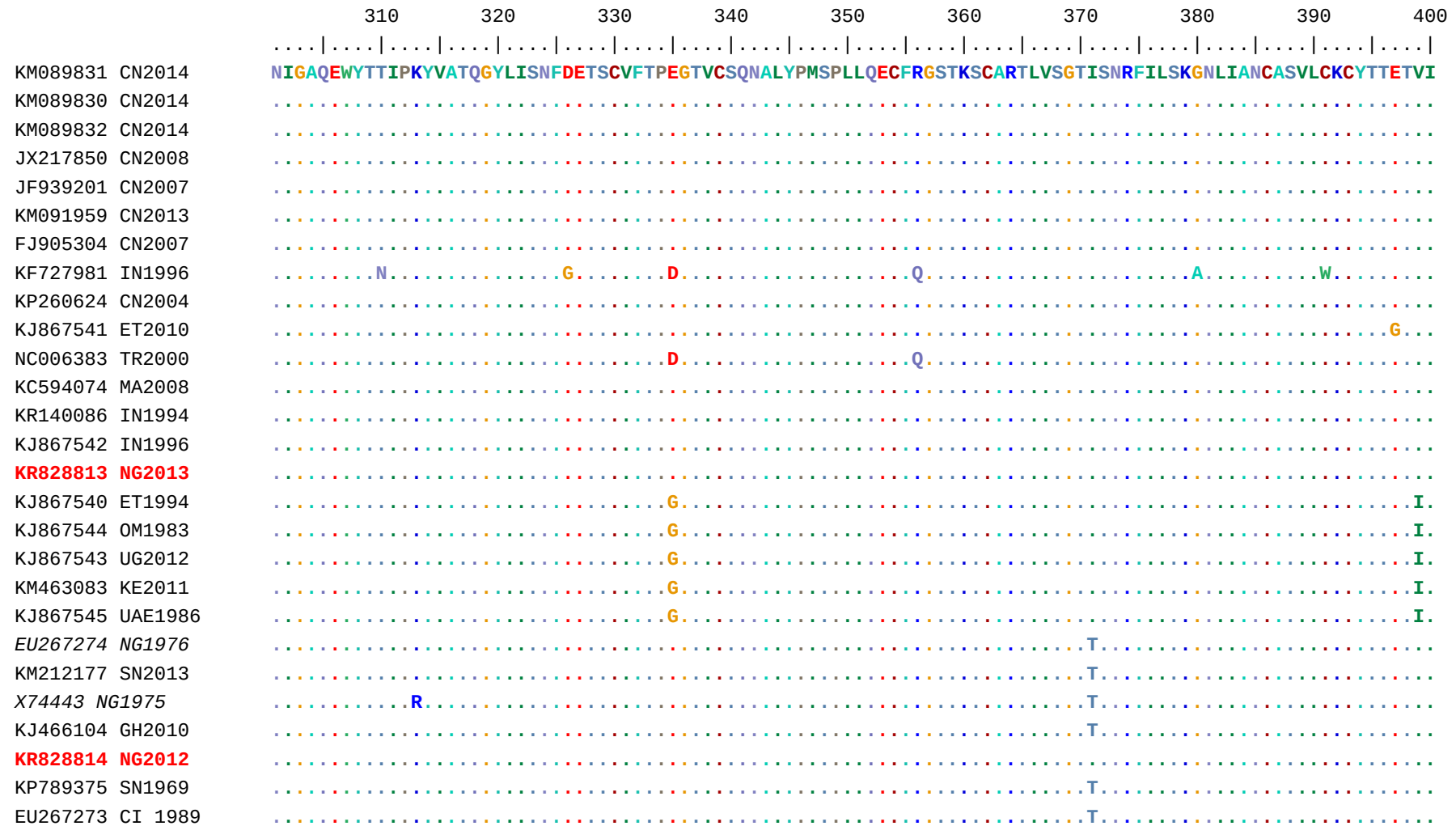
Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities

Appendix 8: F protein sequence alignments of the PPRV from this study and reference strains



		110	120	130	140	150	160	170	180	190	200
											

		210	220	230	240	250	260	270	280	290	300
											
KM089831	CN2014	KLLRYYTEILSIFG	PSLRDPISAEISIQ	ALSVALGGDINKIL	DKLGYSGGDFLAILE	SKGIKARVTYVDTR	DYFIILSIAYPTLSE	IKGVIVHKIEAITY			
KM089830	CN2014							A.....			
KM089832	CN2014										
JX217850	CN2008		A.....								
JF939201	CN2007		A.....								
KM091959	CN2013										
FJ905304	CN2007		A.....								
KF727981	IN1996		A.....	H.....	R.....						
KP260624	CN2004										
KJ867541	ET2010		A.....								
NC006383	TR2000		A.....		R.....						
KC594074	MA2008		A.....								
KR140086	IN1994		A.....								
KJ867542	IN1996		A.....								
KR828813	NG2013		A.....		E.....						
KJ867540	ET1994		A.....			E.....				M.....	VS.....
KJ867544	OM1983		A.....							M.....	VS.....
KJ867543	UG2012		V.....	A.....		E.....				M.....	VS.....
KM463083	KE2011		V.....	A.....		E.....				M.....	VS.....
KJ867545	UAE1986		A.....							M.....	VS.....
<i>EU267274</i>	<i>NG1976</i>		A.....								S.....
KM212177	SN2013		A.....								S.....
<i>X74443</i>	<i>NG1975</i>		A.....								S.....
KJ466104	GH2010		A.....								S.....
KR828814	NG2012		A.....								
KP789375	SN1969		A.....								S.....
<i>EU267273</i>	<i>CI 1989</i>		A.....			E.....					S.....



	410	420	430	440	450	460	470	480	490	500
.....										
KM089831 CN2014	SQDP	DKLLTVVASD	KCPVVEVDGVTIQVGSREYP	DSVYLHKIDLGPAISLEKLDVGTNLGN	AVTRLEN	AKELL	DASDQILKTVKGVPFSGNMYIALA	ACI		
KM089830 CN2014										
KM089832 CN2014		P.....								
JX217850 CN2008								A.....G.....		
JF939201 CN2007								A.....G.....		
KM091959 CN2013										
FJ905304 CN2007								A.....LG.....		
KF727981 IN1996			G.....			S.....	L.....	G.....		
KP260624 CN2004										
KJ867541 ET2010										
NC006383 TR2000									G.....	
KC594074 MA2008								N.....	G.....	
KR140086 IN1994									G.....	
KJ867542 IN1996									G.....	
KR828813 NG2013								I.....	V.....	
KJ867540 ET1994		I.....			I.....				L.....G.....	
KJ867544 OM1983		I.....						L.....L.....G.....		
KJ867543 UG2012		I.....							L.....G.....	
KM463083 KE2011		I.....							L.....G.....	
KJ867545 UAE1986	N.....	I.....							L.....L.....G.....	
<i>EU267274 NG1976</i>	N.....	I.....								
KM212177 SN2013	N.....	I.....								
<i>X74443 NG1975</i>	N.....	I.....		E.....					I.....	
KJ466104 GH2010	N.....	I.....								
KR828814 NG2012										
KP789375 SN1969		I.....							G.....L.....G.....	
<i>EU267273 CI 1989</i>		I.....W.....							LG.....L.....G.....	

	510	520	530	540
				
KM089831 CN2014	GVSLGLVTLIC	CKGRCKNKEI	PTISKINPGL	KPDLTGTSKSYVRS
KM089830 CN2014				
KM089832 CN2014				
JX217850 CN2008				
JF939201 CN2007				
KM091959 CN2013				
FJ905304 CN2007				
KF727981 IN1996	T.....	V.....	R.....	A.E.E.....
KP260624 CN2004				
KJ867541 ET2010	T.....			
NC006383 TR2000		V.....		
KC594074 MA2008				
KR140086 IN1994				
KJ867542 IN1996				
KR828813 NG2013			K.....	
KJ867540 ET1994	R.....	R.....	TP.....	
KJ867544 OM1983		R.....	TP.....	
KJ867543 UG2012	R.....	R.....	TP.....	
KM463083 KE2011	R.....	R.....	TP.....	
KJ867545 UAE1986		R.....	TP.....	
<i>EU267274 NG1976</i>			A.....	
KM212177 SN2013		R.....	A.....	
<i>X74443 NG1975</i>		R.....	A.....	
KJ466104 GH2010		R.....	A.....	
KR828814 NG2012		R.....	A.....	
KP789375 SN1969		R.....	T.....	
EU267273 CI 1989		R.....	T.....	

Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities.

		110	120	130	140	150	160	170	180	190	200																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
KM089831	CN2014	DE	V	G	I	R	I	P	Q	K	F	S	D	L	V	K	F	I	S	D	K	I	K	F	L	N	P	D	R	E	Y	D	F	R	D	L	R	W	C	M	N	P	P	E	R	V	K	I	N	F	D	Q	F	C	E	Y	K	A	A	V	K	S	I	E	H	I	F	E	S	P	L	N	K	S	K	L	Q	S	L	T	L	G	P	G	T	G	C	L	G	R	T	V	T	R	A	H	F	S	E																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
KM089830	CN2014	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.</

		210	220	230	240	250	260	270	280	290	300															
																										
KM089831	CN2014	LTMTLM	DLDEMK	HNVS	SFTV	VEEGL	FGRT	YTVW	RS	DARD	PSTDL	GLIGH	FLRV	FEIG	LVRL	DGLG	PPVF	HMTN	YLT	VNMS	DDYR	RCLL	AVGE	LKLT	ALC	
KM089830	CN2014																									
KM089832	CN2014																									
JX217850	CN2008	..L.													I.											
JF939201	CN2007	..L.													I.											
KM091959	CN2013																									
FJ905304	CN2007	..L.													I.											
KF727981	IN1996	..L.		..L.							I.															
KP260624	CN2004															S.										
KJ867542	IN1996	..L.									P.															
KJ867541	ET2010	..L.		..K.							F.															
NC006383	TR2000	..L.																								
KC594074	MA2008	..L.		I.							F.															
KR140086	IN1994	..L.									P.															
KR828813	NG2013	..L.									L.	IDT.													G.	
KJ867540	ET1994	..L.	L.	..N.		..T.		I.			GNL.	RP.				F.	SA.	S.				S.				
KJ867544	OM1983	..L.	L.	..N.				II.			GNL.	GS.	..N.			F.	SA.				S.				A.	
KJ867543	UG2012	..L.	L.	..N.				I.	G.		Y.	GNL.	AS.	L.		F.	SA.				S.					
KJ867545	UAE1986	..L.	L.	..N.				I.			GNL.	GS.	..N.			F.	SA.				S.				A.	
KM463083	KE2011	..L.	L.	..N.				I.	G.		Y.	GNL.	AS.	L.		F.	SA.				S.					
EU267274	NG1976	..L.									TGK.	SP.				E.	A.	I.			S.					
KM212177	SN2013			..T.				I.			TGK.	S.	D.	Q.		E.	A.	I.			N.					
X74443	NG1975	..L.		..I.							TGK.	SP.				E.	A.	I.			S.					
KJ466104	GH2010	..L.						I.			TGK.	S.	D.	Q.		E.	A.	I.			S.					
KR828814	NG2012	..L.						I.			T.	K.	SPD.	Q.		E.	A.	I.			S.					
KP789375	SN1969	..L.	L.								GN.	..P.	T.			SA.		H.		G.	S.				A.	
EU267273	CI 1989	..L.	L.		..N.			I.			P.	VGN.	I.	S.	A.		A.	L.	..P.	H.	A.		G.	S.		A.

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KM089832	CN2014																									
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JF939201	CN2007			A	K																					
KM091959	CN2013																									
FJ905304	CN2007			A	K																					
KF727981	IN1996	S		G		K		A											E		K		G			
KP260624	CN2004																									
KJ867542	IN1996					K	K																			
KJ867541	ET2010			G		K		A		V									V							
NC006383	TR2000	S		G		K																				
KC594074	MA2008					K													V							
KR140086	IN1994			G		K	K																			
KR828813	NG2013	P				K														S						
KJ867540	ET1994	H				K		A				I	A			F			N							L
KJ867544	OM1983	R		D		K						I	A						N							L
KJ867543	UG2012	H				K		A				I	S			F		N		N						L
KJ867545	UAE1986	R		D		K						I	A						N							L
KM463083	KE2011	H				K		A				I	S			F		N		N						L
EU267274	NG1976	P			I	K													N							I
KM212177	SN2013	P				K				V						H			N							I
X74443	NG 1975	P		S		K						T	PT						N							I
KJ466104	GH2010	P				K				V						H			N							I
KR828814	NG2012	P				K				V						T			N							I
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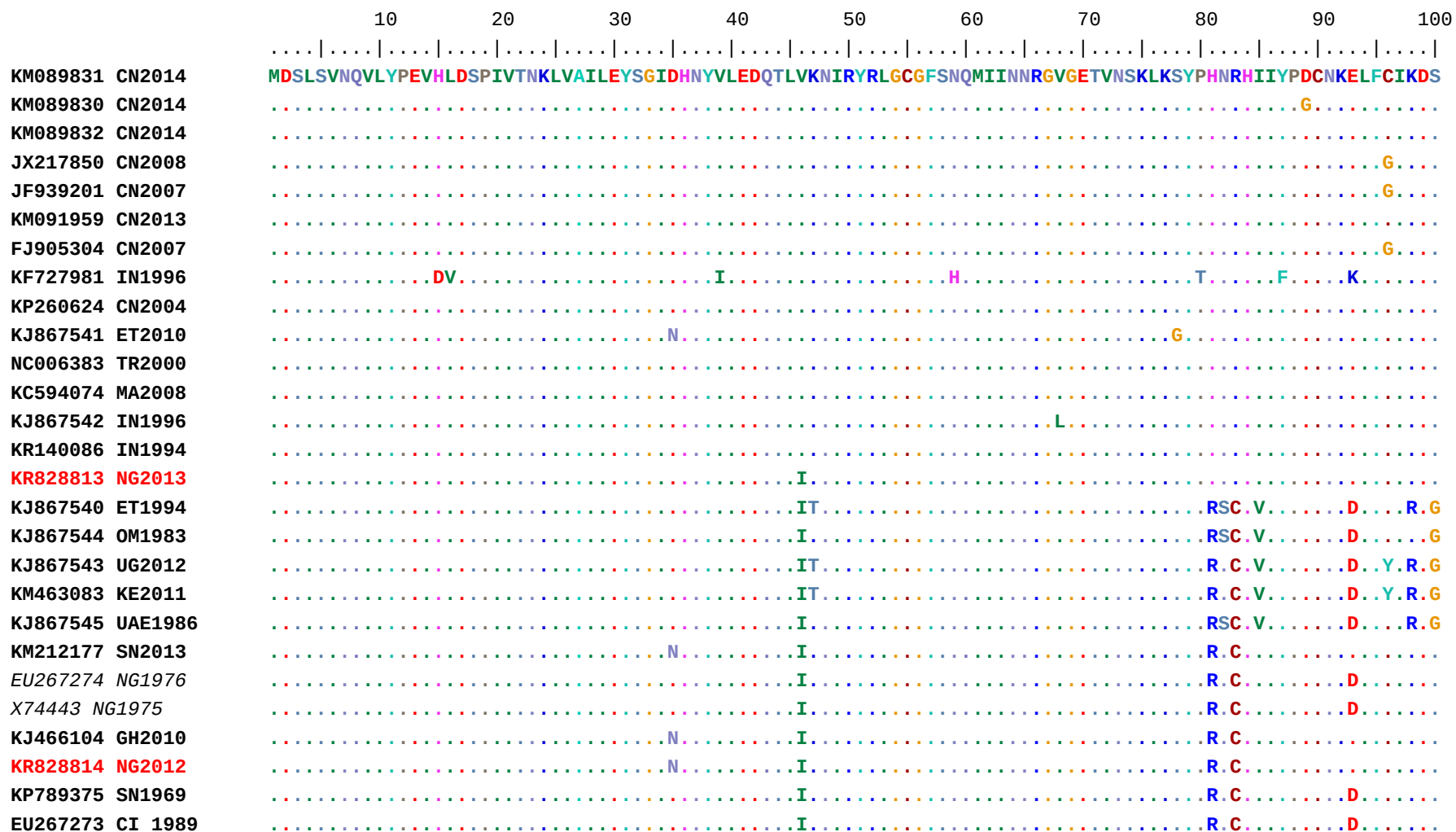
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JF939201	CN2007	G.....R.I.....									
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KF727981	IN1996	G.....A.....GV.H.....R.....R.....P.....									
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NC006383	TR2000	G.....G.....R.....									
KC594074	MA2008	G.....G.....RD.....V.....									
KR140086	IN1994	G.....G.....R.....									
KR828813	NG2013	R.....G.....N.....G.....R.....									
KJ867540	ET1994	V.....G.....R.....A.....L.....									
KJ867544	OM1983	V.....G.....R.....A.D.....T.....L.....									
KJ867543	UG2012	V.....G.....R.....K.....A.S.....L.....									
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EU267274	NG1976	G.....G.....R.A.....D.V.....I.....									
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X74443	NG1975	G.....G.....R.A.....D.....K.....									
KJ466104	GH2010	G.....G.....R.A.....D.....K.....									
KR828814	NG2012	G.....G.....RGA.....D.....K.....									
KP789375	SN1969	G.....S.....Y.....L.....K.....									
EU267273	CI 1989	I.....G.....S.....L.....K.....									

		510	520	530	540	550	560	570	580	590	600
											

	
KM089831 CN2014	G I E V T C N P V
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KM089832 CN2014	
JX217850 CN2008	
JF939201 CN2007	
KM091959 CN2013	
FJ905304 CN2007	
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KP260624 CN2004	
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KC594074 MA2008	
KR140086 IN1994	
KR828813 NG2013	 A
KJ867540 ET1994	
KJ867544 OM1983	
KJ867543 UG2012	
KJ867545 UAE1986	
KM463083 KE2011	
<i>EU267274 NG1976</i>	
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EU267273 CI 1989	 SA

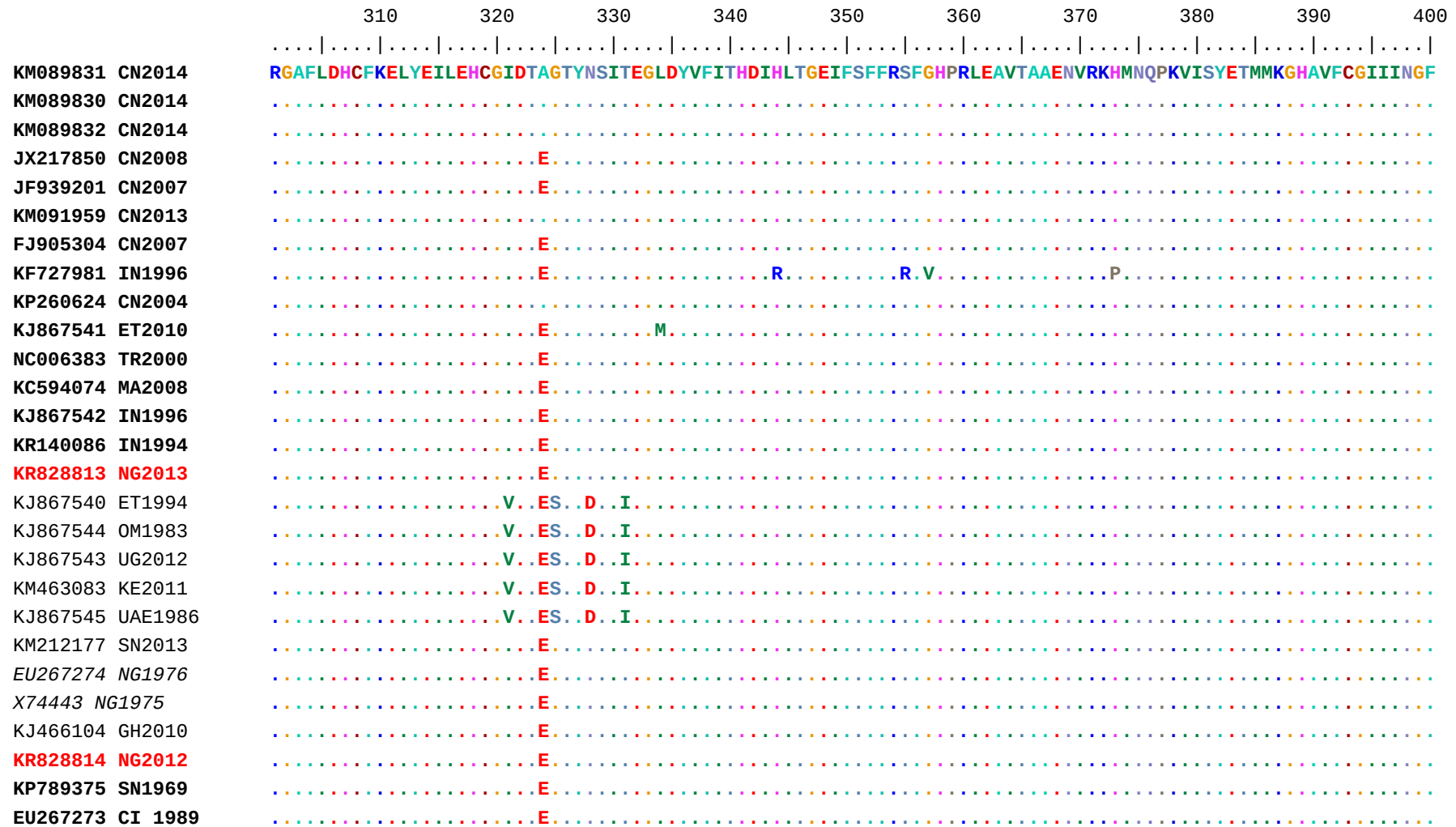
Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities.

Appendix 10: L protein sequence alignments of the PPRV from this study and reference strains



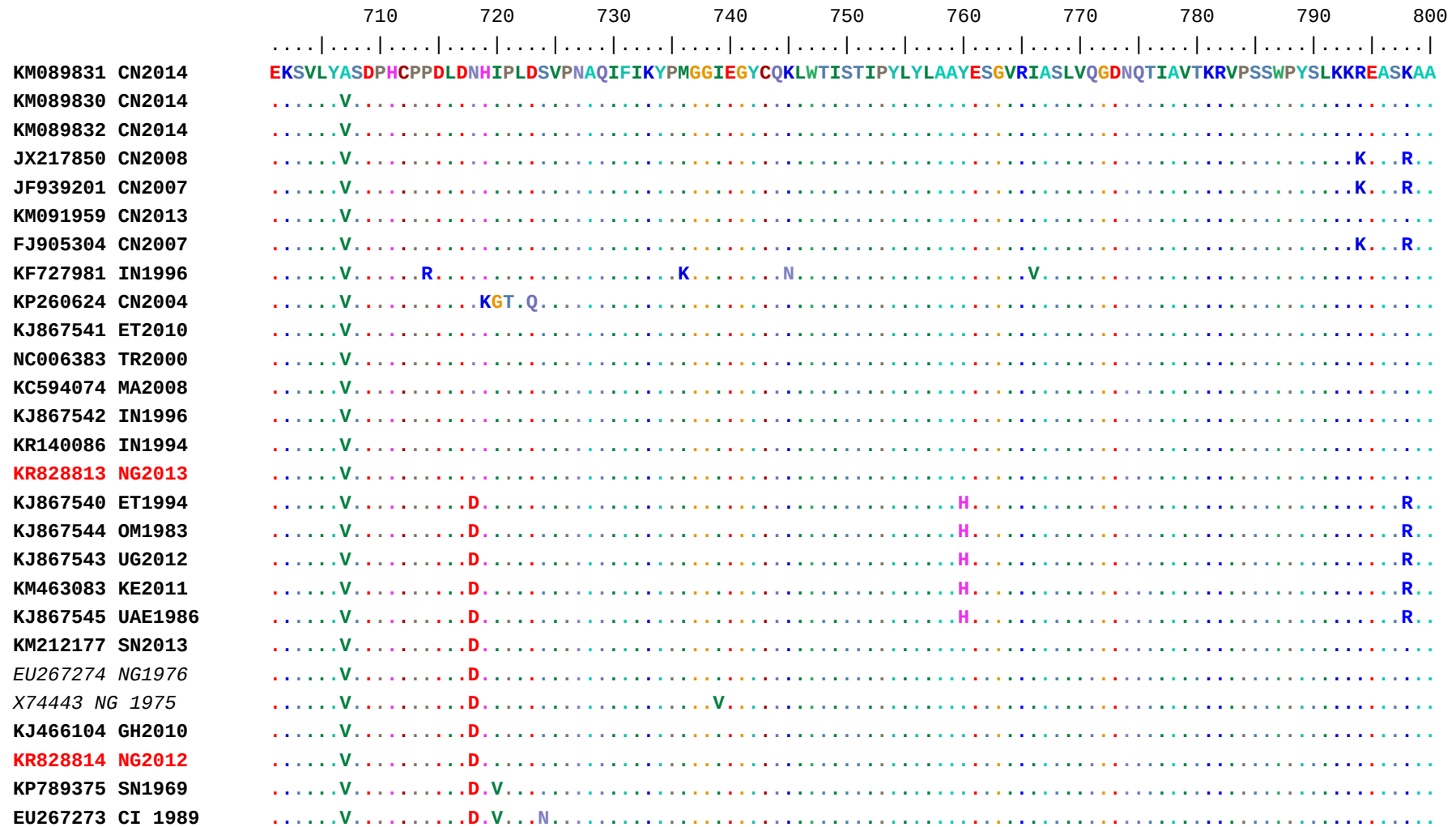
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FJ905304	CN2007	.	.	.	.	.	.	L.	.	I.	.
KF727981	IN1996	.	.	.	.Y.	.	.	L.	.	I.	.
KP260624	CN2004	.	.	.	.	.	.	L.	.	.	.
KJ867541	ET2010	.	.	.	.	.	.	L.	.	K.I.	.
NC006383	TR2000	.	.	.	.Y.	.	.	L.	.	I.	.
KC594074	MA2008	.	.	.	.	.	.	L.	.	K.I.	.
KJ867542	IN1996	.	.	.	.	.	.	L.	.	I.	.
KR140086	IN1994	.	.	.	.	.	.	L.	.	I.	.
KR828813	NG2013	.	R.	.	.	.	.	L.	.	I.	.
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KJ867543	UG2012	R.	.	R.M.S.	.	L.	.	.	.	.	.
KM463083	KE2011	R.	.	R.M.S.	.	L.	.	.	.	.	.
KJ867545	UAE1986	R.	.	R.M.N.	.	L.	.	.	.	.	.
KM212177	SN2013	R.V.	.	N.D.	.	L.	.	.	.	I.	.
EU267274	NG1976	R.	.	.	N.I.L.	.	.	.	.	I.	.
X74443	NG1975	R.K.	.	.	.	L.	.	.	.	I.	.
KJ466104	GH2010	R.V.	.	N.D.	.	L.	.	.	.	I.	.
KR828814	NG2012	R.V.	.	D.	.	L.	.	.	.	I.	.
KP789375	SN1969	R.T.	.	.	.	L.	.	.	.	V.	.
EU267273	CI 1989	R.V.T.	.	.	.	L.	.	.	.	V.	.

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JX217850	CN2008										
JF939201	CN2007										
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KF727981	IN1996								V	L	
KP260624	CN2004										
KJ867541	ET2010										
NC006383	TR2000								V		
KC594074	MA2008										
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KR828813	NG2013								V		
KJ867540	ET1994		C	Y			K		A	V	
KJ867544	OM1983		C	Y			K		A	V	
KJ867543	UG2012	L		T	C	Y		K		A	V
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KJ867545	UAE1986		C	Y			K		A	V	
KM212177	SN2013						N		V		
EU267274	NG1976						N		V		
X74443	NG1975						N		V		
KJ466104	GH2010						N		V		
KR828814	NG2012						N		V		
KP789375	SN1969		A		Y				V		
EU267273	CI 1989		A	G		Y			V		



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KP260624	CN2004																							
KJ867541	ET2010																							
NC006383	TR2000																							
KC594074	MA2008																							
KJ867542	IN1996													I										
KR140086	IN1994																							
KR828813	NG2013										R													
KJ867540	ET1994			V							R													
KJ867544	OM1983			V							R													
KJ867543	UG2012			V							R													
KM463083	KE2011			V							R													
KJ867545	UAE1986			V							R													
KM212177	SN2013										R													
EU267274	NG1976																							
X74443	NG1975										R													
KJ466104	GH2010										R													
KR828814	NG2012										R													
KP789375	SN1969										R													
EU267273	CI 1989										R													

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JX217850	CN2008											
JF939201	CN2007											
KM091959	CN2013											
FJ905304	CN2007											
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KP260624	CN2004											
KJ867541	ET2010											
NC006383	TR2000											
KC594074	MA2008											
KJ867542	IN1996											
KR140086	IN1994											
KR828813	NG2013											
KJ867540	ET1994			E.H								
KJ867544	OM1983			E.H								
KJ867543	UG2012		L	E.H								
KM463083	KE2011		L	E.H								
KJ867545	UAE1986			E.H								
KM212177	SN2013											
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X74443	NG1975											
KJ466104	GH2010											
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KP789375	SN1969											
EU267273	CI 1989											

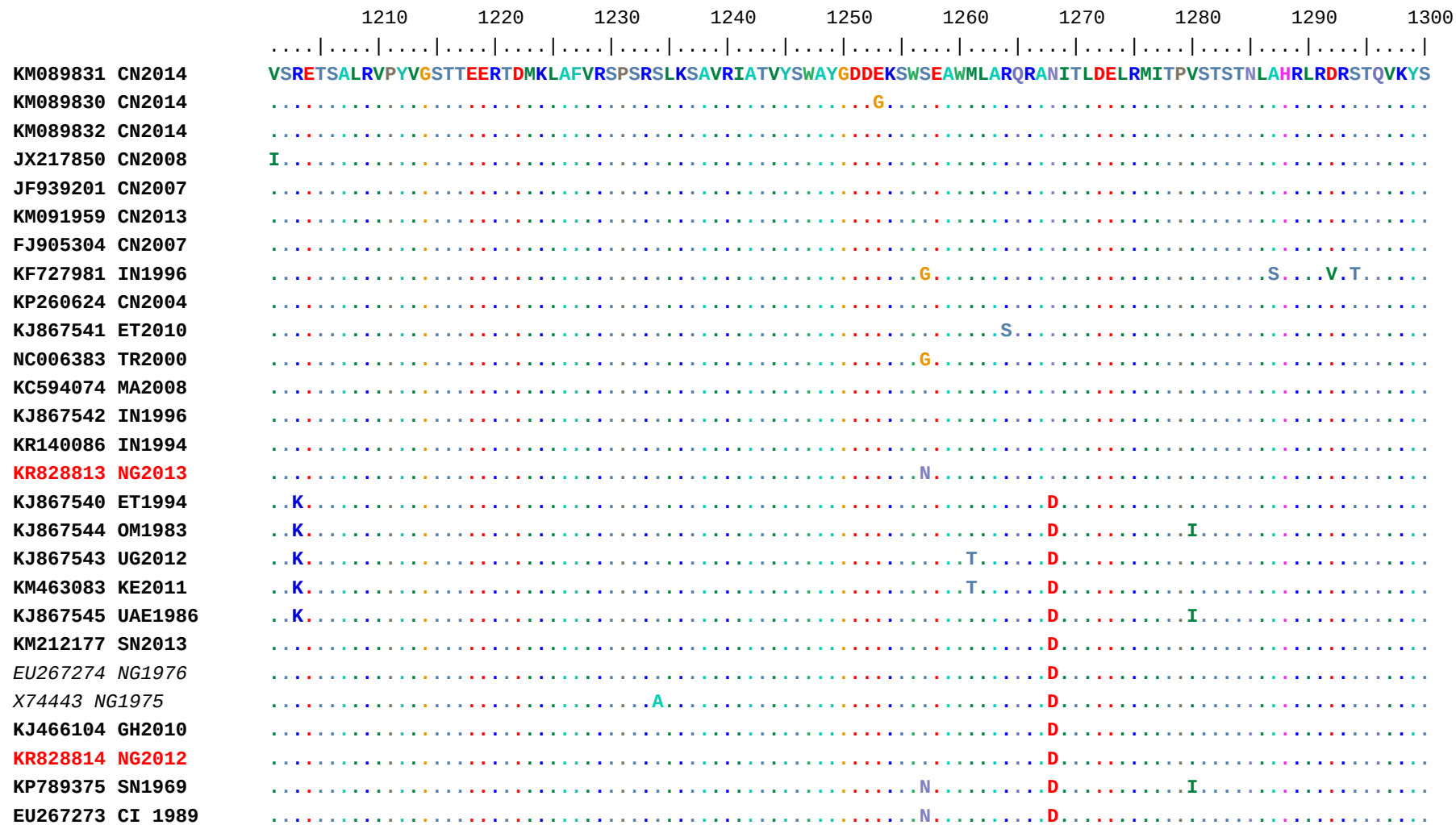


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JF939201	CN2007															
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KC594074	MA2008	R														
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KR828813	NG2013							I								
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EU267274	NG1976							I								
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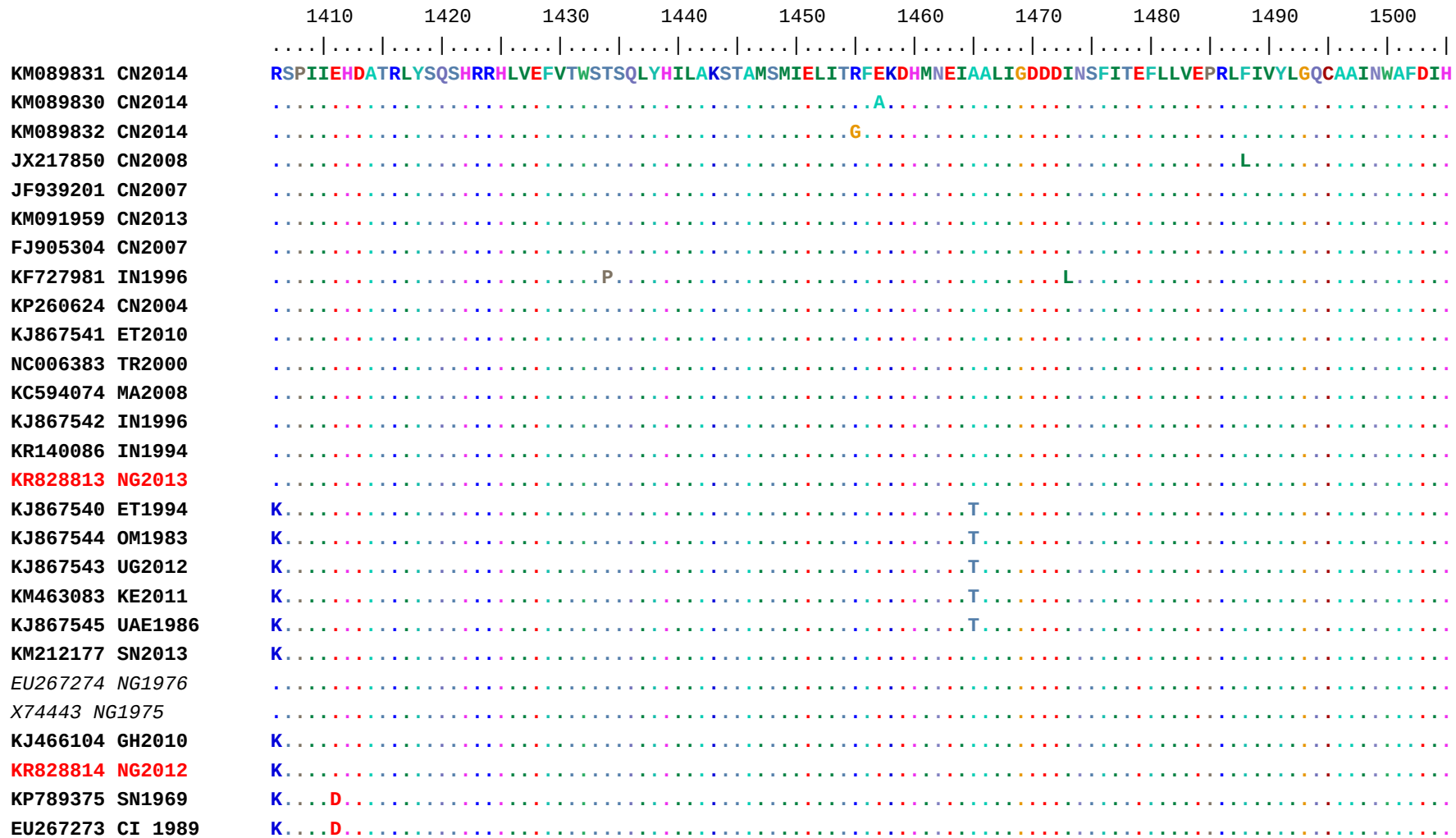
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KR828813	NG2013		K		T		V				
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KR828814	NG2012		V	D							
KP789375	SN1969										
EU267273	CI 1989										

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NC006383	TR2000										
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KR140086	IN1994										
KR828813	NG2013	T.....K..R.....GKN.....E..K.....									
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KP260624 CN2004	K.....									
KJ867541 ET2010	K.....S...R.....I...T.....									
NC006383 TR2000	K.....S...R.....I.....									
KC594074 MA2008	A...K.....S...R.....I.....									
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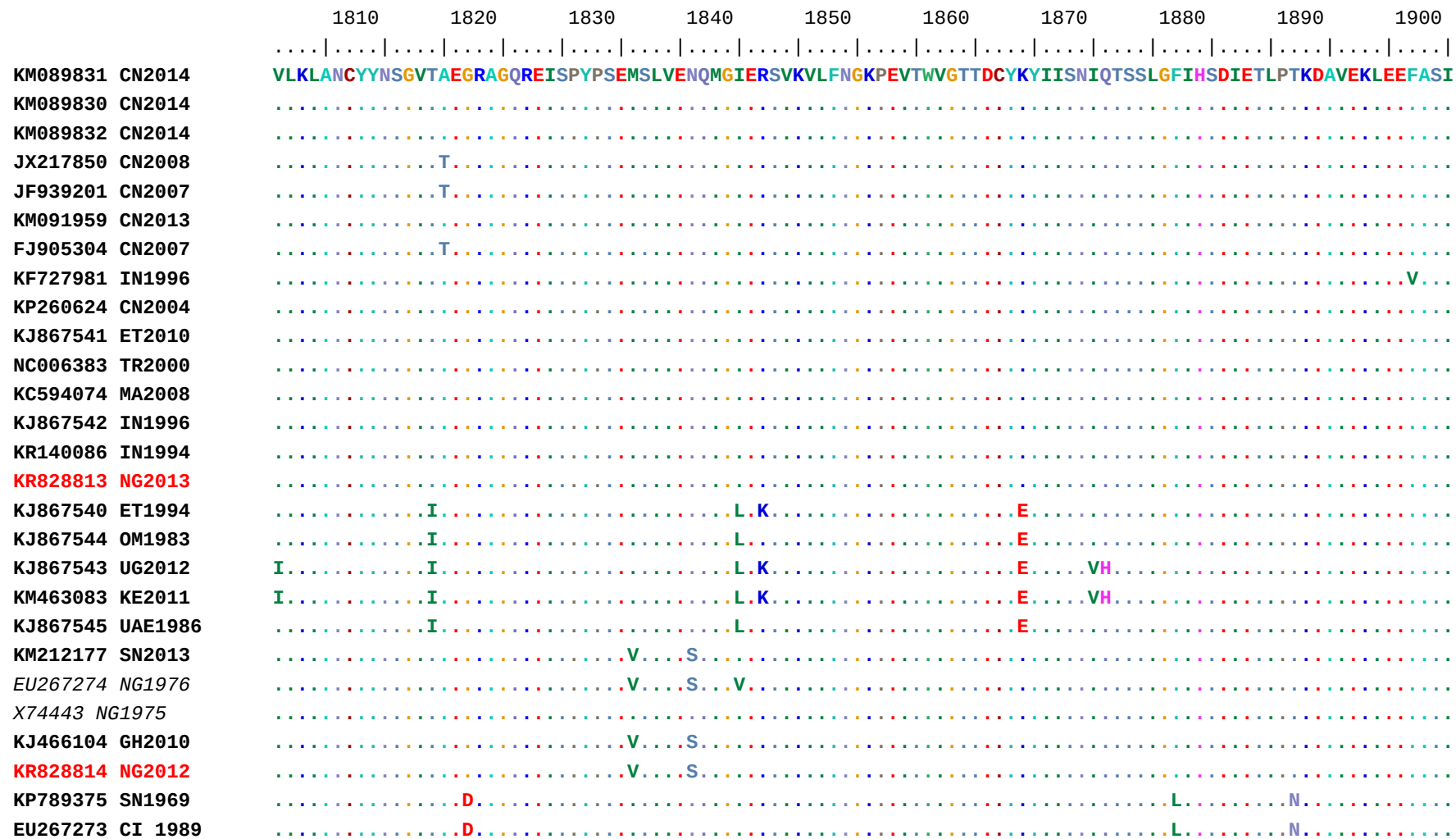
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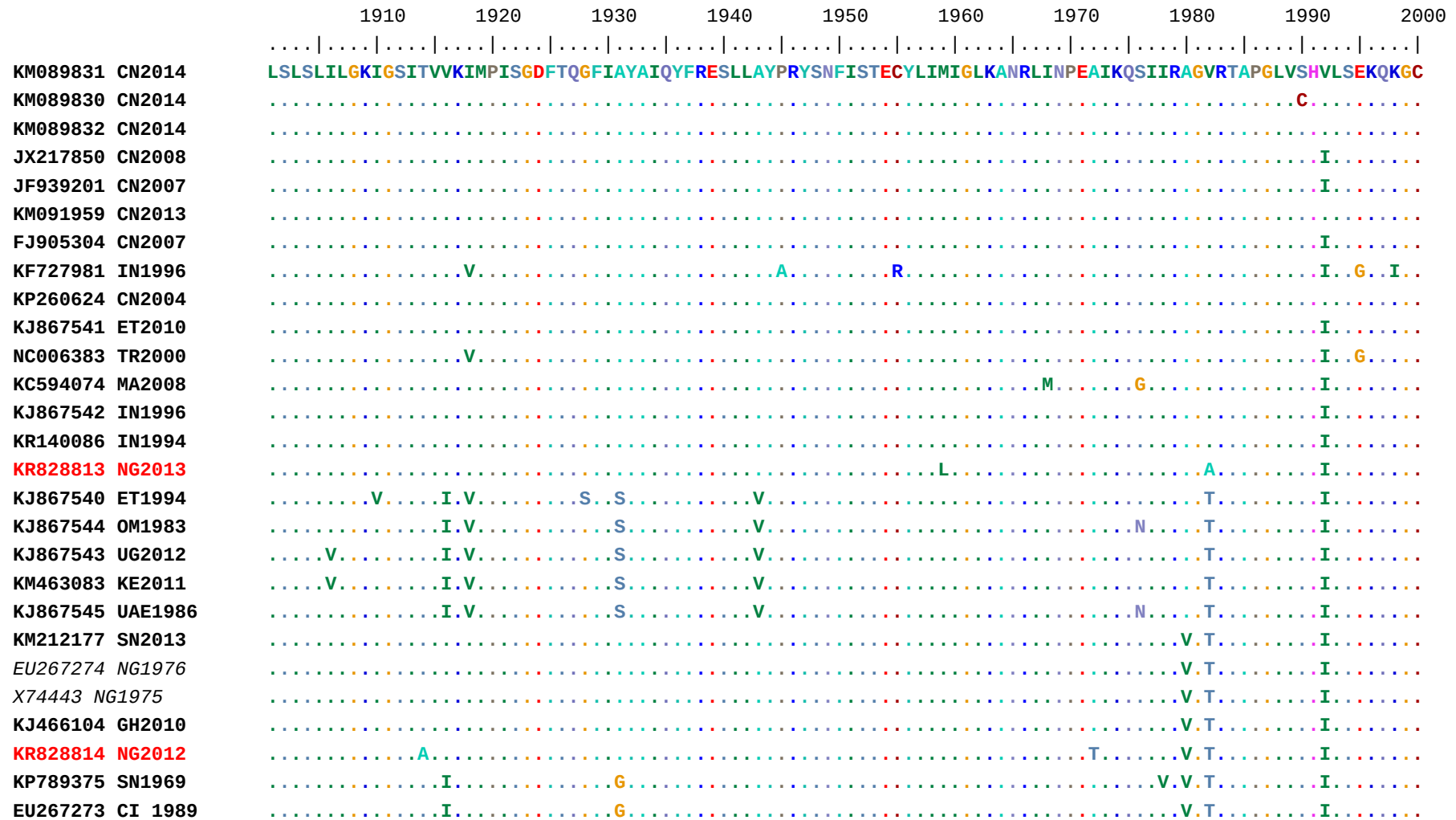


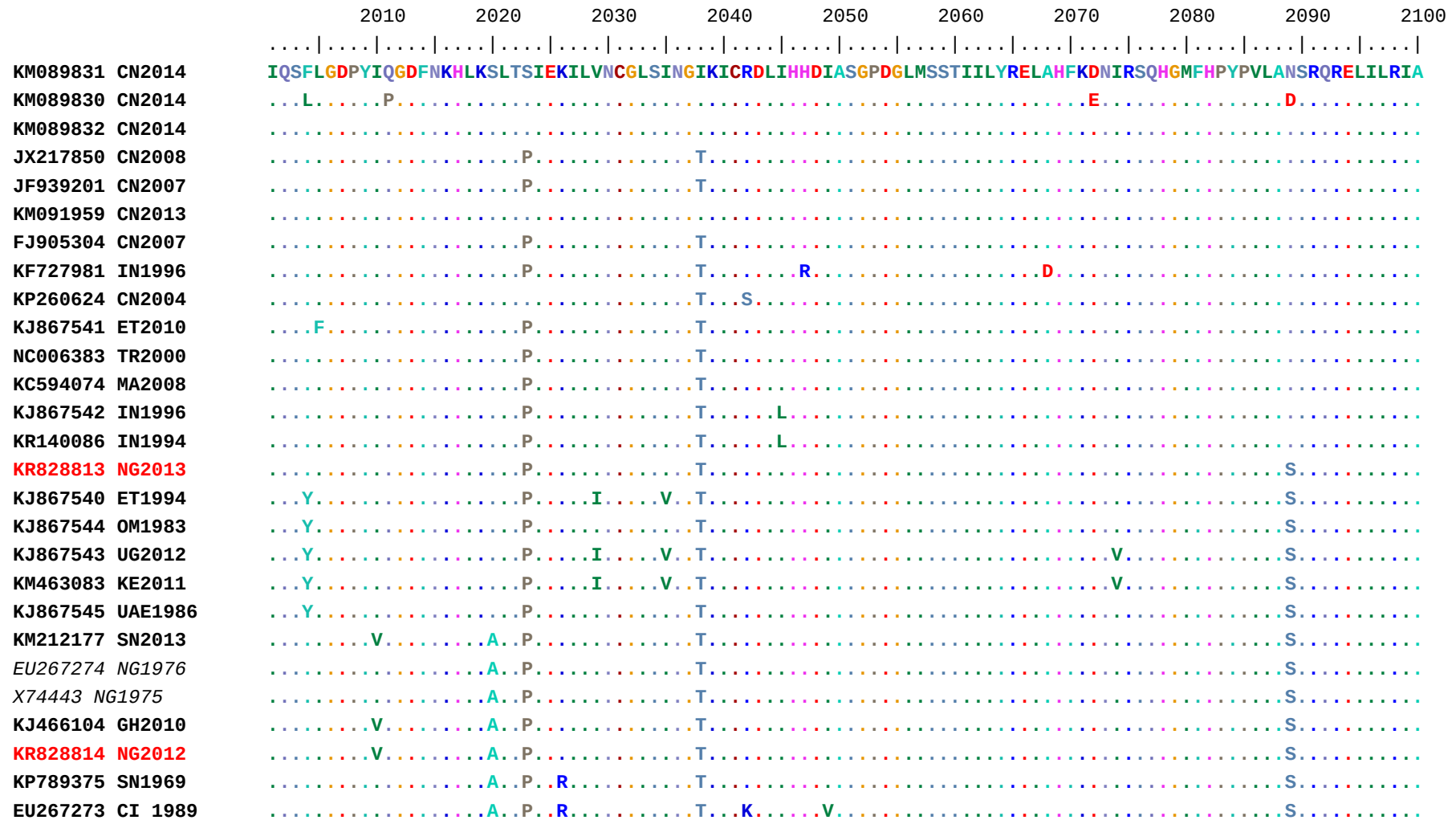
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KC594074 MA2008					V					
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KR828813 NG2013					V					
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KJ867544 OM1983					V				T	
KJ867543 UG2012					V				T	
KM463083 KE2011					V				T	
KJ867545 UAE1986					V				T	
KM212177 SN2013					V				A	
EU267274 NG1976					V				A	
X74443 NG1975					V					
KJ466104 GH2010					V				A	
KR828814 NG2012					V				A	
KP789375 SN1969					T				T	
EU267273 CI 1989					T				T	

		1610	1620	1630	1640	1650	1660	1670	1680	1690	1700
											
KM089831	CN2014	TDRFDNIQAKHLCVLADV	CSSKRCPSIIGMSP	IEKCTILTHYIKGESV	QSPSGT	SWNTDPLVVDHY	SCSLIYLRRG	SIKQIRLRVDP	GFVFEALTDVDF		
KM089830	CN2014							T.....			
KM089832	CN2014		F.....					T.....		L.....	
JX217850	CN2008							T.....			L.....
JF939201	CN2007							T.....			L.....
KM091959	CN2013							T.....			
FJ905304	CN2007							T.....			L.....
KF727981	IN1996	E.V.....		M.....				T.....C.....			
KP260624	CN2004							T.....			
KJ867541	ET2010						I.....	T.....			I.....
NC006383	TR2000							T.....C.....			
KC594074	MA2008							T.....		I.....	
KJ867542	IN1996							T.....			
KR140086	IN1994							T.....			
KR828813	NG2013					I.T.....		T.....			
KJ867540	ET1994					I.....		T.....		I.I.....	
KJ867544	OM1983		K.....			I.....	E.....	T.....		I.T.....	
KJ867543	UG2012					I.....	A.....	T.....		I.V.....	
KM463083	KE2011					I.....	A.....	T.....		I.V.....	
KJ867545	UAE1986		K.....			I.....	E.....	T.....		I.T.....	
KM212177	SN2013						I.....	T.....		I.....	
EU267274	NG1976						I.....	T.....		I.....	
X74443	NG1975						I.....	T.....		I.....	
KJ466104	GH2010						I.....	T.....		I.....	
KR828814	NG2012						I.....	T.....		I.....	
KP789375	SN1969		L.T.....		V.....	I.....	G.....	T.....		I.I.....	
EU267273	CI 1989		L.T.....		V.....	I.....	G.....	T.....		I.I.....	

		1710	1720	1730	1740	1750	1760	1770	1780	1790	1800
											
KM089831	CN2014	KQPRKAKLDISVVGLTDFSPPYDNVGDFLGTINTLRHDLPTGTGVSNEYEVHAYRRIGLNSSACYKAVEISTLIKPSLEVGEHGLFLGEGSGSMLAAYKE									
KM089830	CN2014										
KM089832	CN2014	S.....									
JX217850	CN2008	C.....N.....									
JF939201	CN2007	V.....C.....N.....									
KM091959	CN2013										
FJ905304	CN2007	C.....N.....									
KF727981	IN1996	Y.....C.....N.....R.....E.....									
KP260624	CN2004										
KJ867541	ET2010	C.....N.....									
NC006383	TR2000	C.....N.....R.....									
KC594074	MA2008	C.....N.....Y.....									
KJ867542	IN1996	I.....C.....N.....									
KR140086	IN1994	I.....C.....N.....									
KR828813	NG2013	A.....W.....I.....N.....									
KJ867540	ET1994	NT.V.....W.....I.....R.....N.....									
KJ867544	OM1983	L.....NT.V.....W.....I.....R.....N.....									
KJ867543	UG2012	NTGV.....W.....I.V.....R.....N.....I.....L.....									
KM463083	KE2011	NTGV.....W.....I.V.....R.....N.....I.....L.....									
KJ867545	UAE1986	L.....NT.V.....W.....I.....R.....N.....									
KM212177	SN2013	V.I.....W.....N.....V.....A.....A.....N.....									
EU267274	NG1976	A.I.....W.....N.....S.....D.....									
X74443	NG1975	V.....W.....N.....S.....N.....									
KJ466104	GH2010	V.I.....W.....N.....S.....A.....N.....									
KR828814	NG2012	V.I.....W.....N.....S.....N.....									
KP789375	SN1969	V.....W.....N.....L.....S.....									
EU267273	CI 1989	C.....V.....W.....N.....S.....									







	2110	2120	2130	2140	2150	2160	2170	2180
								
KM089831 CN2014	KKFWGYVLLYSDDPALIRQTIKNLKRNLHFDLH	SNPF	IKGLSKAEKLLVRTSSLRREWLFTLETKEVK	EWFKLVGY	SALIRG			
KM089830 CN2014								
KM089832 CN2014								
JX217850 CN2008					P.	D.		
JF939201 CN2007					P.	A.D.		
KM091959 CN2013								
FJ905304 CN2007					P.	D.		
KF727981 IN1996						D.	S.E.	
KP260624 CN2004								
KJ867541 ET2010						D.		
NC006383 TR2000						D.		
KC594074 MA2008			N.			D.		
KJ867542 IN1996				E.		D.		
KR140086 IN1994						D.		
KR828813 NG2013						D.		
KJ867540 ET1994			N.	R.				V.
KJ867544 OM1983				R.	M.			V.
KJ867543 UG2012			N.	R.				V.
KM463083 KE2011			N.	R.				V.
KJ867545 UAE1986				HSNPF	IKGLSRAEKM.VRTS.LR.EWLFTLETKEVK	EWFKLVGY	SALVRG	
KM212177 SN2013		K.				D.		V.
EU267274 NG1976		K.				D.		V.
X74443 NG 1975		K.				N.		V.
KJ466104 GH2010		K.	S.			D.		V.
KR828814 NG2012		K.	R.		Y.	D.		V.
KP789375 SN1969		R.	G.					V.
EU267273 CI 1989		R.	G.A.			I.		V.

Isolates from this study are labelled in red, older Nigerian isolates are in italics and dots (.) indicates similarities

Appendix 11: Small ruminants PPRV seroprevalence survey from different localities in Nigeria, 2010 – 2013

Province	Town	Latitude	Longitude	Sheep positiv	Sheep negat	Sheep collec	Goat positiv	Goat negativ	Goat collect	Total positiv	Total Collect	Prev_sheep	Prev_goats	Prev_Total
Adamawa State	Gulak	10.806	13.456	5	32	37	11	50	61	16	98	13.51	18.03	16.33
Adamawa State	Mubi	10.300	13.267	3	33	36	13	45	58	16	94	8.33	22.41	17.02
Adamawa State	Njobli	9.227	12.883	11	31	42	17	50	67	28	109	26.19	25.37	25.69
Adamawa State	Numan	9.483	12.050	5	26	31	9	38	47	14	78	16.13	19.15	17.95
Akwa Ibom State	Aka	5.183	7.650	0	1	1	20	68	88	20	89	0.00	22.73	22.47
Akwa Ibom State	Brangaville	4.983	7.833	0	1	1	30	74	104	30	105	0.00	28.85	28.57
Akwa Ibom State	Etobodom	4.800	8.083	0	2	2	22	68	90	22	92	0.00	24.44	23.91
Akwa Ibom State	Uyo	5.017	7.883	1	0	1	21	71	92	22	93	100.00	22.83	23.66
Anambra State	Adazi-Ani	6.064	6.973	0	4	4	17	72	89	17	93	0.00	19.10	18.28
Anambra State	Amada	6.102	6.987	0	5	5	23	76	99	23	104	0.00	23.23	22.12
Anambra State	Eziora	6.070	6.986	0	4	4	18	73	91	18	95	0.00	19.78	18.95
Anambra State	Umuchi	6.025	7.084	0	4	4	16	71	87	16	91	0.00	18.39	17.58
Cross Rivers State	Biase	5.602	8.121	0	0	0	16	82	98	16	98		16.33	16.33
Cross Rivers State	Gabu	6.267	8.600	0	0	0	9	58	67	9	67		13.43	13.43
Cross Rivers State	Ikot Eneobong	5.056	8.372	0	0	0	7	36	43	7	43		16.28	16.28
Cross Rivers State	Ikot-Omin	5.050	8.357	0	0	0	11	76	87	11	87		12.64	12.64
Cross Rivers State	Mariam	4.975	8.337	0	0	0	13	71	84	13	84		15.48	15.48
Imo State	Eziama-Obaire	5.797	7.144	0	0	0	29	71	100	29	100		29.00	29.00
Imo State	Iho	5.577	7.115	0	0	0	22	70	92	22	92		23.91	23.91
Imo State	Oguta	5.705	6.806	1	1	2	23	78	101	24	103	50.00	22.77	23.30
Imo State	Owerri	5.477	7.025	1	3	4	26	54	80	27	84	25.00	32.50	32.14
Kano State	Dambatta	12.426	8.509	16	27	43	12	40	52	28	95	37.21	23.08	29.47
Kano State	Dogora	12.013	8.521	12	29	41	15	44	59	27	100	29.27	25.42	27.00
Kano State	Gaya	11.867	8.467	13	21	34	14	41	55	27	89	38.24	25.45	30.34
Kano State	Kano	11.539	8.158	11	28	39	13	43	56	24	95	28.21	23.21	25.26
Kwara State	Baruten	9.550	3.231	9	27	36	14	47	61	23	97	25.00	22.95	23.71
Kwara State	Illorin	8.474	4.619	12	27	39	19	44	63	31	102	30.77	30.16	30.39
Kwara State	Kaima	9.604	3.947	9	15	24	8	41	49	17	73	37.50	16.33	23.29
Kwara State	Offa	8.217	4.700	13	29	42	17	51	68	30	110	30.95	25.00	27.27
Ogun State	Abeokuta	7.050	3.317	3	11	14	14	68	82	17	96	21.43	17.07	17.71
Ogun State	Ijebu-Ode	6.783	3.967	2	7	9	11	78	89	13	98	22.22	12.36	13.27
Ogun State	Iwo	6.650	4.150	2	10	12	18	69	87	20	99	16.67	20.69	20.20
Ogun State	Owode	6.948	3.505	2	14	16	15	55	70	17	86	12.50	21.43	19.77
Ondo State	Akure	7.253	5.192	1	14	15	14	58	72	15	87	6.67	19.44	17.24
Ondo State	Fajaw	6.450	4.967	0	17	17	15	56	71	15	88	0.00	21.13	17.05
Ondo State	Idanre	7.151	5.109	0	14	14	19	66	85	19	99	0.00	22.35	19.19
Ondo State	Ikare	7.533	5.667	1	15	16	21	68	89	22	105	6.25	23.60	20.95
Plateau State	Jos	9.882	8.969	7	37	44	9	42	51	16	95	15.91	17.65	16.84
Plateau State	Pankshin	9.327	9.443	6	33	39	16	36	52	22	91	15.38	30.77	24.18
Plateau State	Rukuba	9.882	8.885	5	34	39	11	42	53	16	92	12.82	20.75	17.39
Plateau State	Shendam	8.877	9.527	8	33	41	17	43	60	25	101	19.51	28.33	24.75
Sokoto State	Bafarawa	13.317	6.650	10	34	44	14	42	56	24	100	22.73	25.00	24.00
Sokoto State	Bodinga	12.875	5.167	4	33	37	14	39	53	18	90	10.81	26.42	20.00
Sokoto State	Goronyo	13.433	5.667	12	37	49	10	38	48	22	97	24.49	20.83	22.68
Sokoto State	Sokoto	13.033	5.267	9	33	42	11	39	50	20	92	21.43	22.00	21.74
Taraba State	Garbabi	7.838	11.035	9	22	31	14	41	55	23	86	29.03	25.45	26.74
Taraba State	Kassa	8.822	11.643	14	28	42	23	46	69	37	111	33.33	33.33	33.33
Taraba State	Maihula	7.989	10.995	10	26	36	17	33	50	27	86	27.78	34.00	31.40
Taraba State	Wukari	7.866	9.765	7	27	34	14	48	62	21	96	20.59	22.58	21.88
TOTAL				101	377	478	170	489	659	271	93.5	21.15	25.59	23.74

Appendix 12: Nigerian individual State PPRV seroprevalence data based on species of small ruminant, 2010 – 2013

Province	SheepPos	SheepNeg	SheepTotal	Prev_sheep	GoatsPos	GoatsNeg	GoatTotal	Prev_goat	TotalPos	TotalNeg	Total	Prevalence
Anambra	0	13	13	0.00	74	292	366	20.22	74	305	379	19.53
Akwa Ibom	1	7	8	12.50	94	277	371	25.34	95	284	379	24.80
Adamawa	24	122	146	16.44	52	181	233	22.32	76	303	379	20.05
Imo	2	4	6	33.33	100	273	373	26.81	102	277	379	26.91
Taraba	40	103	143	27.97	66	170	236	27.97	106	273	379	28.50
Plateau	26	137	163	15.95	53	163	216	24.54	79	300	379	20.84
Kano	52	105	157	33.12	54	168	222	24.32	106	273	379	27.97
Ogun	9	42	51	17.65	58	270	328	17.68	67	312	379	17.68
Kwara	43	95	138	31.16	58	183	241	24.07	101	278	379	26.65
Ondo	2	60	62	3.23	69	248	317	21.77	71	308	379	18.73
Cross River	0	0	0		56	323	379	14.78	56	323	379	14.78
Sokoto	35	137	172	20.35	49	158	207	23.67	84	295	379	22.16

Appendix 13: PPRV seroprevalence data based on category of small ruminant in Nigeria, 2010 – 2013

Province	SMApos	SMYpos	SFApos	SFYpos	GMApos	GMYpos	GFApos	GFYpos	SMAneg	SMYneg	SFAneg	SFYneg	GMAneg	GMYneg	GFAneg	GFYneg	Prev_SMA	Prev_GMA	Prev_SMY	Prev_GMY	Prev_SFA	Prev_GFA	Prev_SFY	Prev_GFY
Anambra	0	0	0	0	17	12	24	21	8	0	5	0	69	66	85	73	0.00	19.77		15.38	0.00	22.02		22.34
Akwa Ibom	1	0	0	0	11	23	36	24	3	0	3	1	31	61	120	65	25.00	26.19		27.38	0.00	23.08	0.00	26.97
Adamawa	8	3	12	1	15	5	18	14	48	11	52	11	54	22	71	34	14.29	21.74	21.43	18.52	18.75	20.22	8.33	29.17
Imo	1	0	1	0	29	18	32	21	1	0	3	0	73	56	77	67	50.00	28.43		24.32	25.00	29.36		23.86
Taraba	13	6	16	5	21	7	26	14	36	11	40	16	50	17	72	29	26.53	29.58	35.29	29.17	28.57	26.53	23.81	32.56
Plateau	8	2	12	4	16	7	21	9	45	12	57	23	48	17	68	30	15.09	25.00	14.29	29.17	17.39	23.60	14.81	23.08
Kano	14	8	21	9	14	9	19	12	32	15	40	18	38	27	70	33	30.43	26.92	34.78	25.00	34.43	21.35	33.33	26.67
Ogun	5	0	3	1	11	8	27	12	14	2	21	5	50	25	69	26	26.32	18.03	0.00	24.24	12.50	28.13	16.67	31.58
Kwara	11	4	16	12	21	9	23	5	32	12	45	6	68	25	68	22	25.58	23.60	25.00	26.47	26.23	25.27	66.67	18.52
Ondo	1	0	1	0	13	8	32	16	17	11	20	12	71	49	81	47	5.56	15.48	0.00	14.04	4.76	28.32	0.00	25.40
Cross Rive	0	0	0	0	11	18	23	4	0	0	0	0	76	73	120	54		12.64		19.78		16.08		6.90
Sokoto	12	4	13	6	14	5	22	8	52	12	58	15	45	17	70	26	18.75	23.73	25.00	22.73	18.31	23.91	28.57	23.53
Total	74	27	95	38	193	129	303	160	288	86	344	107	673	455	971	506	20.44	23.89	21.64	26.21	22.29	22.09	23.78	24.02