Peste des petits ruminants in Nigeria: identification and variations of lineage II and lineage IV in sheep and goats

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Summary
Peste des petits ruminants virus (PPRV) is the aetiologic agent of Peste des petits ruminants (PPR), an important viral disease of sheep and goats. PPR is endemic in Nigeria and leads to social and economic losses. The objective of this study was to determine the prevalence of PPR infection and genetically characterize PPRV strains obtained from sheep and goats in three States of Southeast Nigeria. A total of 285 nasal swab samples collected in 2017-2018 were processed for PPRV genome detection by RT-PCR. Sixty-five (22.81%) of the samples were positive for PPRV. Sequence and phylogenetic analyses revealed that the PPRV belonged to lineages II (11/38, 28.9%) and IV (27/38, 71.1%). The N gene fragment sequence showed a 99.77%-100% and 99.98%-100% identity among the strains of lineages II and IV, respectively. Fourteen amino acid substitutions, previously unreported in PPRV strains from Nigeria, were recorded. This study confirms the circulation of PPRV lineages II and IV in Southeast Nigeria, the dominance of strains belonging to lineage IV in recent years, and their close genetic relationship with those previously reported in other parts of Nigeria and neighboring countries.

Introduction

Peste des petits ruminants (PPR) is a transboundary viral disease of small ruminants, whose clinical signs are characterized by pneumonia, ocular and nasal discharges, conjunctivitis, necrotic stomatitis and diarrhoea (Zahur *et al.* 2011, Gibbs *et al.* 1979). The disease associated with high morbidity (100%) and mortality (up to 90%), particularly among naïve populations of sheep and goats, might cause severe social and economic losses amongst rural households/small ruminant farmers (Abu Elzein *et al.* 1990). Following its first report in Côte d'Ivoire (Gargadennec and Lalanne 1942), the disease has spread and has been reported in several countries across African, the Near and Middle East, Asia and, more recently, Europe (Parida *et al.* 2015, Donduashvili *et al.* 2018).

PPR is caused by Peste des petits ruminants virus (PPRV), taxonomically classified by the International Committee on Taxonomy of Viruses (ICTV) as specie small ruminant morbillivirus (SRMV) (Amarasinghe *et al.* 2019), a member of the *Morbillivirus* genus, belonging to the family *Paramyxoviridae*, subfamily *Orthoparamyxovirinae* (Gibbs *et al.* 1979). PPRV is a single-stranded negative-sense RNA virus, whose genome consists of approximately 15-16 Kb in size, encoding for six structural proteins, namely

nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin (H) and large (L), and two nonstructural (V and C) proteins (Bailey *et al.* 2005).

Although PPRV has only one serotype, PPRV isolates are genetically grouped into four distinct lineages (I, II, III and IV), based on sequence analysis of the fusion (F), nucleocapsid (N) or haemagglutinin (H) protein genes (Forsyth and Barrett 1995, Dhar *et al.* 2002, Couacy-Hymann *et al.* 2002, Kumar *et al.* 2014). Lineage I, which was circulating in West African countries in 1988-1994 (Kwiatek *et al.* 2007), has not been identified at molecular level in Africa since 2001 (Tounkara *et al.* 2018). Lineage II has been identified in Central and Western Africa; lineage III in Eastern Africa and southern part of the Middle East while lineage IV circulates across the Middle East, including Israel and Turkey, part of Asian continent and Northern, Western, Central and Eastern Africa (Kwiatek *et al.* 2007, Banyard *et al.* 2010, Munir *et al.* 2013, Cosseddu *et al.* 2013, Parida *et al.* 2015, Mantip *et al.* 2016, Woma *et al.* 2016, Baazizi *et al.* 2017). Lineages III and IV co-circulation in the United Arab Emirates and Qatar (Kwiatek *et al.* 2007, Libeau *et al.* 2014) has been reported. Because of these previous reports, it has become necessary to provide further epidemiological and genetic data on the spread of PPRV among sheep and goats in Nigeria. This study was therefore conducted to determine the prevalence of PPRV infection and genetic characteristics of PPRV strains circulating in South-East Nigeria.

Materials and methods

Samples collection

A multistage sampling method was performed with three hierarchical stages and the size of the sample used in this study was estimated using the formula according to Thrusfield (Thrusfield 2005), as described by Chukwudi and colleagues (Chukwudi *et al.* 2020). Nasal swabs were collected within a period of seven months (from December 2017 to June 2018) from 285 randomly selected sheep (n = 172) and goats (n = 113) at local markets, households, abattoirs and veterinary clinics in three randomly selected states: Anambra, Ebonyi and Enugu (latitudes 7°07′N and 3°90′N and longitudes 6°51′E and 8°30′E), located in South-East Nigeria (Figure 1). The animals had no history of vaccination and the sick animals showed one or a combination of the following clinical signs: nasal and ocular discharges, coughing, diarrhoea, ulcerative stomatitis, weakness, emaciation (Supplementary Table). The samples were properly packaged in dry ice and sent to the Transboundary Animal Disease Laboratory, Agricultural Research Council (ARC) - Ondersteport Veterinary Research Institute (OVI), South Africa, for processing, analysis, and sequencing.

RNA extraction and RT-PCR

Sterile phosphate-buffered saline (PBS) (500 µl) was added into each tube containing the swab sample. This was centrifuged at 10,000 x g for 3-5 minutes (+ 4 °C) and supernatants (swab extract) was decanted into a sterile tube. Viral RNA was extracted from 140 µl of swab homogenate using the commercial viral RNA kit, QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instruction. Presence of PPRV RNA was evaluated using the forward primer NP3 and reverse primer NP4 amplifying a 351-bp fragment of the nucleocapsid (N) gene (Couacy-Hymann *et al.* 2002). Reverse transcription-polymerase chain reaction was carried out in 25 µl volume reaction mix using the Qiagen® OneStep Ahead RT-PCR Kit (Qiagen) consisting of 10 µl of 2.5x OneStep Ahead RT-PCR Master mix, 1 µl of 25x OneStep Ahead RT-Mix, 1.5 µl of 10 pmol (each) of forward and reverse primers, 5 µl of 5x Q solution, 3.8 µl dH_2O, 0.2 µl of 125x OneStep Ahead Master Mix Tracer and 2 µl of template RNA. Aliquot of molecular grade water was used as the negative control while the positive control consisted of an established positive sample (a clone of the live-attenuated PPRV vaccine derived from the Nigeria/75/1 strain, grown on VERO cell, and imported from The Pirbright Institute, Pirbright, United Kingdom). Amplification was conducted under the following thermal conditions: 50 °C for 10 min to activate the reverse transcriptases, 95 °C for 5 min to activate the DNA polymerases followed by 40 cycles of 95 °C for 10 sec, 55°C for 10 sec, 72 °C for 10 sec and a final extension of 72 °C for 2 min. Each amplicon (5 µl)

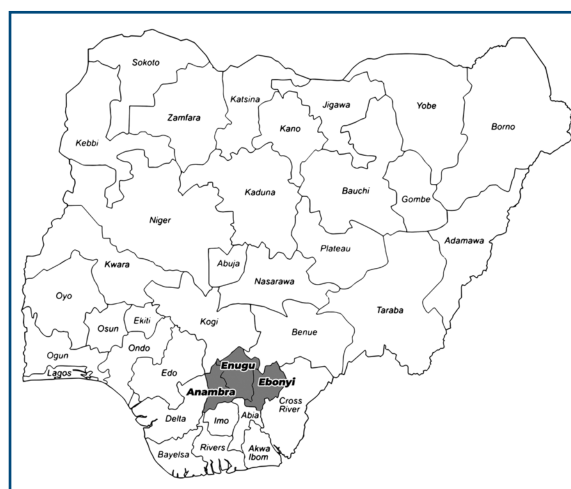

Figure 1. *Map of Nigeria showing the three States sampled (marked with gray background).*

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was analysed by electrophoresis at 120 volt/80 mA for 45 min on a 1.5% agarose gel stained with ethidium bromide, examined and photographed under ultraviolet light using a gel documentation system (BioRAd Gel DocTM xRT model no. Universal HoodII, BioRad, USA).

Sequencing

Amplicons were purified with the commercial kit Qiaquick® Gel Extraction kit (Qiagen), according to manufacturer's instruction. The concentration and purity of the DNAs were quantified using spectrophotometer (NanoDrop ND-1000, PeQlab Biotech Gmbh), according to manufacturer's protocol. Amplicon that had more than 20 ng DNA load were stored at - 20 °C and then submitted to Inqaba Biotech™ (Pretoria, South Africa) for direct Sanger sequencing in both forward and reverse directions with primer pair NP3/NP4. Sequences were viewed and reversed using Chromas ver 2.6.4 (Technelysium Pty Ltd, South Brisbane, Australia) and low-quality sequences were not included in the further analyses. Sequences were then assembled and analysed using BioEdit ver 7.2.5 software and submitted to the nBLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search related sequences in public domain databases. These sequence data have been submitted to the DDBJ/EMBL/GenBank databases under accession number as presented in Supplementary Table.

Phylogenetic analysis

To elucidate the genetic relationships of the analysed PPRV strains, a phylogenetic tree based on the partial N gene sequences was constructed with the MEGA X software. The model selection was performed using the best-fit model of nucleotide substitution with MEGA X software. The phylogenetic tree was constructed using the maximum-likelihood method, according to the Kimura 2-parameter (K2) model with invariant sites (I) and bootstrap analysis with 1,000 replicates.

Statistical analysis

The infection prevalence (total positive/total sample) was calculated. Pearson's Chi-squared test was used to determine if there were associations in the number of positive and negative test results within each variable (area/location, species, sex, breeds, and months of sampling). Significance was accepted at $P < 0.05$.

Results

Detection of PPRV viral genome by RT-PCR

Sixty-five (22.81%) of the 285 nasal swab samples were positive for PPRV by RT-PCR assay. The highest infection rate of 27.73% was obtained from Enugu State while 21.73% and 16.21% were recorded for Anambra and Ebonyi States, respectively. Infection rate was higher in sheep (23.26%) than in goats (22.12%) while more females (24.5%) than males (20.8%) tested positive for PPRV. Based on breed distribution, Uda, Yankass and West African Dwarf breeds of sheep had infection prevalence of 33.3%, 13.3% and 23.5%, respectively, while Red Sokoto and West African Dwarf breeds of goats had 25% and 20.8%, respectively. Each analysed parameter is presented in Table I. There were no significant associations ($p > 0.05$) between the infection prevalence and state/location, species, sex or breed of animals sampled. Based on the month of sampling, the highest infection prevalence (68.8%) was obtained in March while the least (5.6%) was obtained in June. There was a significant association ($p < 0.05$) between the infection prevalence and the months of sampling (Figure 2).

Sequence analysis

Among the 65 samples that tested positive for PPRV by RT-PCR, 38 good quality sequences (303 nts

Table I. *Presence of the Pestes des petit ruminants virus in sheep and goats in South-East Nigeria according to state, species, breed and sex.*

State	No. positive	No. negative	Total samples
Enugu	33	86	119
Anambra	20	72	92
Ebonyi	12	62	74
Total	65	220	285
Species	**No. positive**	**No. negative**	**Total samples**
Sheep	40	132	172
Goat	25	88	113
Total	65	220	285
Breed	**No. positive**	**No. negative**	**Total samples**
Uda	4	8	12
Yankassa	2	13	15
WAD sheep	34	111	145
Red Sokoto	9	27	36
WAD goat	16	61	77
Total	65	220	285
Sex	**No. positive**	**No. negative**	**Total samples**
Male	27	103	130
Female	38	117	155
Total	65	220	285

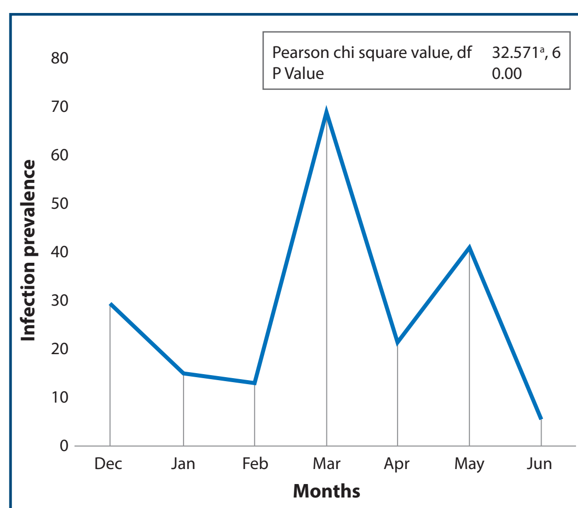

Figure 2. *Trend of PPR virus infection prevalence in small ruminants in South-East Nigeria.*

N gene fragments, by excluding the primers sequences; sequences ranged from nucleotide 1205 to 1507 of the N gene sequence acc. no. KR828813) were obtained by Sanger sequencing. Based on the amino acid (aa) residues, the 38 sequences clustered into lineage II (11/38, 28.9%) and IV (27/38, 71.1%). The N gene fragment showed 99.77%-100% and 99.98%-100% nt identities among the strains of the lineages II and IV, respectively.

When compared with the reference strain NGKW2012-MSLN (acc. no. KR828814), a number of amino acid substitutions previously reported among Nigerian PPRV strains belonging to lineage II (Mantip *et al.* 2016, Woma *et al.* 2016), were also found among the 11 strains in this study. However, two of these strains varied from previous lineage II strains from Nigeria by additional substitutions I→V and P→A at

Table II. *Amino acid changes of PPRV Lineage II strains collected in Nigeria.*

Strain/Isolate id.	Acc. no.	Year	Amino acid residue											
			403	424	425	435	442	451	456	459	469	473	477	482
NGKW2012-MSLN[b]	KR828814	2012	E	I	G	K	I	I	P	P	L	P	V	G
N67-127-GYM31KD-Oyo[b]	KJ124767	2011	D	T	D	R	I	T	S	P	P	Q	L	S
Vaccine Nigeria/75/1[b]	KY628761	1975	D	T	D	R	I	T	S	P	P	Q	L	S
NGEN_038 CA 2018	MT038903	2018	D	T	D	R	I	T	S	P	P	Q	L	S
NGEN_228 OV 2018	MT038904	2018	D	T	D	R	I	T	S	**A**	P	Q	L	S
NGEN_003 OV 2017	MT038905	2017	D	T	D	R	**V**	T	S	P	P	Q	L	S
Other[c]	Reference	2010-2013	E/D	I/T	G/D	K/R	I	I/T	P/S	P	L	P	V	G/S

[b]Reference strain; [c]Other previous PPRV (Peste des petit ruminants virus) sequences from Nigeria (Mantip *et al.* 2016, Woma *et al.* 2016); Bolded are amino acid changes reported for the first time among all the PPRV sequences collected in Nigeria

Table III. *Amino acid changes of PPRV Lineage IV strains collected in Nigeria.*

Strain/Isolate id.	Acc. no.	Year	Amino acid residue													
			415	423	429	432	434	437	442	443	454	462	470	472	478	483
NGYO2013-2162[d]	KR828813	2013	A	R	S	P	T	G	I	S	T	E	E	M	S	Q
PPRV/Cameroon/ CMRS6/2017[d]	MH447983	2017	A	K	S	P	T	G	I	P	T	E	E	T	S	Q
SRMV/Niger/12/2017[d]	MK673131	2017	-	K	L	P	T	G	I	P	T	E	E	T	S	Q
Nig12:IM21[d]	KF479428	2012	A	K	S	P	T	G	I	P	T	E	E	T	S	Q
Nig12:TR10[d]	KF479417	2012	A	K	S	P	T	G	I	S	T	E	E	M	S	Q
NGAN_150 CA 2018	MN271590	2018	A	K	S	P	T	G	I	P	T	E	E	T	S	Q
NGAN_084 OV 2018	MN271599	2018	A	K	S	P	A	G	I	P	T	E	E	T	S	Q
NGEN_137 OV 2018	MN271587	2018	A	K	S	P	T	G	I	P	A	E	E	T	S	Q
NGEN_255 OV 2018	MN271601	2018	A	K	S	P	T	E	I	P	T	E	E	T	P	Q
NGAN_217 OV 2018	MN271594	2018	A	K	S	P	T	G	I	P	T	E	E	T	S	K
NGEN_146 CA 2018	MN271593	2018	A	K	S	P	T	G	T	S	T	E	D	M	S	Q
NGAN_087 OV 2018	MT038906	2018	A	K	S	P	T	G	T	S	T	K	D	M	S	Q
NGAN_156 CA 2018	MN271592	2018	V	K	L	L	T	G	I	P	T	E	E	T	S	Q
Other[e]	Reference	2010-2013	A/T	R/K	S	P	T/I	G/E	I	S/P	T/P	E	E	M/T	S/L	Q

[d]Reference strain; [e]Other previous PPRV (Peste des petit ruminants virus) sequences from Nigeria (Mantip *et al.* 2016, Woma *et al.* 2016); Bolded are amino acid changes reported for the first time among all the PPRV sequences collected in Nigeria

positions 442 and 459, respectively (Table II). When compared with PPRV sequences previously analysed and available in public domain databases, the N gene sequences of the analysed lineage II strains showed the highest nt identities (99.00%-100%) with the vaccine strain 75/1, collected in 1975 in Nigeria (acc. no. DQ840160 and KY628761), and with the strain N67-127-GYM31KD-Oyo, collected in 2011 in Oyo State (Nigeria) (acc. no. KJ124767, Woma *et al.* 2016).

A number of amino acid substitutions were equally observed among the sequences of the 27 PPRV strains belonging to lineage IV. Based on the deduced amino acid profiles, the strains were assigned into eight haplotypes or groups. Group 1 (n = 2; strain no. 9, 53) which consisted of strains with amino acid profiles similar to those of reference strains (acc. no. MH447983 and KF479428) or those previously reported in Nigeria (Mantip *et al.* 2016, Woma *et al.* 2016) while Groups 2 (n = 5; strain no. 3, 19, 20, 48, 49), 3 (n = 2; strain no. 46, 180), 4 (n = 1; strain no. 55) and 5 (n = 4; strain no. 12, 14, 16, 52) consisted of strains with single amino acid substitutions T434A, T454A, S478P and Q483K, respectively. Amino acid profiles of the representative of each of the eight haplotypes are shown in Table III. The amino acid changes recorded for the seven haplotypes (groups 2-8) have not been reported in PPRV strains from Nigeria. Sequences clustered in haplotypes 1, 3, 4 and 8 showed 99.33%-97.35% nt identities with the strain PPRV/Cameroon/CMRS6/2017 (acc. no. MH447983), collected in Cameroon in June 2017, and 99.21%-97.63% with the isolate SRMV/Niger/12/2017 (acc. no. MK673131), collected in Niger in January 2017. While sequences clustered in haplotypes 2, 5 and 6, 7 showed 99.66% and 99.34%-98.01% nt identities with the strain Nig12:IM21 and with the strain Nig12:TR10 (acc. no. KF479428 and KF479417, Woma *et al.* 2016), collected in Nigeria in 2012, respectively.

Phylogenetic analysis

The N gene nucleotide sequence of the PPRV strain representing each of the haplotypes was used for phylogenetic analysis. A phylogenetic tree constructed based on the N gene sequences of the representative PPRV strains in our study and other PPRV viruses representing the four lineages is shown in Figure 3. The PPRV strains obtained in this study clustered with the PPRV strains of the lineage II (11 strains) and IV (27 strains). The 27 PPRV strains that clustered with the Asian lineage IV were collected from all the States sampled (Enugu, Anambra, and Ebonyi). These strains were 76%-85% related to PPRV isolates from Imo, Anambra, Plateau, Taraba and Ondo States of Nigeria and isolates from Cameroon and Niger. The 11 PPRV strains that clustered with lineage II were 99% related to Nigeria 75/1 vaccine strain and all were obtained from Enugu State.

Discussion

Peste des petits ruminants is a transboundary viral disease of small ruminants, associated with high morbidity and mortality rates, leading to social and economic losses amongst rural households/small ruminant farmers (Abu-Elzein *et al.* 1990). Because of the dramatic economic consequences it may cause, particularly in developing Countries, the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) supported a campaign for global eradication of PPR (https://www.oie.int/eng/ppr2015/background.html - http://www.fao.org/ag/againfo/programmes/en/empres/news_271014.html).

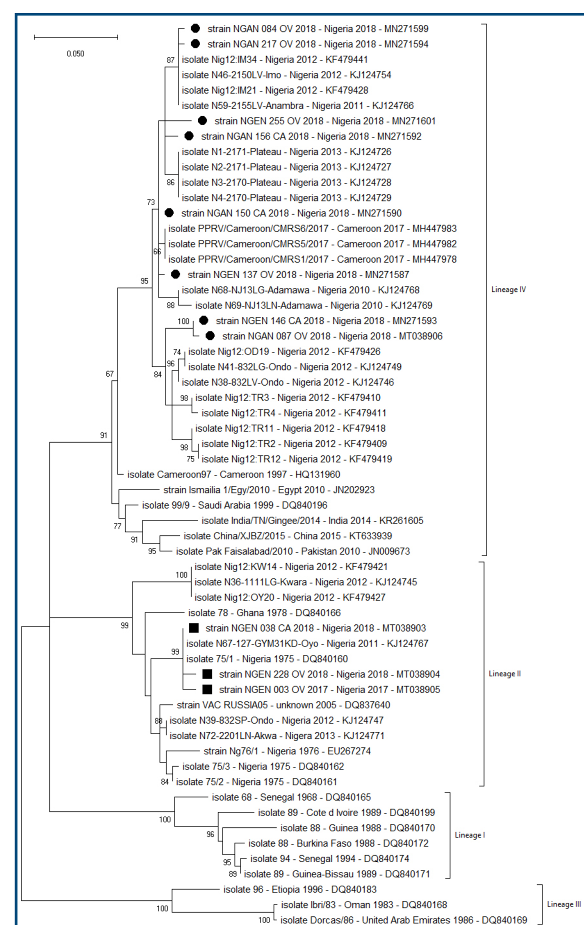

Figure 3. *Unrooted maximum-likelihood tree based on 59 partial N gene sequences of peste des petits ruminants virus strains (bootstrap 1,000 replicates; bootstrap values greater than 65 are shown)*. Black squares (■) and black dots (●) markings indicate PPRV strains of lineage II and IV, respectively, analysed in this study. Each sequence is indicated with strain/isolate name, country and year of collection, and accession number.

Although PPR was first described in Nigeria in 1975 (Taylor and Abegunde 1979), it is only in the last decade that molecular analyses on PPRV strains circulating in Nigeria were provided. These studies, based on the N gene (Mantip *et al.* 2016, Woma *et al.* 2016) or the F gene (Luka *et al.* 2011) sequence analyses, characterized strains collected from 2007 to 2013 in many but not all States of Nigeria, highlighting the circulation of the lineage II and, since 2010, of the lineage IV of PPRV (Mantip *et al.* 2016, Woma *et al.* 2016).

In the present study the molecular characteristics of PPRV strains circulating in Nigeria in 2017-2018 were determined and compared with those previuosly collected in Nigeria and other countries. The analysis was based on the N gene sequence of PPRV strains collected from States of Nigeria not included in previous studies (Ebonyi State) or with none or few tested positive samples (Anambra and Enugu States). PPRV was detected by RT-PCR in 65 (22.8%) of the 285 animals tested, confirming the current circulation of PPRV in South-East Nigeria (Anambra, Ebonyi and Enugu States). Different infection rates from clinical samples (9.7%, Mantip *et al.* 2016; 42%, Woma *et al.* 2016; 51.52%, Luka *et al.* 2016) were reported in previous studies conducted in other States of Nigeria. The differences in detection rates recorded may be related to the sample size, sampling method, season of the year, management practice and detection method (Couacy-Hymann *et al.* 2005, Devi *et al.* 2016) as well as the clinical status of the animals at the period of sampling.

The analysis of the results revealed that there were no significant associations between PPRV detection rate and location/states, species, sex and breeds of the animal sampled. These results suggest that PPRV affects equally both sheep and goats, males and females, and also all breeds of the animals studied. However, our findings differ with those of other investigators such as Anderson and colleagues (Anderson *et al.* 1991) and Al-Majali and colleagues (Al-Majali *et al.* 2008) in Northern Jordan. Additionally, Lefevre and Diallo (Lefevre and Diallo 1990) also reported that goats are more sensitive to PPRV. Previous studies reported the variability on susceptibility among domestic species but the PPRV spread is thought to be related also to the relationship of virus sequence and host species (Banyard *et al.* 2010).

The highest PPR infection rate observed in March (68.8%) coincides with the harmattan period. Similar observation has been reported (Ezeibe *et al.* 2008, Okoli 2003, Wosu 1994, Wosu *et al.* 1990, Ezeokoli *et al.* 1986, Obi *et al.* 1983). This higher infection rate during the harmattan period has been attributed to the dry, cold and dusty weather, together with the poor nutrition as a result of inadequate pasture (Ezeibe *et al.* 2008, Wosu *et al.* 1990).

Genetic characterization of the circulating PPRV strains has largely been based on the comparison of partial or complete N or, less frequently, F gene sequences. According to the sequence analysis, PPRV strains could be clustered in four different lineages (I-IV). Due to the divergence in the N gene, the analysis of this gene highlights the relationship of the circulating strains more clearly (Kwiatek *et al.* 2011). Woma and colleagues (Woma *et al.* 2016) and Mantip and colleagues (Mantip *et al.* 2016) first described the spreading of the lineage IV in the years 2010 to 2013 in Nigeria, where the lineage II was, for a long time, the prevalent genetic type. In the present study both lineages II and IV were detected in samples collected from sheep and goats in South-East Nigeria, with lineage IV being the dominant genotype. Thus, this study further confirms the wide circulation in Nigeria of PPR viruses belonging to the Asian lineage IV.

This study allowed to depict the prevalence of the lineages and their genetic features in selected areas. Mantip and colleagues (Mantip *et al.* 2016) obtained negative results from the samples collected in Enugu State while Woma and colleagues (Woma *et al.* 2016) obtained only two positive samples from Anambra State, both belonging to the lineage IV. Genetically, the PPRV strains in this study had a close relationship with previous strains collected in other States of Nigeria and Cameroon. However, amino acid changes reported for the first time were found in strains belonging to both lineage II and lineage IV. Lack of recent further genetic analyses in Nigeria or neighboring countries makes it difficult to ascertain if these changes are due to the recent introduction of different but closely related strains from other territories or if they represent potential local adaptative genetic changes. The genetic close connection of the most prevalent haplotype of the lineage IV with PPRV strains collected in the same years in neighboring Cameroon and Niger suggests the potential introduction of the related strains from other countries. Indeed, in the phylogenetic tree depicted in Figure 3, the lineage IV viruses described in this study cluster in a sub-clade together with strains collected in Cameroon in 2017 (unpublished) and Niger in 2015/2017 (Souley *et al.* 2019). This close genetic relationship between PPRV strains from neighboring Countries (Nigeria, Niger, Cameroon) suggests a common origin, probably connected to the transboundary movements of animals due to the absence of natural barriers or the commercial routes of animal trade (Tounkara *et al.* 2017, Souley *et al.* 2019). The varying amino acid changes observed among the lineage IV strains in this study indicate the strains circulating in Nigeria are genetically diverse and reflect the geographical differences between the different parts of Africa (Dundon *et al.* 2020).

The high degree of sequence similarity in PPRV lineage II with the Nigeria 75/1 vaccine strain recorded in our study has also been reported by other authors in Nigeria (Mantip *et al.* 2016, Woma *et al.* 2016), Sierra Leone (Munir *et al.* 2012), Tanzania (Misinzo *et al.* 2015, Mahapatra *et al.* 2015), China (Zhou *et al.* 2018), India (unpublished, acc no. DQ176750) and Iran (unpublished; acc. no. KC534492), although Dundon and colleagues (Dundon *et al.* 2020) posited that some of these previously reported cases of sequence similarity are most likely as a result of laboratory contamination during sample processing or due to circulating strains that are very similar to or variants of the vaccine strain Nigeria 75/1.

Although homologous PPR vaccine available in Nigeria for immunization of small ruminant is of Nigeria PPRV 75/1 origin (Sen *et al.* 2010), a recent study reported lack of awareness of PPR vaccination among small ruminant farmers and non-availability of PPR vaccine at veterinary establishments in Southeast Nigeria (Chukwudi *et al.* 2020). There is not a documented evidence that animals tested in this study have been vaccinated against PPRV but the molecular analysis of the 11 lineage II strains collected from Enugu State in this study shows their close relationship with a vaccine strain. Therefore, albeit hypothesized, a clear origin of these viruses cannot be pointed out and necessarily this limitation deserves further evaluation or specific assays for discrimination between Nigeria PPRV 75/1-based vaccines and field strains of PPRV. The limited data available makes this recent debate still open and requires further studies, in the direction of which this study goes, to be definitively documented. Moreover, a sequence study based on multiple genes or the total genome, together with the pursuit of good laboratory practices, may contribute to clarify these hypotheses. Indeed, the partial N gene sequence was commonly used as an effective target for PPR detection and lineage identification (Kwiatek *et al.* 2011, Tounkara *et al.* 2019), aiming mainly to understand the epidemiology and transmission dynamics of PPR (Tounkara *et al.* 2019), as in the present study.

This study reports vast diversity in the amino acid data among the PPRV lineages IV and II circulating in South-East Nigeria,and confirms the predominance of the lineage IV in Nigeria. These data support the need for further molecular epidemiological investigation in order to better comprehend the spread of the PPRV strains and their transboundary circulation, to support the future control strategies.

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Annex 1

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Supplementary Table. *Details of tested positive samples. —cont'd*

S/NO	ST/NO	State	Species	Breed	Sex	Vaccination status	Anamnesis	Date of collection	Accession number and sequence ID
1	1	EN	OVI	UDA	F	Unk	Nd, Od, Cou. Dia, Du	21/12/2017	
2	3	AN	OVI	WAD	M	Nv	Nd, Cou. Dia, Du, RHC, Wk, Us	25/01/2018	MN271599, NGAN_084 OV 2018
3	7	EN	CAP	WAD	F	Nv	Nd, Od, Cou. Du, Ema, RHC, Wk	13/03/2018	
4	8	EN	CAP	WAD	F	Nv	Nd, Od	14/03/2018	
5	9	AN	CAP	WAD	F	Nv	Nd, Od, Dia, Us	19/03/2018	MN271590, NGAN_150 CA 2018
6	10	AN	CAP	WAD	F	Nv	Nd, Od, Cou. Dia, Du, RHC, Wk, Se	26/03/2018	MN271592, NGAN_156 CA 2018
7	12	AN	OVI	WAD	F	Nv	Nd, Dia, Cou	16/04/2018	MN271594, NGAN_217 OV 2018
8	13	AN	OVI	WAD	F	Nv	Nd, Od, Cou. Du, Wk	16/04/2018	
9	14	AN	CAP	WAD	M	Nv	Nd, Cou. Du, Us, RHC	16/04/2018	
10	16	EN	OVI	UDA	F	Nv	Apparently healthy	22/12/2017	
11	17	EN	CAP	RS	F	Unk	Nd	09/01/2018	
12	19	EN	OVI	WAD	M	Nv	Severe mucoprulent Nd, Du	17/01/2018	
13	20	AN	OVI	WAD	F	Nv	Nd, Dia, Us, Cou. Du, RHC	25/01/2018	
14	26	EN	CAP	WAD	M	Nv	Nd, Cou. Dia, Du, RHC, Ema, Wk, Se	12/03/2018	MN271593, NGEN_146 CA 2018
15	27	EN	CAP	WAD	F	Nv	Nd, Cou. Du, RHC, Ema	19/03/2018	
16	28	AN	CAP	WAD	F	Nv	Nd, Od, Cou. Du, RHC, Ema	26/03/2018	
17	29	AN	CAP	WAD	F	Nv	Nd, Cou. Dia, Us, Du, RHC, Ema	26/03/2018	
18	32	EB	OVI	WAD	F	Nv	Nd, Od, Cou. Du, RHC	11/04/2018	
19	33	EN	OVI	WAD	M	Nv	Mucopurulent Nd, Us, Cou, Du	21/04/2018	
20	35	EB	OVI	WAD	M	Nv	Mucopurulent Nd, Us, Cou, Du	23/05/2018	
21	46	EN	OVI	WAD	M	Nv	Apparently healthy	17/02/2018	MN271587, NGEN_137 OV 2018
22	48	EB	OVI	WAD	F	Nv	Mucopurulent Nd, Us, Cou, Du	06/04/2018	
23	49	EB	OVI	WAD	F	Nv	Mucopurulent Nd, Du	11/04/2018	
24	52	AN	OVI	WAD	M	Nv	Nd, Dia, Cou	16/04/2018	
25	53	EN	OVI	WAD	M	Nv	Mucopurulent Nd, Cou, Du	21/04/2018	
26	55	EN	OVI	WAD	M	Nv	Mucopurulent Nd, Du	28/05/2018	MN271601, NGEN_255 OV 2018
27	60	AN	OVI	WAD	F	Nv	Nd RHC, Du	25/01/2018	MT038906, NGAN_087 OV 2018
28	61	EN	CAP	RS	F	Unk	Apparently healthy	09/01/2018	MT038903, NGEN_038 CA 2018
29	64	AN	OVI	WAD	M	Nv	Apparently healthy	30/05/2018	
30	66	EN	OVI	WAD	M	Nv	Nd	21/04/2018	
31	70	EN	CAP	RS	M	Unk	Nd, Du Wk	17/01/2018	
32	71	AN	OVI	WAD	M	Nv	Mucopurulent Nd, Cou	25/01/2018	
33	75	EN	CAP	WAD	M	Nv	Mucopurulent Nd, Dia, Od, Cou. Du, RHC, Us, Ema	17/01/2018	
34	85	AN	OVI	YAN	M	Nv	Nd, Cou	30/05/2018	

continued

S/NO = Sample number; ST/NO = Strain number; State of collection: EN = Enugu, AN = Anambra, EB = Ebonyi; Specie of collection: OVI = Ovine, CAP = Caprine; Breed: WAD = West African Dwarf, RS = Red Sokoto, YAN = Yankassa; Sex: M = Male, F = Female; Vaccination status: Nv = Not vaccinated, Unk = Unknown; Anamnesis: Nd = Nasal discharge, Od = Ocular discharge, Cou = Coughing, Ema = Emaciated, RHC = Rough hair coat, Wk = Weak, Du = Dull, Dia = Diarrhea, Se = Sunken eyeball, Us = Ulcerative stomatitis.

Annex 1

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Supplementary Table. *Details of tested positive samples. —cont'd*

S/NO	ST/NO	State	Species	Breed	Sex	Vaccination status	Anamnesis	Date of collection	Accession number and sequence ID
35	90	AN	CAP	WAD	M	Nv	Nd, Du, Cou	16/04/2018	
36	93	EN	OVI	WAD	F	Nv	Nd, Cou, Du	23/01/2018	
37	97	EN	CAP	RS	F	Unk	Nd, RHC, Du,	21/12/2017	
38	108	EN	OVI	WAD	F	Nv	Nd, RHC, Du	23/01/2018	
39	116	EN	OVI	WAD	M	Nv	Nd, Du	28/05/2018	
40	118	EN	OVI	WAD	M	Nv	Nd	21/04/2018	MT038904, NGEN_228 OV 2018
41	122	AN	CAP	WAD	F	Nv	Nd, Cou, Du	26/03/2018	
42	123	AN	CAP	WAD	F	Nv	Nd Du, Cou	26/03/2018	
43	127	EN	CAP	RS	F	Unk	Apparently healthy	28/12/2017	
44	131	EN	OVI	UDA	F	Unk	Nd	09/01/2018	
45	132	EB	OVI	WAD	F	Nv	Nd, Cou, Du	23/05/2018	
46	137	EB	OVI	WAD	M	Nv	Nd Du, Cou	23/05/2018	
47	141	EB	OVI	WAD	M	Nv	Nd, Cou	05/06/2018	
48	143	EN	CAP	RS	F	Unk	Nd	09/01/2018	
49	144	EB	OVI	WAD	F	Nv	Nd	11/04/2018	
50	149	AN	OVI	WAD	F	Nv	Nd, Du, Wk	30/05/2018	
51	150	AN	OVI	WAD	M	Nv	Nd, Cou, RHC	30/05/2018	
52	159	EN	OVI	WAD	M	Nv	Nd, Cou, Du	17/01/2018	
53	160	EN	CAP	WAD	F	Nv	Mucopurulent Nd, Cou	22/05/2018	
54	161	EN	OVI	YAN	M	Unk	Apparently healthy	17/02/2018	
55	165	AN	OVI	WAD	M	Nv	Apparently healthy	30/05/2018	
56	168	EN	CAP	WAD	F	Nv	Nd, Du	13/03/2018	
57	169	EN	CAP	RS	F	Unk	Apparently healthy	28/12/2017	
58	170	EN	OVI	UDA	F	Nv	Apparently healthy	20/12/2017	MT038905, NGEN_003 OV 2017
59	172	EB	OVI	WAD	F	Nv	Nd	06/04/2018	
60	174	EB	CAP	WAD	F	Nv	Apparently healthy	06/04/2018	
61	175	EB	OVI	WAD	M	Nv	Apparently healthy	06/04/2018	
62	177	EN	CAP	RS	F	Unk	Apparently healthy	28/12//2017	
63	178	EN	CAP	RS	F	Unk	Apparently healthy	22/12/1017	
64	179	EN	OVI	WAD	M	Nv	Du	21/04/2018	
65	180	EB	OVI	WAD	F	Nv	Apparently healthy	17/02/2018	

S/NO = Sample number; ST/NO = Strain number; State of collection: EN = Enugu, AN = Anambra, EB = Ebonyi; Specie of collection: OVI = Ovine, CAP = Caprine; Breed: WAD = West African Dwarf, RS = Red Sokoto, YAN = Yankassa; Sex: M = Male, F = Female; Vaccination status: Nv = Not vaccinated, Unk = Unknown; Anamnesis: Nd = Nasal discharge, Od = Ocular discharge, Cou = Coughing, Ema = Emaciated, RHC = Rough hair coat, Wk = Weak, Du = Dull, Dia = Diarrhea, Se = Sunken eyeball, Us = Ulcerative stomatitis.