

SHORT COMMUNICATION

Detection and Genome Analysis of a Lineage III Peste Des Petits Ruminants Virus in Kenya in 2011

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Summary

In May 2011 in Turkana County, north-western Kenya, tissue samples were collected from goats suspected of having died of peste des petits ruminant (PPR) disease, an acute viral disease of small ruminants. The samples were processed and tested by reverse transcriptase PCR for the presence of PPR viral RNA. The positive samples were sequenced and identified as belonging to peste des petits ruminants virus (PPRV) lineage III. Full-genome analysis of one of the positive samples revealed that the virus causing disease in Kenya in 2011 was 95.7% identical to the full genome of a virus isolated in Uganda in 2012 and that a segment of the viral fusion gene was 100% identical to that of a virus circulating in Tanzania in 2013. These data strongly indicate transboundary movement of lineage III viruses between Eastern Africa countries and have significant implications for surveillance and control of this important disease as it moves southwards in Africa.

Introduction

The peste des petits ruminants virus (PPRV) is the cause of a highly infectious transboundary animal disease that primarily affects sheep, goats and small wild ruminants. Morbidity and mortality rates for peste des petits ruminant (PPR) can be as high as 80%, and for this reason, it is classified as a notifiable disease by the OIE (World Organization for Animal Health). Sheep and particularly goats contribute considerably to the household and cash income and nutrition of small farmers in many countries so the control of PPR is considered an essential element in the fight for global food security and poverty alleviation. Indeed, it is for this reason that PPR is presently being considered as the next animal disease for global eradication (Albina et al., 2013).

There are presently four genetic lineages of PPRV circulating globally. The lineages are defined based on the comparison of a fragment of either the nucleoprotein (N) or fusion protein (F) gene of the virus. All four lineages have been

reported in Africa, and currently, lineages II, III and IV are actively circulating (Banyard et al., 2010; Dundon et al., 2014a; Libeau et al., 2014). The last report of lineage I PPRV was in Senegal in 1994, and it remains to be seen whether this virus still circulates in Africa (Diop et al., 2005). In Kenya, the presence of PPR was first suspected in 1992 (Kihu et al., 2012) and was confirmed serologically in 1995 (Wamwayi et al., 1995). The first evidence of disease, however, was not until 2006 in Turkana County, north-western Kenya. The presence of PPR in Kenya was officially confirmed to the OIE in 2007. During 2006 and 2008, the disease spread rapidly to 16 of 46 districts with mortality rates varying according to the age of the animals infected. The highest mortality (100%) was seen in kids and the lowest (10%) in adult animals. It has been estimated that during this period, over 2.5 million animals died as a result of PPRV infection in Kenya and caused significant economic and food security hardships on local communities. Vaccination and quarantine programmes were implemented in an attempt to stop the spread of the disease (Banyard et al., 2010).

This report describes a more recent outbreak in Turkana County in 2011 confirming the persistence of the virus in Kenya. Clinical manifestation data are presented together with a phylogenetic and complete genome sequence analysis of the virus (i.e. PPRV KN5/2011) associated with the outbreak.

Materials and Methods

Study area and background information

This study was carried out in May 2011 in the Kakuma administrative division of Turkana County in Kenya on a specific village herd named Lotakaa (GPS position, N 03°38'39.0, E 034°50'98.7). Local pastoralists reported that 21 kids, 24 adult goats and one sheep had died from suspected PPR 2 weeks prior to the study visit.

Sample collection and processing

Organ samples were collected from the carcasses of three goats suspected of having died of PPR (Table 1). The symptoms of the animals prior to death were depression, diarrhoea, emaciation, serous and mucopurulent nasal discharges, respiratory difficulties and elevated temperatures of 41°C. The samples were transported on ice to the University of Nairobi, stored at -80°C and, in May 2014, were shipped to APHL for further characterization.

PPRV RNA extraction and amplification

Upon arrival in Vienna, the tissue samples were ground with sterile quartz beads to make a 10% homogenate in medium Dulbecco's modified Eagle's medium-high glucose medium (DMEM-HG). Homogenates were clarified at 1200 g for 5 min. The supernatants were collected, and aliquots were submitted for total RNA extraction using the RNeasy kit (Qiagen, Hilden, Germany). The extracted RNA samples were analysed by RT-PCR using the One-Step RT-PCR kit (Qiagen) to amplify a 351 bp fragment of the PPRV N gene with primers NP3 and NP4 (Couacy-Hymann et al., 2002; Dundon et al., 2014a). PCR amplicons

were gel-purified and sent for sequencing using standard Sanger methods at LGC Genomics (Berlin, Germany).

Genome sequencing

The full-genome sequence of PPRV KN5/2011 (GenBank KM463083) was generated according to Dundon et al. (2014a). The full set of primers used is available upon request.

Phylogenetic analysis

The MEGA4 program (Tamura et al., 2007) was used to construct phylogenetic trees comparing the nucleotide sequences generated using primers NP3 and NP4 (as described above), and a 372-bp segment of the F gene of KN5/2011 with those of other PPRVs in GenBank. The neighbour-joining method with 1000 bootstrap replicates was chosen for the construction of the trees.

Results

RT-PCR results

All six of the tissue samples tested by RT-PCR using primers NP3 and NP4 were positive for PPRV (Table 1). Amplicons from PPRV KN1/2011, PPRV KN3/2011 and PPRV KN5/2011, representing the three animals, were purified and sent for sequencing. The sequences have been deposited in GenBank under accession numbers KP100649, KP100650 and KP100651.

Phylogenetic analysis

Phylogenetic analysis of the sequences generated from PPRV KN1/2011, PPRV KN3/2011 and PPRV KN5/2011 using the MEGA4 program revealed that they all belonged to lineage III (Fig. 1). The sequences clearly clustered with other lineage III viruses from Ethiopia (KJ867540), Oman (KJ867544), United Arab Emirates (UAE) (KJ867545) and Sudan (DQ840159). They showed 100% identity to a sequence from Uganda (KJ867543) (Muniraju et al., 2014).

Table 1. Sample description

Species	Animal number	Sample	Sex	Age ^a	Organ	RT-PCR	GenBank
Goat	1	KN1/2011	F	6	Lung	+	KP100649
Goat	1	KN2/2011	F	6	Mesenteric lymph node	+	ns
Goat	2	KN3/2011	M	6	Mesenteric lymph node	+	KP100650
Goat	2	KN4/2011	M	6	Lung	+	ns
Goat	3	KN5/2011	F	6	Lung	+	KP100651
Goat	3	KN6/2011	F	6	Mesenteric Lymph node	+	ns

^aapprox. age in months and ns, not submitted.

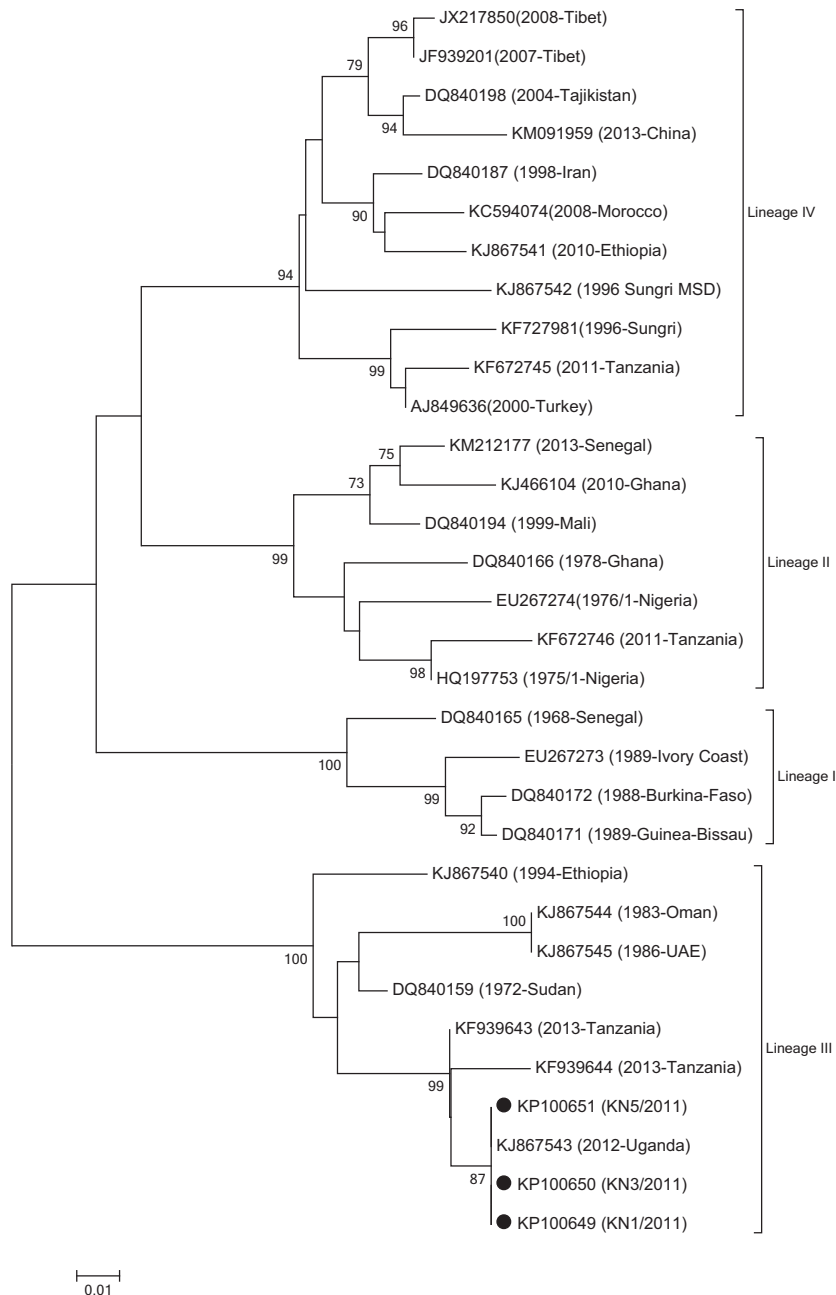


Fig. 1. Phylogenetic analysis using the MEGA4 software (neighbour joining) of the nucleotide sequences generated using primers NP3 and NP4 (GenBank KP100649 to KP100651; filled circles). The numbers indicate the bootstrap values calculated from 1000 bootstrap replicates.

In addition, there was high similarity between the PPRV KN1/2011, PPRV KN3/2011 and PPRV KN5/2011, and sequences generated from goat samples collected from northern and eastern Tanzania in 2012–2013 (KF939643 and KF939643) (Kgotlele et al., 2014).

A comparison of the 372 bp segment of the F gene of PPRV KN5/2011 (KM463083) with sequences in GenBank provided a similar picture (Fig. 2). Again, PPRV KN5/2011 clustered with lineage III viruses from Ethiopia, Oman and

UAE but was 100% identical to viruses from Uganda (HQ407501 and HQ407502) and Tanzania (FN995114) identified in 2007 and 2010, respectively.

Genome analysis

Sample PPRV KN5/2011 was selected for full-genome sequencing as previously described (Dundon et al., 2014b). The organization of the PPRV KN5/2011 genome

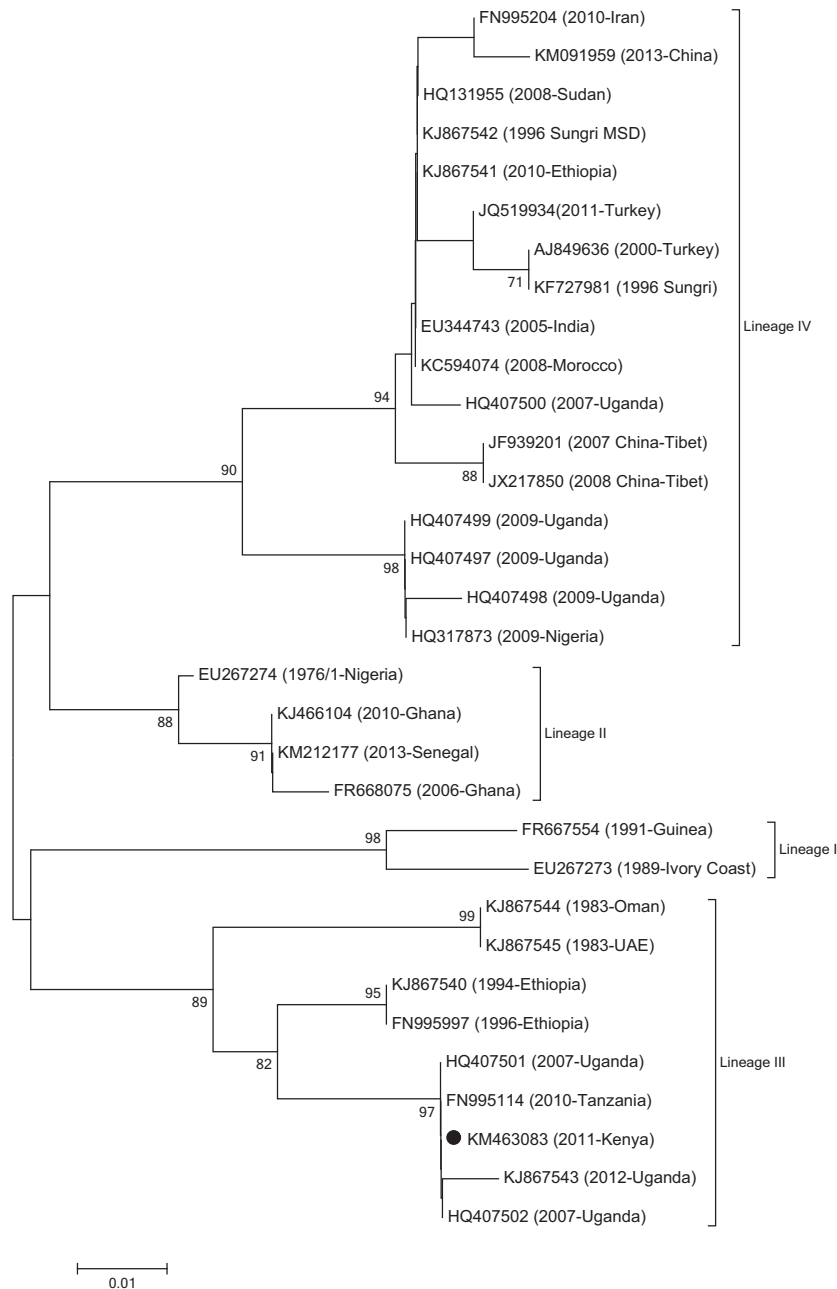


Fig. 2. Phylogenetic analysis using the MEGA4 software (neighbour joining) of the nucleotide sequences for the F gene. The peste des petits ruminants virus (PPRV) KN5/2011 is shown with a filled circle. The numbers indicate the bootstrap values calculated from 1000 bootstrap replicates.

(15 948 bp) consists of a 107 nt genome promoter region at the 3' end followed by the transcription units for the N protein, phosphoprotein (P), matrix protein (M), F protein, haemagglutinin (H) and the RNA-dependent RNA polymerase (L) and the anti-genome promoter at the 5' end. From Table 2, it can be seen that PPRV KN5/2011 shows the highest nucleotide identity with other lineage III viral genomes ranging from 95.7% with PPRV Uganda

2012 (KJ867543) and 93.5% with both PPRV Oman 1983 (KJ867544) and PPRV UAE 1986 (KJ867545). The lowest nucleotide identity (84.3%) is with the lineage IV PPRV Ethiopia 2010 (KJ867541).

Analysis of the N protein revealed that it is 100% identical to PPRV Uganda 2012 (Table 2). In addition, the protein contains both the nuclear export signal motif (4-LLKSLALF-11) and the nuclear localization signal (70-TGVMISML-77)

Table 2. Comparison of genome characteristics of peste des petits ruminants virus (PPRV) KN5/2011 and other PPRV genome sequences available in public databases

Genome	Accession number	Country/Date	Lineage	Nucleotide identity (%)	Protein identity (%)							
					N	P	V	C	M	F	H	L
Uganda 2012	KJ867543	Uganda 2012	III	95.7	100	98.2	99.7	99.4	99.3	99.6	99.8	99.9
Ethiopia 1994	KJ867540	Ethiopia 1994	III	94.2	98.1	91.6	87.5	86.7	99.1	98.5	96.8	98.8
Oman 1983	KJ867544	Oman 1983	III	93.5	96.7	93.7	89.2	94.2	98.8	98.2	94.9	98.2
UAE 1986	KJ867545	UAE 1986	III	93.5	96.9	93.7	89.2	94.2	98.8	98.0	94.9	98.2
NK1/2010	KJ466104	Ghana 2010	II	86.5	94.1	85.2	76.2	80.7	97.3	94.7	88.7	95.2
SnDk11 13	KM212177	Senegal 2013	II	86.6	94.5	85.0	76.2	80.1	97.6	94.7	88.3	95.2
Nigeria 76/1	EU267274	Nigeria 1976	II	87.6	93.3	85.2	76.7	80.7	97.9	94.7	89.3	95.5
Nigeria 75/1	HQ197753	Nigeria 1975	II	86.8	93.5	86.6	80.0	82.1	97.0	94.3	89.1	95.7
Turkey 2000	AJ849636	Turkey 2000	IV	84.5	93.7	84.3	77.1	79.4	95.4	93.8	89.4	95.0
Morocco 2008	KC594074	Morocco 2008	IV	84.5	93.7	85.0	77.9	78.7	97.6	93.8	88.7	94.8
China/Tibet/Geg/07-30	JF939201	Tibet 2007	IV	84.5	92.7	84.4	76.7	81.4	96.4	94.0	88.9	95.1
Tibet/Bharal/2008	JX217850	Tibet 2008	IV	84.5	92.7	84.4	76.7	81.4	96.4	94.2	89.3	95.0
Ethiopia 2010	KJ867541	Ethiopia 2010	IV	84.3	93.1	83.6	76.7	80.1	97.3	93.8	88.3	94.6
China/XJYL/2013	KM091959	China 2013	IV	85.0	92.5	84.5	n/a	n/a	96.7	94.2	88.9	95.1
Sungri 1996 MSD	KJ867542	India 1996	IV	85.0	93.3	85.0	77.9	81.4	93.3	94.2	88.9	94.9
ICV89	EU267273	Cote d'Ivoire 1989	I	85.4	93.1	84.7	78.8	80.1	97.6	93.6	88.2	94.8

n/a, not annotated in original sequence.

motifs which are involved in the transport of the N protein to the nucleus of the host cell. The N protein also contains 8 of 9 putative phosphorylation sites recently identified by Sugai et al. (2014) in the measles virus (MV).

The P protein of PPRV KN5/2011 only shows 98.2% identity with PPRV Uganda 2012 and as little as 80.8% with PPRV Sungri/96. The soyuz 1 motif (4-EQAYHVNKGLECI KSL-20) as described by Karlin and Belshaw (2012) is conserved in PPRV KN5/2011 as is the serine at residue 151 which has been shown to be involved in the regulation of viral transcription through changes in its phosphorylation status. There is also a unique G at aa residue 146 in PPRV KN5/2011 that is not present in other PPRV P proteins sequenced to date.

The C and V proteins of PPRV KN5/2011 are highly conserved between PPRV KN5/2011 and PPRV Uganda 2012 with 99.4% and 99.7% identity between them, respectively. There is only 1 aa difference between the V protein of PPRV Uganda 2012 and PPRV KN5/2011. The V protein of PPRV KN5/2011 only shows between 87.5 and 89.2% identity with other lineage III viruses from Oman, UAE and Ethiopia isolated in the mid-80s and mid-90s, respectively. The M protein is also highly conserved between PPRV KN5/2011 and PPRV Uganda 2012 although it is not 100% identical due to a single D to E change at position 230. The F protein cleavage site GRRTRR (aa position 108), where host cell proteases cleave the F₀ inactive precursor protein into two disulphide linked subunits F₁ (438–546) and F₂ (1–108), is present in PPRV KN5/2011, while the haemagglutinin (H) is 99.8% identical to PPRV Uganda 2012 with only one aa

difference (e.g. E69K) at position 69. There are just two aa differences (99.9%) between the L of PPRV KN5/2011 and PPRV Uganda 2012 at position 613 (T/A) and 625 (S/R), respectively. All of the motifs that have previously been identified in the lineage II virus PPRV Nigeria 75/1 are also present in the L of PPRV KN5/2011 (Minet et al., 2009). These include the QGDNQ (aa position 771–775) and GDDD (aa position 1464) motifs associated with RNA polymerase activity, motifs involved in methyl-transferase activity (e.g. K1766, D1881, K1917, E1954, 1788-GEGSGSM-1974, 1809-YNSG-1812, 1855-TWVG-1858), the VLYPEVH LDSPIV motif believed to be an interaction domain for the P protein, the RNA-binding motif 535-KEKEIKETGRLEFA KMTYKM-553 and the putative purine nucleotide-binding element 1766-KX₁₇GLFLGEGSGSM-1794.

Discussion

This study describes the characterization of a lineage III PPRV associated with an outbreak in Kenya in 2011. Although PPR has been circulating in the country since the early 1990s and possibly even before then, this is the first in-depth analysis of PPRV in Kenya. The complete genome sequence of the PPRV KN5/2011 genome was released in GenBank in September 2014 and, at the time of release, it was the only lineage III genome available (Dundon et al., 2014b). However, very shortly after this release, the complete genomes of three other lineage III viruses (Muniraju et al., 2014) were released allowing for a more comprehensive and in-depth analysis of KN5/2011 as described in this report.

To date, lineage III PPRVs have been confined to a specific geographic location. The first lineage III PPRVs were identified in East Africa (Sudan and Ethiopia) in 1972 and 1996, respectively, while on the Arabian Peninsula, lineage III PPRV was identified in Oman in 1983 and UAE in 1986 (Banyard et al., 2010; Muniraju et al., 2014). More recently, lineage III viruses have been identified in Uganda (Muniraju et al., 2014) and Tanzania (Kgotlele et al., 2014).

A phylogenetic characterization of PPRVs using the F gene collected in 2007 and 2008 in Uganda incorrectly classified them as belonging to lineages I, IV and III (Luka et al., 2012). A reanalysis of Luka et al.'s data in this report reveals that there was cocirculation of different viruses in Uganda between 2007 and 2008 but that the virus circulating belonged to lineages IV and III only (Fig. 2). Indeed, comparison of the sequences with the F sequence PPRV KN5/2011 shows that the two sequences (HQ407501 and HQ407502 from viruses identified in July 2007) are 100% identical. Also, an F gene sequence in GenBank (FN995114) from a virus identified in 2010 in Tanzania also shows 100% identity to PPRV KN5/2011.

In addition to the circulation of lineage III viruses in Tanzania (Kgotlele et al., 2014), the presence of lineages II and IV viruses has also been recently reported in the country (Misonzo et al., 2015). Misonzo and colleagues collected and characterized samples in 2011 from Tandahimba which is a region of southern Tanzania that is very close to the border with Mozambique. When these sequences were used in a phylogenetic analysis using the N gene they clustered with lineage IV (KF672745) and lineage II (KF672746) viruses, respectively (Fig 1). Although the lineage II PPRV virus was highly similar to the live attenuated vaccine Nigeria 75/1, the authors argue that there was no history of PPR vaccination in Tandahimba at the time of sampling and that, therefore, their data confirm the circulation of a lineage II virus in Tanzania. However, it is intriguing that the lineage II virus identified by Misonzo and colleagues is more similar to Nigeria 75/1 (isolated in 1975) than to the recent lineage II viruses isolated in Ghana (KJ466104) and Senegal (KM212177) in 2010 and 2013, respectively (Fig 1).

There is a particularly high sequence identity (99.7%) between the V protein of PPRV KN5/2011 and PPRV Uganda 2012. The V protein is usually one of the least conserved of the PPRV proteins and so this high identity indicates a close relationship between PPRV KN5/2011 and PPRV Uganda 2012. Indeed, the location of the collection point of the Kenyan samples (i.e. Kakuma, Turkana County) is <50 km from the Ugandan border where it is presumed that there is regular cross-border trade of animals between the two countries.

Although the first PPRVs identified in Ethiopia in 1994 were of lineage III, more recent PPR isolates are from lineage IV (Muniraju et al., 2014; our unpublished data).

Likewise lineage IV has been reported in Angola (OIE 2012) and in the Democratic Republic of Congo (our unpublished data). It will be interesting to determine whether the southward movement of lineage IV PPRV will displace the predominant lineage III viruses currently in East Africa and, if so, what effect this will have on the spread, incidence and severity of PPRV outbreaks in the region. Indeed, there is an increasing apprehension among the Southern Africa Development Community (SADC) that PPRV will spread to other PPR free SADC member states (currently Zambia, Mozambique, Malawi, Zimbabwe, Botswana, Namibia, Lesotho, Republic of South Africa and Swaziland) potentially devastating the livelihoods and food security of millions of vulnerable smallholder farmers and agropastoralists (Chazya et al., 2014).

In conclusion, the data generated from this study strongly suggest that the viruses detected in Uganda, Kenya and Tanzania are of the same origin and that there is evident transboundary movement of a lineage III PPRV between these three countries.

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