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Short communication

Peste des petits ruminants viruses of lineages II and III identified in the Democratic Republic of the Congo

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ABSTRACT

Understanding the molecular epidemiology and evolution of peste des petits ruminants virus (PPRV), the causative agent of Peste des petits ruminants, can assist in the control of the transboundary spread of this economically important disease. To date, despite having been reported in the majority of northern and central African countries, no molecular epidemiological data on PPRVs are available for the Democratic Republic of the Congo (DRC). This study reports the collection and analysis of 11 samples collected from three provinces of the DRC in 2016 and 2018. Sequence analysis identified two (i.e. II and III) of the four known lineages of PPRV in the country providing important information that will assist in the global eradication of PPR.

1. Introduction

Peste des petits ruminants (PPR), caused by the peste des petits ruminants virus (PPRV), is a highly infectious transboundary animal disease that affects mainly sheep, goats and small wild ruminants (Baron et al., 2016). The disease is characterized clinically by ocular and nasal discharges, diarrhoea and erosive lesions of different mucous membranes. Morbidity and mortality rates can be as high as 70–80% and, because sheep and particularly goats contribute considerably to the cash income and nutrition of small farmers in many countries, the control of PPR is considered as an essential element in the fight for global food security and poverty alleviation. For this reason, PPR has been selected by the World Organization of Animal Health (OIE) and the Food and Agricultural Organization of the United Nations (FAO) as the next candidate animal virus for global eradication (FAO, 2017).

PPRV can be divided into four genetic lineages based on the comparison of a sequence fragment for the nucleocapsid protein (N) and/or fusion protein (F) genes (Forsyth and Barrett, 1995; Couacy-Hymann et al., 2002). To date Lineage I and II isolates have been confined, mainly, to Western Africa (e.g. Mali, Senegal, Benin, Burkina Faso, Guinea, Côte d'Ivoire, Niger and Nigeria) while Lineage III has been detected in Eastern Africa (e.g. Burundi, Ethiopia, Kenya, Sudan, Tanzania and Uganda) (Parida et al., 2015; Baron et al., 2016; Niyokwishimira et al., 2019). Lineage IV, which is the predominant lineage globally, is found primarily in Asia and the Middle East (Libeau et al., 2014; Baron et al., 2016). More recently however, it has also been detected in several African countries namely Algeria, Angola, Cameroon, Central African Republic, Egypt, Ethiopia, Gabon, Morocco, Nigeria, Niger, Sudan, and Tunisia (Kwiatek et al., 2011; Baron et al., 2016).

PPR is steadily moving southwards in Africa; there is an increasing apprehension among the Southern Africa Development Community (SADC) that the disease will spread to other SADC member states potentially devastating the livelihoods and food security of millions of vulnerable smallholder farmers and agropastoralists (Chazya et al., 2014).

In the Democratic Republic of the Congo (DRC), the presence of PPR was officially reported in 2012 (FAO, 2012; OIE, 2012a) and resulted in high mortalities of small ruminants throughout the country estimated at between 75,000 and 300,000. Emergency vaccination was employed by

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Table 1
Description of the samples analysed in this study.

Sample	Collection Year	Animal	Tissue/Swab	Location	GPS	GenBank #	Lineage
PPRV/DRC/G1/2016	2016	Sheep	Swab	North Kivu_Goma	1° 41'36" S 29°13'31"E	MN243715	II
PPRV/DRC/4/2018	2018	Goat	Swab	Kinshasa _N'sele	4°23' 28 S 15°27'33"E	MN243716	III
PPRV/DRC/5/2018	2018	Goat	Swab	Kinshasa _N'sele	4°23' 28 S 15°27'33"E	MN243717	III
PPRV/DRC/6/2018	2018	Goat	Lung	Kinshasa _N'sele	4°23' 28 S 15°27'33"E	MN243718	III
PPRV/DRC/7/2018	2018	Goat	Lung	Kinshasa _Maluku	4°20' 24 S 15°24'16"E	MN243719	III
PPRV/DRC/8/2018	2018	Goat	Swab	Kinshasa _Maluku	4°20' 24 S 15°24'16"E	MN243720	III
PPRV/DRC/9/2018	2018	Goat	Swab	South Kivu_Kalehe	2° 07' S 26°05'E	MN243721	III
PPRV/DRC/10/2018	2018	Goat	Swab	South Kivu_Kalehe	2° 07' S 28°58'E	MN243722	III
PPRV/DRC/11/2018	2018	Goat	Swab	South Kivu_Kalehe	2° 08' S 34°15'E	MN243723	III
PPRV/DRC/12/2018	2018	Goat	Swab	South Kivu_Kalehe	2° 08' S 34°15'E	MN243724	III
PPRV/DRC/13/2018	2018	Goat	Swab	South Kivu_Kalehe	2° 07' S 28°42'E	MN243725	III

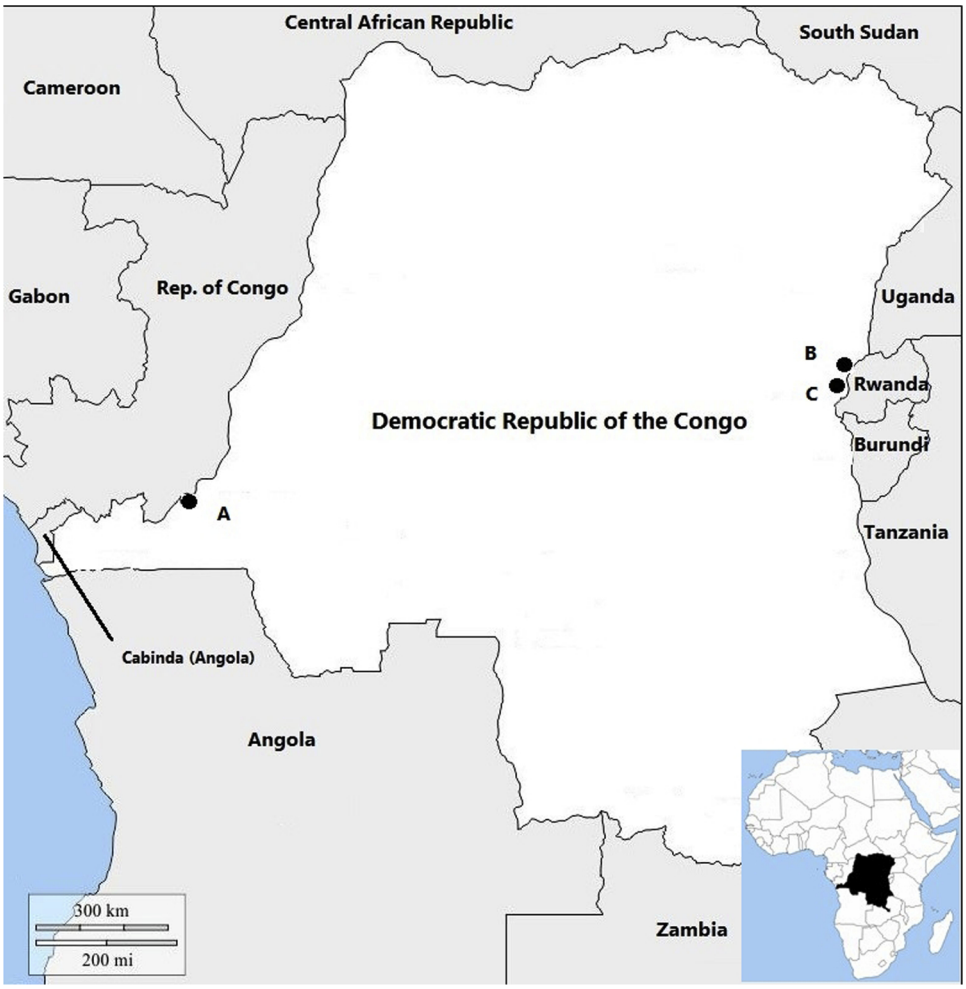

Fig. 1. Map of DRC showing sampling areas indicated by black circles and a letter (A, Kinshasa; B, North-Kivu; C, South-Kivu). Adapted from www.d-maps.com.

national and international organizations (Annonymous, 2012,) in the regions considered at high risk but this has not been maintained. In 2016, further outbreaks of PPR were reported in the east of the country but, due to regional conflict, no measures were taken to control the disease or limit the movement of animals from neighbouring countries. Over 50,000 animals perished due to PPR in Nord-Kivu in 2017 according to national reporting and in 2018 further outbreaks were reported.

Molecular epidemiology provides important information on the transboundary movement of viruses such as PPRV. In fact, molecular epidemiological investigations satisfy one of the recommendations of the global Strategy for the Control and Eradication of PPR by 2030 (FAO, 2017) which states that each country needs to define and implement robust monitoring plans to gain a good understanding of the circulation (or non-circulation) of PPRV in their country. To date, there has been no molecular characterization of the virus involved in any of the PPR outbreaks in the DRC. This report, therefore, describes the first molecular epidemiological study of PPRVs circulating in the DRC.

2. Material and methods

2.1. Sample collection

Lung and nasal swab samples were collected from herds following reports of disease by individual farmers in 2016 and 2018 (Table 1) (Fig. 1). Each herd consisted of local breeds of between 8–15 animals.

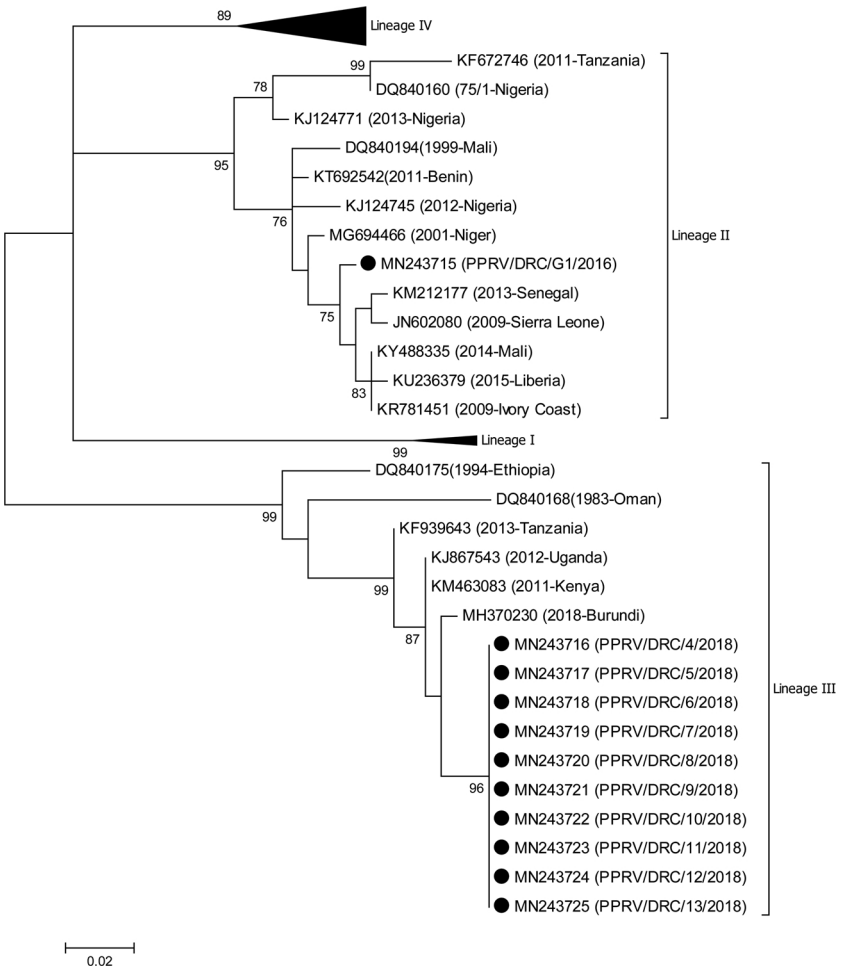

Fig. 2. ML phylogenetic tree of partial nucleotide sequences (219 bp) of the N gene from the PPRV samples in this study together with representative sequences available from the GenBank. The sequences from this study are indicated with a black circle. The numbers indicate the bootstrap values calculated from 500 bootstrap replicates.

The clinical symptoms reported were indicative of PPR; difficulty in breathing, coughing, diarrhoea, erosive lesions of the mouth, refusal to graze and death within 5 to 7 days. Samples were transferred in viral transport medium on ice to the Central Veterinary Laboratory, Kinshasa where they were stored at -80 °C until further processing.

2.2. RNA extraction and RT-PCR amplification

Total RNA was extracted directly from swabs and ground tissues with an RNAeasy Mini Kit (Qiagen, Germany). RT-PCR reactions were performed using the One Step RT-PCR kit (Qiagen) and primers NP3 (5′ TCTCGGAAATCGCCTCACAGACTG 3′) and NP4 (5′ CCTCCTCCTGGTC CTCCAGAATCT 3′) for the N gene with thermocycling conditions as previously described (Couacy-Hymann et al., 2002).

2.3. Sequencing and phylogenetic analysis

PCR amplicons were purified using the QIAquick PCR purification Kit (Qiagen) and sent for sequencing using standard Sanger methods at LGC genomics (Berlin, Germany). Sequences have been deposited in GenBank under accession numbers MN243715 to MN243725. The Staden Package (http://staden.sourceforge.net/) was used to assemble the generated sequences while the MEGA6 programme (Tamura et al., 2013) was used to construct phylogenetic trees which were estimated using the Maximum Likelihood (ML) method employing the Kimura 2-parameter model of nucleotide substitution and 500 bootstrap replications

3. Results and discussion

A segment of the N gene (219 bp) was amplified and sequenced from 11 PPRV positive samples collected in three separate locations in the DRC in 2016 and 2018. Phylogenetic analysis confirmed that 10 of the samples belonged to lineage III, and 1 to lineage II (Fig. 2).

The lineage III viruses were identical to each other and were collected from the eastern part of the country close to the borders with Uganda, Burundi, Rwanda and Tanzania and in the capital city, Kinshasa, in the west of the country. Lineage III PPRVs are predominant in Eastern Africa and have been identified in Burundi, Ethiopia, Sudan, Tanzania and Uganda (Kwiatek et al., 2007; Kgotlele et al., 2014; Muniraju et al., 2014; Dundon et al., 2017; Niyokwishimira et al., 2019). Of note, however, was that the partial N gene sequences of the lineage III DRC viruses were significantly different (only 98.1% nucleotide identity) from their closest lineage III relative from Burundi suggesting that they are not directly related and that they are not the result of recent transboundary movement between the two countries. The presence of lineage III viruses in Kinshasa located in the west of the country is explained by the fact that there is regular and documented transport of goats from the east of the DRC to Kinshasa for sale or in order to improve productivity and repopulate herds in the city.

The single lineage II virus was collected in 2016 in Goma, North Kivu in the east of the country. From the phylogenetic analysis it can be

seen that this virus is most related to viruses from West Africa (e.g. Senegal, Mali, Sierra Leone). Nevertheless, and like the lineage III, there is nucleotide sequence diversity (1.4%) between the N sequence of this lineage II virus and its nearest relative (e.g. Mali and Côte D'Ivoire). This would indicate that any shared origin of these viruses is not recent.

Interestingly, there are two reports that describe the identification of lineage II PPRVs in neighbouring Tanzania. A partial N gene sequence (GenBank KF672746) from a lineage II PPRV was identified in a goat in southern Tanzania in 2011 (Misinzo et al., 2015). In addition, there is a report of lineage II PPRVs identified in sheep in northern Tanzania in 2014 although the sequences are presently not publicly available (Mahapatra et al., 2016). An analysis of sequence KF672746, however, shows that it is very similar (98.4% nucleotide identify) to the Nigeria 75/1 vaccine strain (see Fig. 2). Likewise, the phylogenetic tree presented by Mahapatra et al. (2016) indicates a high similarity between their sequences and sequence KF672746. This suggests that there may be a PPRV circulating in Tanzania that is very similar to, or even a variant of, the vaccine strain Nigeria 75/1 vaccine strain or that the results from these studies are due to laboratory contamination as has been proposed for other studies (Liu et al., 2018).

The lineage II PPRV identified in this study, however, is more closely related to viruses from Mali, Côte d'Ivoire, Niger, Senegal, Liberia and Sierra Leone (98.6 to 98.1% nucleotide identity) than to Nigeria 75/1 (92.2% nucleotide identity) (Fig. 2). How a lineage II virus, normally confined to Western Africa, reached eastern DRC is unclear. There is no official trading of live animals between these two regions of the continent. The identification of further lineage II viruses in DRC and the generation of full genome sequences may clarify the exact origin of this lineage II virus.

It is strongly suspected that lineage IV PPRVs are circulating (or have circulated in the past) in the DRC. Libeau et al (2014) indicated that lineage IV PPRVs were present in the DRC in 2012 although they did not provide any supporting molecular data. However, the PPRV reported in Angola in 2012 belonged to lineage IV (GenBank KP189203) and itis believed to have been introduced to the Angolan enclave of Cabinda via "a herd of 55 sheep and goats brought from DRC" (OIE, 2012b) and then to the rest of the country. In addition, lineage IV has been identified in the Central African Republic which shares a long border with northern DRC (Banyard et al., 2010) and so the transboundary introduction of lineage IV viruses into DRC would not be surprising. Further investigations are required to determine whether lineage IV viruses are present or not in the DRC.

Finally, the co-circulation of different lineages of PPRVs is not unique to the DRC. In Nigeria, for example, both lineage IV and II were identified by Woma et al. (2016) while in neighbouring Niger three lineages (I, II and IV) have been identified (Tounkara et al., 2018). The consequences of having more than one PPRV lineage circulating in a country are unknown. There is no data available that indicate that any one lineage is more virulent than the others or that different ruminant species are more susceptible to individual lineages. Although it is known that the vaccine most commonly used in Africa (e.g. Nigeria 75/1) provides complete protection against all four lineages, the level of antigenic drift (if any) of different viral lineages due to vaccination pressure is also unknown.

In summary, this report confirms the circulation of PPRV in the DRC and provides the first sequence information and phylogenetic analysis of circulating viruses. This data will be of assistance in the development of national and regional control programmes for PPR that will contribute to the global eradication of this disease.

Ethical approval

No animal work was performed for this study.

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