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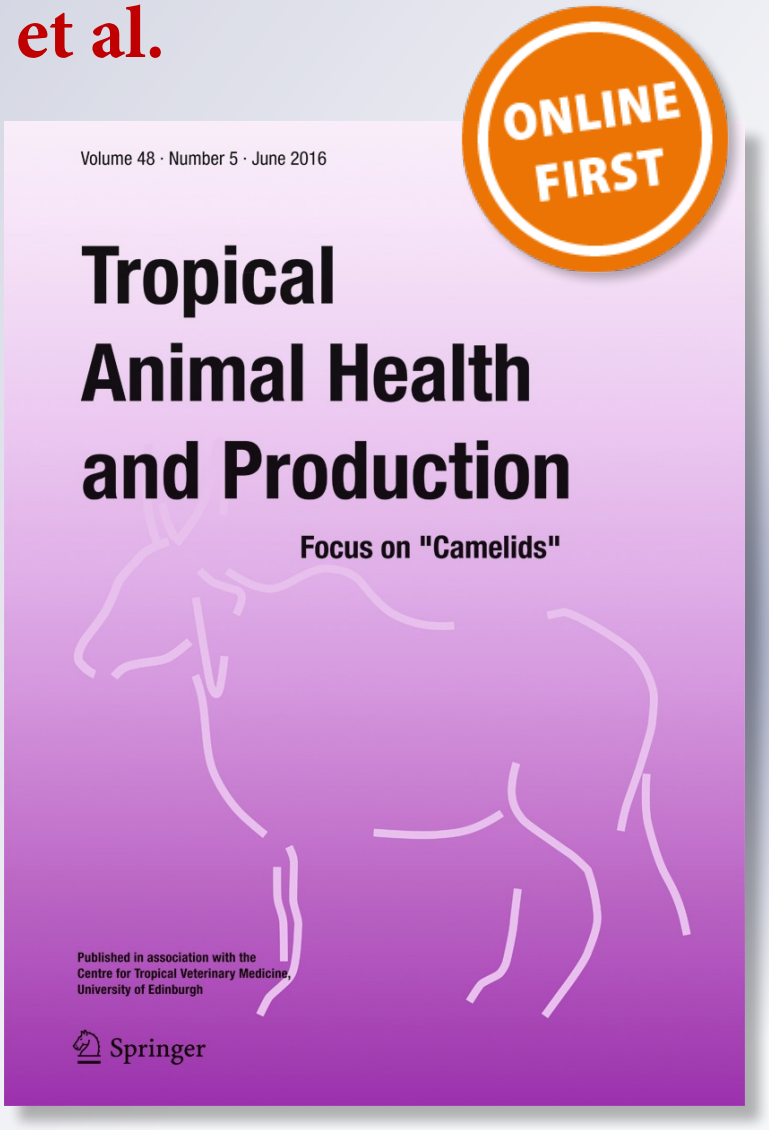

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SHORT COMMUNICATIONS

First report and characterization of peste des petits ruminants virus in Liberia, West Africa

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Peste des petits ruminants (PPR) is a contagious and often fatal disease affecting sheep and goats that is currently endemic in Africa, the Middle East, the Indian subcontinent and China. Understanding the molecular epidemiology and evolution of PPR virus (PPRV) can assist in the control of the transboundary spread of this economically important disease. Here we report the isolation of a PPRV from pathological and swab samples collected from goats in Liberia, West Africa in July 2015. The full genome of one of the isolates was sequenced and phylogenetic analysis showed that it clustered within viral lineage II. The full genome revealed a 99.2 % identity at the nucleotide level with the full genome of a PPRV isolated in neighbouring Côte d'Ivoire in 2009 indicating a common origin of the viruses.

Peste des petits ruminants (PPR) is a highly contagious infectious viral disease of domestic and wild small ruminants. The control of PPR is considered an important element in the fight for global food security and poverty alleviation and it is for this reason that the disease has been chosen as the next animal disease for global eradication. In April 2015, in Abidjan, Côte d'Ivoire, the Food and Agricultural Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) met with high-level authorities from affected countries to agree on a global plan to eradicate PPR by 2030 (FAO 2015).

The causative agent, the peste des petits ruminants virus (PPRV), is a member of the family *Paramyxoviridae*, genus *Morbillivirus* that includes rinderpest virus (RPV), measles virus (MV), canine distemper virus (CDV), phocine distemper virus (PDV), dolphin morbillivirus (DMV) and feline morbillivirus (Woo et al. 2012; Baron et al. 2016). The non-segmented single-stranded, negative-sense RNA genome of PPRV is 15,948 nucleotides in size and encodes 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) (Baron et al. 2016). Based on partial sequences of the N and F genes, PPRV strains have been classified into four genetically distinct lineages (I, II, III and IV) even though the virus is serologically monotypic (Libeau et al. 2014; Parida et al. 2015). PPRV strains that were first identified in Africa belong to lineages I, II and III while viruses belonging to lineage IV have been found in Asia including the Middle East (Libeau et al. 2014; Parida et al. 2015).

Although, PPRV has been reported in several West African countries there has been no report of the virus in Liberia to

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date. In early July 2015, a joint African Union-Inter Bureau for Animal Resources (AU-IBAR) and African Union-Pan African Veterinary Vaccine Centre (AU-PANVAC) mission was organized in response to the Liberian government's request for emergency technical assistance as a result of significant mortalities in sheep and goats in the country. The suspected outbreaks with associated mortalities began around mid-April 2015 in Nimba county, Sinequanelli district in the north-central part of the country, close to the border with Guinea. The outbreaks were officially reported to the Liberian national veterinary authorities on 25 May, 2015. The clinical symptoms described by farmers were fever, cough, nasal and lachrymal discharge, laboured breathing, abundant diarrhoea and death within 3–4 days of symptom onset. Goats were more susceptible than sheep showing between 95–100 % and 70–75 % mortality, respectively.

During the expert mission a new disease outbreak was identified in Ganta city, Nimba county (GPS: 7° 14′ 15″ N 8° 58′ 53″ W) (Fig. 1). Twelve goats from a herd of 15 (80 %) that had been purchased in the cross-border livestock market of N'Zerekole in neighbouring Guinea had succumbed to the disease. The clinical signs and the post mortem lesions of the remaining goats indicated a strong suspicion of PPR. Whole blood, swab samples (e.g. nasal, ocular and buccal) and organs (e.g. liver, lymph nodes, lung, duodenum, ileum, spleen, heart and kidney) were collected and shipped back to AU-PANVAC in Ethiopia for processing.

Nasal swab, lung and lymph node tissues collected from one of the sacrificed goats were processed for viral isolation using CHS-20 cells as previously described (Adombi et al. 2011). The cells were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated foetal bovine serum, 1 % mixed antibiotic-antimycotic solution, and 600 μg/ml of hygromycin B at 37 °C in a 5-% (*v*/*v*) CO_2 incubator. All three samples resulted in cytopathic effect on the CHS-20 and the cell supernatant was collected and stored. The virus (Lib/2015) originating from the lung samples was chosen for further characterization.

Total RNA was extracted from the CHS-20 cell supernatant infected with Lib/2015 using an RNeasy kit (Qiagen). The extracted RNA sample was analyzed by RT-PCR using the One-Step RT-PCR kit (Qiagen) to amplify a 351 bp fragment of the N gene with the primer pair (NP3: 5′-TCT CGG AAA TCG CCT CAC AGA CTG-3′ and NP4: 5′-CCT CCT CCT GGT CCT CCA GAA TCT-3′) (Couacy-Hymann et al. 2002). The phylogenetic analysis of this partial sequence of the N gene is commonly used to classify PPRV into one of four genetic lineages (Libeau et al. 2014; Parida et al. 2015). The following thermal profile was used: reverse transcription at 50 °C for 30 min, initial denaturation at 95 °C for 15 min and then 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, followed by a final elongation at 72 °C for 5 min.

The positive RT-PCR amplicon was purified using a Wizard SV Gel and PCR Clean-up system (Promega) and was sent to LGC Genomics (Berlin, Germany) for sequencing. The sequencing primers were the same as those used in the RT-PCR assay described. The Staden Package (http://staden.sourceforge.net/) was used to assemble the generated sequences.

A phylogenetic tree of N gene segments from a representative selection of PPRV sequences available in GenBank was estimated using the maximum likelihood (ML) method available in MEGA6 employing the Kimura-2 parameter model of nucleotide substitution and 1000 bootstrap replications. The phylogenetic analysis

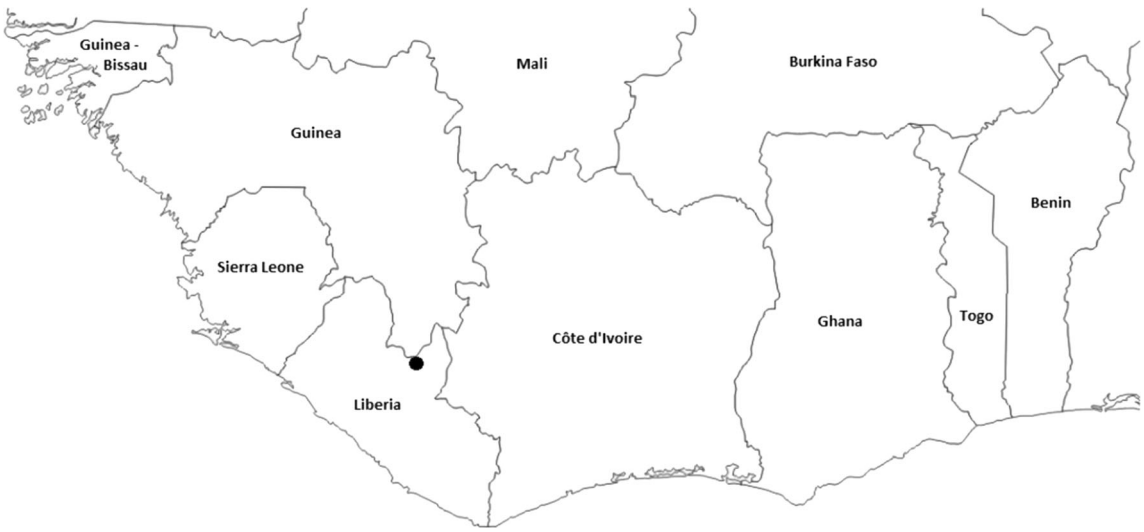

Fig. 1 Map of Liberia and neighbouring countries. The location of the sampling site (Ganta city) is shown by a *black circle* (adapted, with permission, from www.d-maps.com)

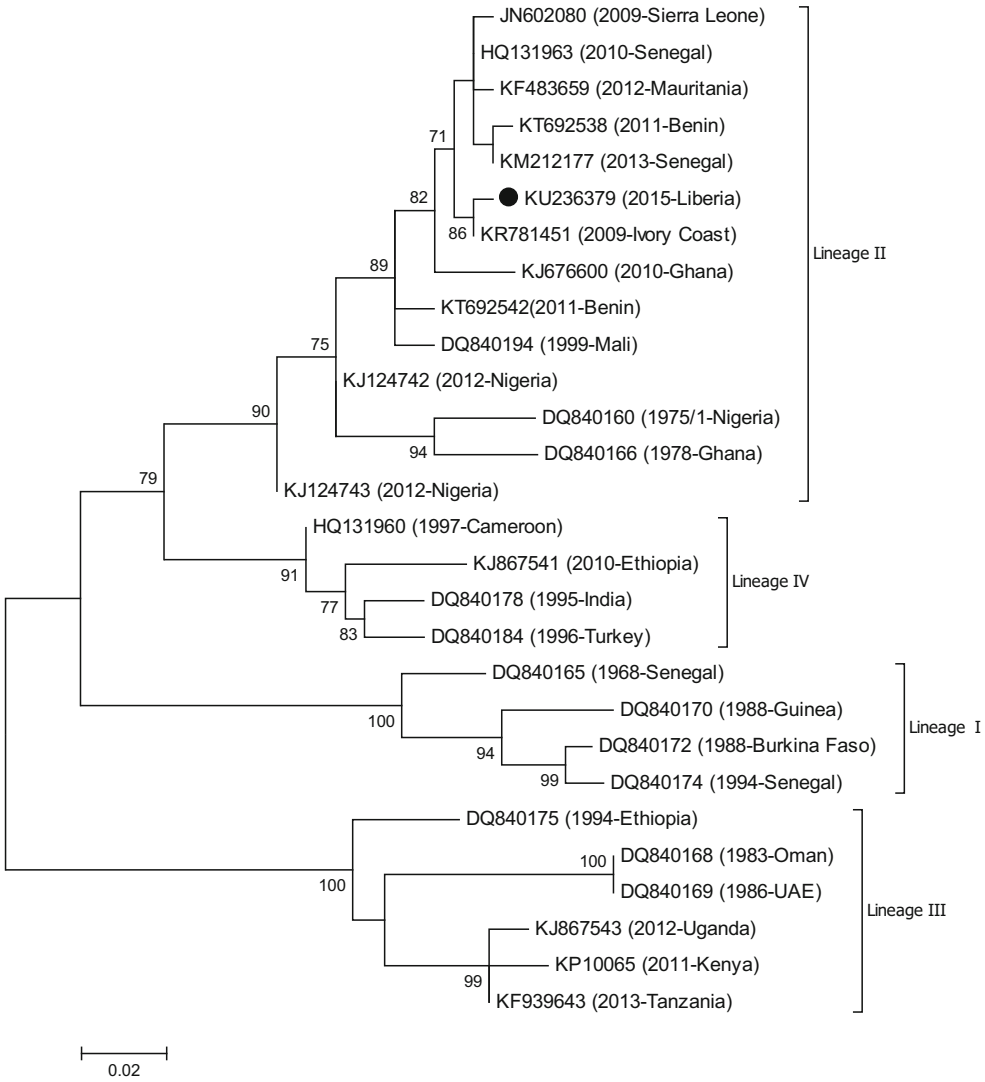

Fig. 2 ML phylogenetic tree of partial N gene sequence from the PPRV sampled in Liberia combined with similar sequences available in GenBank. The virus from Liberia is shown by a *filled black circle*. Bootstrap support values (>70 %) are shown

revealed that the PPRV isolate from the goat lung sample collected on 8 July 2015 in Ganta belonged to lineage II (Fig. 2). Notably, the sequence was closest to that of an isolate collected in neighbouring Côte d'Ivoire in 2009 (Adombi in preparation) than to any other West African isolate characterized to date.

The full genome sequence was generated as previously described using 30 primers sets (available on request) (Dundon et al. 2014a). The sequence has been deposited in GenBank under accession number KU236379. Analysis of the full genome sequence of Lib/2015 revealed that it consisted of 15,948 bp with a 107 nt genome promoter region

Table 1 Comparison of genome characteristics of PPRV Lib/2015 and other lineage II PPRV genome sequences available in public databases

Genome	Accession number	Country and date	Nucleotide identity with PPRV Lib/2015 (%)	Protein identity (%)							
				N	P	V	C	M	F	H	L
CIV/01P/2009	KR781451	Côte d'Ivoire, 2009	99.2	100	99.0	98.6	98.9	99.4	99.6	99.3	99.5
SnDk/11|13	KM212177	Senegal, 2013	98.8	99.8	98.2	97.6	98.3	100	99.6	99.0	99.5
Ghana/NK1/2010	KJ466104	Ghana, 2010	98.6	99.0	98.2	97.3	98.9	99.7	99.6	99.0	99.6
Benin/10/2011	KR781449	Benin, 2011	97.9	99.2	96.6	95.6	98.3	100	99.3	97.5	99.2
Nigeria 76/1	EU267274	Nigeria 1976	96.5	97.9	94.8	91.6	92.4	99.7	99.1	96.2	98.7
Benin/B1/1969	KR781450	Benin 1969	96.2	98.5	94.8	91.6	93.0	99.7	99.1	96.3	98.6
Nigeria 75/1	HQ197753	Nigeria 1975	95.7	97.5	94.3	91.6	92.4	98.5	98.3	95.8	98.4

at the 3′ end followed by the transcription units for the N, P, M, F, H and L proteins and the anti-genome promoter at the 5′ end as seen previously for other PPRVs (Dundon et al. 2014a, b, 2015a, b; Adombi et al. 2016). Comparison of the nucleotide sequence revealed the highest identity of 99.2 % with CIV/01P/2009 confirming the phylogenetic analysis shown in Fig. 2. Table 1 provides a detailed comparison at both the nucleotide and amino acid level of Lib/2015 with other lineage II viruses. Again, the strong relationship between Lib/2015 and CIV/01P/2009 can be seen. Indeed, the amino acid sequences of the N protein of both viruses are identical (the nucleotide differences in the N gene between Lib/2015 and CIV/01P/2009 are due to synonymous mutations) while the percentage similarities of the other proteins range from 98.6 % for the V protein to 99.6 % for the F protein. A number of the amino acid motifs previously identified in other PPRV proteins are present in Lib/2015 (Dundon et al. 2014a, b, 2015a, b; Adombi et al. 2016). These include nuclear export and localization signals and the F protein cleavage site GRRTRR.

Liberia imports live animals for human consumption from Guinea, Côte d'Ivoire and Mali all of which have previously reported PPR (Koikoi 2011). It is evident from this study that there is a close relationship between the virus isolated in Côte d'Ivoire in 2009 and that from Liberia in 2015 which indicates a common origin. Other countries in the region in which PPRV has been partially characterized are Sierra Leone, Guinea, Mali, Ghana, Benin and Senegal (Munir et al. 2012; Dundon et al. 2014a; Salami et al. 2014; Adombi et al. 2016) but none of these viruses reveal the same similarity to Lib/2015 as the virus from Côte d'Ivoire does. Indeed, a segment of the N gene of an isolate which was collected in 2009 in Sierra Leone (Munir et al. 2012) only shows 98.1 % identity with the N gene of Lib/2015. The only PPRV isolates characterized from Guinea and Mali date back to 1989 and 1999, respectively, and belong to lineage I, so comparisons with Lib/2015 are difficult to make.

In conclusion, this study strongly indicates that the PPRV isolated from Côte d'Ivoire (2009) and Liberia (2015) is of the same origin and suggests transboundary movement of animals between the two countries. However, given the present lack of information on PPRV in Guinea, transboundary movement between Guinea and Liberia cannot be excluded. Indeed, the diseased goats identified in Ganta city were originally purchased at a cross-border livestock market in Guinea.

Although the PPRV from Sierra Leone is less related to Lib/2015 it is possible that different lineage II subclades are circulating in the country which may be more similar to Lib/2015; a situation that has recently been reported in Benin and Nigeria (Woma et al. 2015; Adombi et al. 2016). Therefore, in order to clarify the present PPR situation in Liberia, further similar studies should be carried out in neighbouring Guinea and Sierra Leone given the high cross-border movement of live animals for trade. Such a regional approach is also needed for the effective prevention and control of PPR in the region.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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