



Molecular epidemiological update of Peste des Petits Ruminants virus (PPRV) in Ethiopia

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ABSTRACT

Peste des Petits ruminants (PPR) is a devastating disease of small ruminants with high morbidity and mortality rates among susceptible animals. The disease is endemic in much of Africa, the Middle East and Asia and constitutes one of the major hurdles to the improvement of small-ruminant production in these countries. The causal agent of PPR, the Small Ruminant Morbillivirus (SRMV), previously known as PPR virus (PPRV) belongs to the genus *Morbillivirus* within the family *Paramyxoviridae*. SRMV can be categorized into four genetically distinct lineages (I to IV). Suspicion of PPR was first reported in Ethiopia in 1977 and since then genetic characterization of circulating viruses has identified lineages III and IV in the country. This study was undertaken to provide an update on the molecular epidemiology of PPR in Ethiopia by analysing animal tissue samples collected between 2011 and 2017. PPR positive samples were identified in four regions of the country. Sequence and phylogenetic analysis of fourteen RT-PCR positive amplicons revealed that all of the SRMV in the samples from 2010 to 2017 belong to sub-clade II of clade I of lineage IV. No lineage III viruses were identified.

1. Introduction

Peste des Petits Ruminants (PPR) is an acute, highly contagious disease affecting small domestic (sheep and goats) and wild ruminants. PPR has an impact on small ruminant production in most infected countries, especially in rural areas where sheep and goats are important for livelihoods. Mortality and morbidity due to PPR can reach 50–90 % and 10–90% respectively, particularly in naïve populations of sheep and goats (Libeau et al., 2014). Since the first description of PPR in Côte d'Ivoire in 1942 (Gargadennec and Lalanne, 1942), the disease has spread across most African countries (with the exception of the southern part of the continent), the Middle East, Eurasia (Bulgaria, Georgia and Turkey) and Asia (Libeau et al., 2014; Parida et al., 2015; Donduashvili et al., 2018). PPR is characterised by pyrexia, purulent ocular nasal discharges, and erosive lesions in mucous membranes particularly in the mouth, pneumonia and diarrhoea with severe

dehydration, often leading to death. The causative agent of PPR, recently renamed as Small Ruminant Morbillivirus (SRMV), is a member of the family *Paramyxoviridae*, genus *Morbillivirus* (Gibbs et al., 1979; Parida et al., 2015; Amarasinghe et al., 2017). The SRMV exists as a single serotype but can be differentiated into four genetically distinct lineages I, II, III and IV based on partial sequences of the C-terminus of the nucleoprotein (N) gene and the fusion protein (F) gene (Banyard et al., 2010). Further subdivisions of lineage IV have been proposed by Kumar et al. (2014) who identified two clades (CI and CII) and four subclades (SCI to SCIV) based on the nucleoprotein (N) gene.

All four lineages have been identified across Africa. In some countries more than one lineage has been found. For example, lineages II, III and IV have been reported in Tanzania (Misinzoo et al., 2015; Mahapatra et al., 2016); lineages III and IV in Uganda (Luka et al., 2012). In Ethiopia, SRMV genetically characterized from confirmed outbreaks in 1994 and 2010 and were identified as lineages III and IV, respectively

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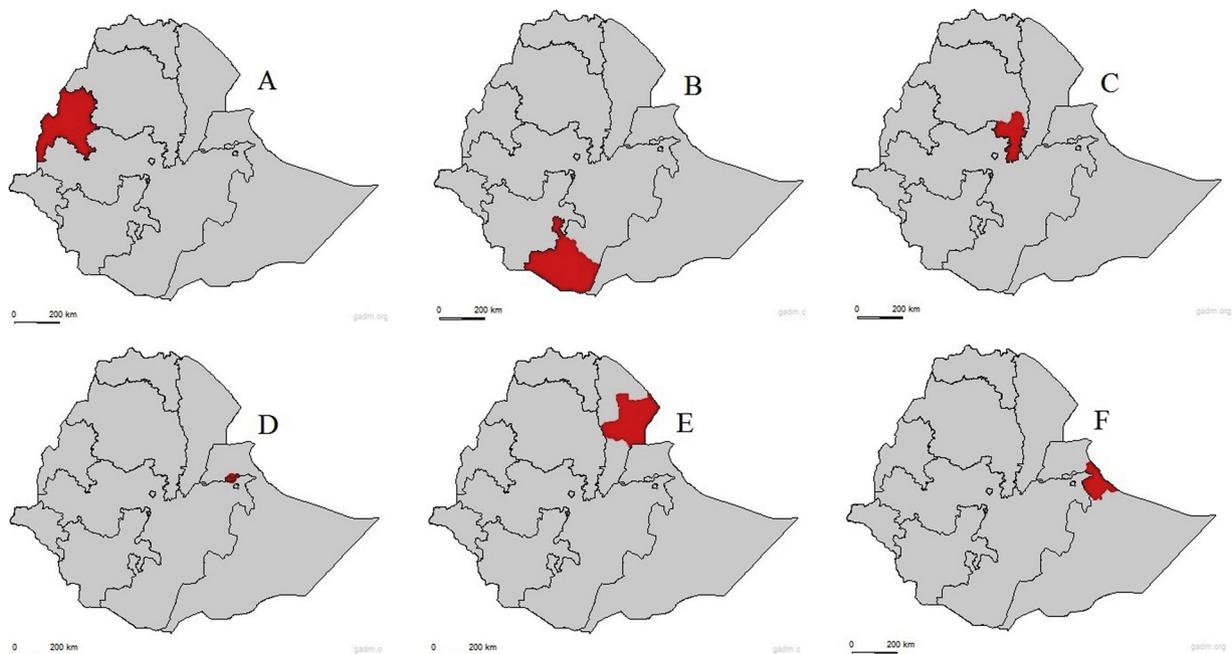


Fig. 1. Zones/Regions of Ethiopia where the study was conducted (A) Benshangul-Gumaz region, (B) Borana zone (Oromia region), (C) Debre-Berhan (Amhara Region), (D) Direh Dawa (Federal city), (E) Semra (Afar region), (F) Jijiga (Somali region), adapted from www.d-maps.com.

(Roeder et al., 1994; Kwiatek et al., 2007; Banyard et al., 2010; Muniraju et al., 2014, 2016). Since then, no studies on SRMV in the country have been published. With this in mind, this study undertook the phylogenetic analysis of SRMVs identified in Ethiopia from confirmed PPR outbreaks between 2010 and 2017 in order to update the molecular epidemiology data on SRMV in Ethiopia.

2. Material and methods

2.1. PPR outbreak samples

In May 2011, a PPR diagnostic investigation was carried out on 60 male goats (aged less than 12 months) purchased from Bulbula city (9°12'56.3"N 38°30'01.0"E) located in the Oromia Region at 34 km North-West of Addis-Ababa (the capital of Ethiopia). These animals developed PPR clinical signs two weeks after their introduction into the African Union Pan Veterinary vaccine Centre (AU-PANVAC) animal facilities. Six animals died and tissue samples were collected following the post-mortem examination for PPR confirmation.

In 2017, tissue samples (n = 59) from PPR suspected infected animals were collected in six regions and one federal city in Ethiopia (Fig. 1) during a survey conducted for PPR investigation. Samples were from Borana zone (5°00'00.0"N 38°15'00.0"E) in the Oromiya region, Debre-Berhan (9°40'46.34"N, 39°31'57.43"E) located in the Semien Shewa Zone of the Amhara Region, about 120 km north east of Addis-Ababa, Benshangul-Gumaz (10°46'00.0"N 35°32'00.0"E), the chartered city of Direh Dawa (9°36'00.0"N 41°52'00.0"E), the Mekelle zone (13°29'00.0"N 39°28'00.0"E) of the Tigray region, Jijiga (9°21'00.0"N 42°48'00.0"E) located in the Faafan zone of the Somali region, Semra (11°47'32.0"N 41°00'31.0"E) in located the Administrative Zone 1 of the Afar region. Similarly, in December 2017, suspicions of a PPR outbreak in 109 goats purchased in December 2017 from Debre-Birhan were investigated at the AU-PANVAC laboratories. Tissue samples from dead animals (n = 41) were collected following mortalities a week after arrival of these animals at AU-PANVAC laboratories.

A total of 100 tissue samples collected during the 2017 outbreaks and 6 tissue samples from 2011 were used for this study.

In addition, a SRMV isolated in 2014 by the National Animal Health Diagnostic and Investigation Center (NAHDIC) from a PPR outbreak in

Bati (11°11'00.0"N 40°01'00.0"E), a town in Debub Wollo zone (in North-Central part of Ethiopia) in Amhara Region was included in the study.

2.2. Detection of SRMV RNA and partial genome sequencing for phylogenetic analysis

All tissue samples were disrupted in phosphate buffered saline using the TissueLyser LT (Qiagen, Germany) following the manufacturer's instructions. Tissue lysate suspensions were used for total RNA extraction using the RNeasy mini kit (Qiagen, Germany) as per the manufacturer's instructions. The specific SRMV primers NP3; 5'-TCT CGG AAA TCG CCT CAC AGA CTG-3' and NP4; 5'-CCT CCT CCT GGT CCT CCA GAA TCT-3' were used to amplify a 351 bp fragment of the N gene (Couacy-Hymann et al., 2002). RT-PCR amplicons were purified using Wizard SV Gel and PCR Clean-up kit (Promega) and sent to LGC Genomics (Berlin, Germany) for sequencing using primers NP3 and NP4. All sequences generated in this study have been submitted to the GenBank under accession numbers (MK571524 to MK571537) (Table 1). The Staden Package (<http://staden.sourceforge.net/>) was used to assemble the generated sequences. The lengths of the sequences analysed were 255 bp for partial N gene. Sequence alignment was performed using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>) with default settings, incorporating all the sequences generated here combined with those available in GenBank (accession numbers are shown in the tree figure). Of particular note are three GenBank submissions (KX816961 to KX816963) from an unpublished molecular epidemiological study of SRMV in the Eastern Amhara region of Ethiopia in 2014 which were included in the analysis along with the partial sequence of the N gene of an Ethiopian SRMV isolate (GenBank KJ867541) from an outbreak reported in 2010 at the National Veterinary Institute of Ethiopia (Muniraju et al., 2016). Additionally, partial N gene sequences from lineage III viruses identified in Ethiopia in 1994 and 1996 were included in the analysis (Kwiatek et al., 2007; Muniraju et al., 2014). Phylogenetic trees were estimated using the Maximum Likelihood (ML) method available in MEGA 6 (Tamura et al., 2013), employing the Kimura 2-parameter model of nucleotide substitution and 500 bootstrap replications

Table 1
Description of the samples analysed in this study.

Sample	Year	Species	Organ	Location	Region	GenBank #
PPRV/Ethiopia/1	2011	Goat	Lung	Bulbulla	Oromia	MK571524
PPRV/Ethiopia/KD16	2011	Goat	Kidney	Bulbulla	Oromia	MK571525
PPRV/Ethiopia/LG3	2011	Goat	Lung	Bulbulla	Oromia	MK571526
PPRV/Ethiopia/1	2014	Goat	Unknown	Bati	Amhara	MK571527
PPRV/Ethiopia/4	2017	Goat	Spleen	Debre Berhan	Amhara	MK571528
PPRV/Ethiopia/5	2017	Goat	Lung	Semra	Afar	MK571529
PPRV/Ethiopia/6	2017	Goat	Lung	Benshangul	Gumaz	MK571530
PPRV/Ethiopia/7	2017	Goat	Lung	Debre Berhan	Amhara	MK571531
PPRV/Ethiopia/8	2017	Goat	Lymph node	Debre Berhan	Amhara	MK571532
PPRV/Ethiopia/9	2017	Goat	Lymph node	Debre Berhan	Amhara	MK571533
PPRV/Ethiopia/10	2017	Goat	Lung	Borana zone	Oromia	MK571534
PPRV/Ethiopia/11	2017	Goat	Lung	Debre Berhan	Amhara	MK571535
PPRV/Ethiopia/12	2017	Goat	Lymph node	Dire Dawa	Federal city	MK571536
PPRV/Ethiopia/13	2017	Goat	Lung	Semra	Afar	MK571537

2.3. Virus isolation

PPR virus isolation was performed using the cell line CHS-20 (Adombi et al., 2011) with a slight procedure modification. Briefly, tissue lysate suspensions prepared with Tissue Lyser (Qiagen) were directly inoculated on to the cells at 80% confluence. The cells were maintained in Glasgow Minimum Essential Medium (GMEM) supplemented with 10% heat-inactivated foetal bovine serum, 1% mixed antibiotic-antimycotic solution and 200 µg/ml of hygromycin B. The flasks were incubated at 37 °C in a 5% (v/v) CO₂ incubator and inspected daily for cytopathic effects.

3. Results and discussions

The RT-PCRs on outbreak samples from 2011, 2014 and 2017 were confirmed positive for PPR. Out of the 100 tissue samples collected in 2017 and analysed by RT-PCR, 29 were found to be positive for SRMV. Out of the six regions where samples were collected in 2017, four regions were confirmed positive namely, Debre-Berhan in the Amhara Region, the Benshangul-Gumaz region, Semra in the Afar region, the Borena zone of the Oromia region and the administrative city of Direh Dawa. Similarly, tissue samples from the 2011 outbreaks in Bulbulla city (Oromia region) and from the goats from Debre-Birhan purchased by AU-PANVAC in 2017 were also positive.

A total of fourteen positive samples (ten samples from 2017, three samples from 2011 and the positive isolate from 2014) presented in Table 1 and showing strong bands with RT-PCR were selected for SRMV isolation performed on the CHS-20 cell line (Adombi et al., 2011). Microscopic observations of culture inoculated with tissue lysate suspensions showed clear cytopathic effects within five days on CHS-20 cells. Infected cultures were aliquoted and stored at –80 °C.

For the phylogenetic analysis of the partial N sequences, the same fourteen positive samples were used (Table 1, Fig. 2). Data showed that all SRMVs identified in 2011, 2014 and 2017 belonged to sub-clade II of clade I of lineage IV. Sub-clade II consists of viruses from Africa, Pakistan, Saudi Arabia and Israel (Kumar et al., 2014). Of note is that the sequences were not identical to each other with percentage identity ranging from 96.7 to 100%. Interestingly the viruses are grouped according to year of identification (i.e. 2011, 2014 and 2017) indicating that, as expected for an RNA virus, SRMVs are continuously evolving.

What is evident from this study is that none of the samples collected over the six-year study period belong to lineage III. Indeed, since its first identification in Ethiopia (Roeder et al., 1994) the SRMV lineage III has not been reported in the country. Similar observations regarding the replacement of one lineage by another have been reported in neighbouring Sudan. A study conducted from 2000 to 2009 indicated a progressive substitution of lineage III with lineage IV in a large area of the country encompassing the eastern, northern, Blue Nile, and

Khartoum regions (Kwiatek et al., 2011). While the epidemiology of PPR in Ethiopia appears to be similar to Sudan, the situation is different when compared to other Eastern-Africa countries such as Kenya, Uganda, Tanzania and Burundi which continue to report the circulation of lineage III viruses only (Niyokwishimira et al., 2019; Dundon et al., 2017; Luka et al., 2012; Misinzo et al., 2015; Mahapatra et al., 2016). Nevertheless, although this study did not identify the circulation of lineage III SRMV in Ethiopia, due to the relatively small samples number, our findings do not imply that lineage III virus is not currently present in the country.

In addition to the shift in SRMV lineage in Ethiopia, it was also observed that PPR outbreak occurred regularly over the years in different regions in Ethiopia since the first description of the lineage IV in 2010 (M. Muniraju et al., 2014). This increase of PPR outbreak might be related to the virulence of the lineage IV as also reported in Sudan (Kwiatek et al., 2011).

The Ethiopian government in collaboration with the Food and Agricultural Organization of the United Nations (FAO) has developed a National PPR Progressive Control and Eradication Strategy funded by the European Union (EC-SHARE). The strategy is aligned with the Global strategy for the Control and Eradication of PPR (FAO, 2017). Currently, over 8 million of sheep and goat have been vaccinated against the PPR (FAO, 2018) using the Nigeria 75/1 vaccine (produced by the National Veterinary Institute, Debre-Zeit, Ethiopia), from a total small ruminant population estimated at 60.9 million (Beyene et al., 2018).

The possible explanations for the apparent dominance of lineage IV SRMVs over lineage III SRMVs in Ethiopia can be the following; the current level of PPR vaccination coverage with the SHARE programme in the country is around 13% and more susceptible animal can be affected by the circulating SRMV lineage IV which seems more virulent to the lineage III as indicated above, despite the fact that it is believed that the vaccine strain Nigeria 75/1 provides complete protection across genetic lineages (Kumar et al., 2017) the vaccination pressure might have resulted in the antigenic drift of lineage IV viruses while limiting the spread of lineage III viruses. A more in-depth genomic (i.e. full genome sequencing) and antigenic analysis of the viruses circulating in Ethiopia may determine whether this assumption of antigenic drift is true or not.

Finally, an important observation from this work is that SRMV is widely distributed in Ethiopia posing a significant risk to sheep and goat production. As part of the planned global eradication of PPR by the year 2030 (FAO, 2017), the determination and generation of more recent sequence data of all SRMVs circulating in the country will be of importance to veterinary authorities involved in the implementation and/or optimization of PPR control programmes at both national and regional levels.

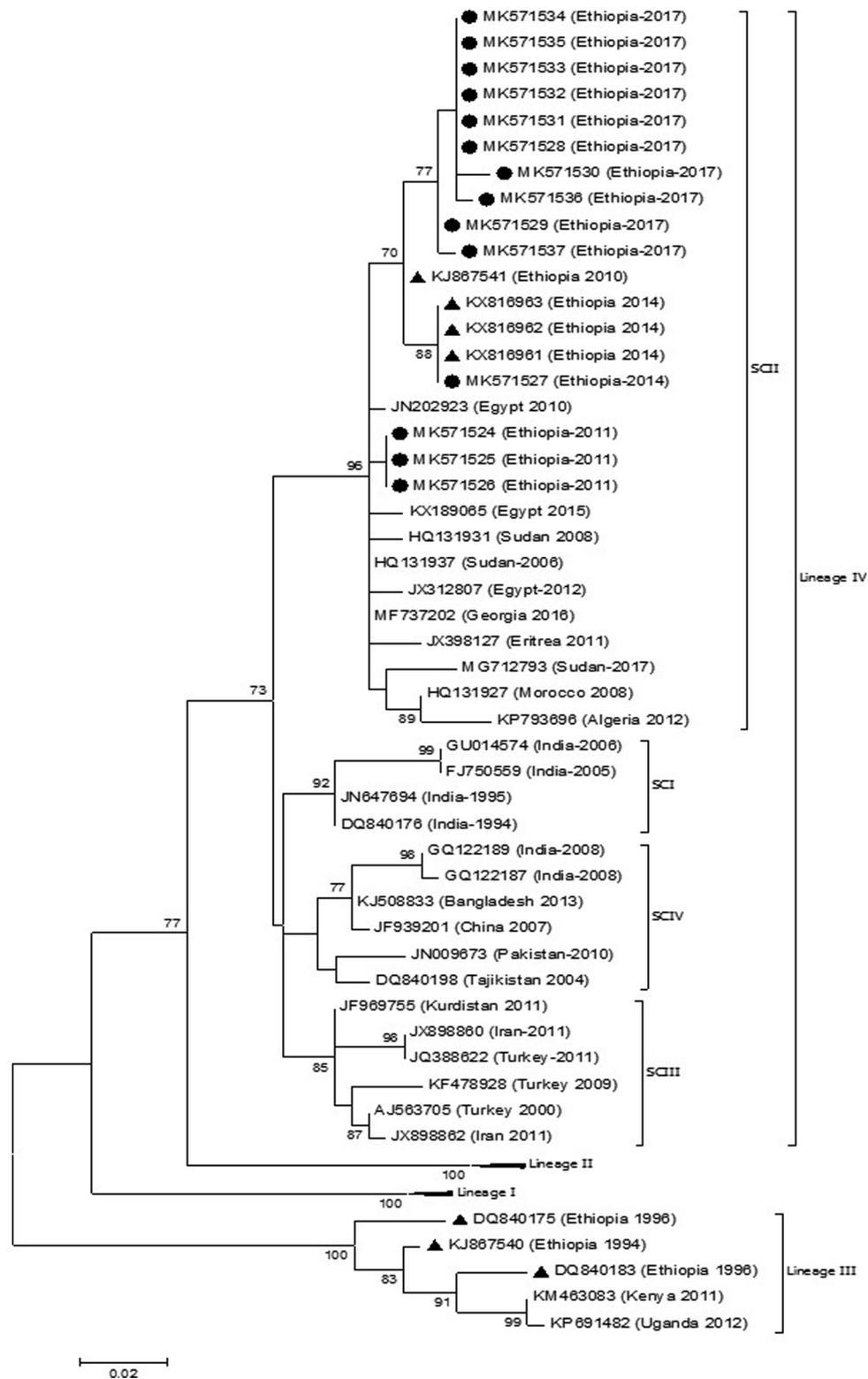


Fig. 2. ML phylogenetic tree of partial nucleotide sequences (255 bp) of the N gene from the PPRV samples in this study together with representative sequences available from the GenBank. The PPR viral sequences from Ethiopia in this study are indicated with black dots while those from previous studies are indicated with black triangles. The numbers indicate the bootstrap values calculated from 500 bootstrap replicates. The different sub-clades and lineages are shown according to Kumar et al., 2014.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable, National, and Institutional guidelines for the care and use of animals were followed. AU-PANVAC is a specialised Technical Agency of the African Union Commission which has signed a headquarters agreement with the Government of the Federal Democratic Republic of Ethiopia. All laboratory activities are conducted in accordance with the laws and regulations of Ethiopia. Animal manipulations are conducted under the AU-PANVAC Quality Management System.

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