Emergence of Lineage IV Peste des Petits Ruminants Virus in Ethiopia: Complete Genome Sequence of an Ethiopian Isolate 2010

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Summary
Isolates of peste des petits ruminants virus (PPRV) can be segregated genetically into four lineages. For decades, lineages I–III have been reported across Africa whilst lineage IV has predominantly circulated across Asia. However, the lineage distribution is currently changing in Africa. Importantly, full genome sequence data for African field isolates have been lacking. Here, we announce the first complete genome sequence of a field isolate of peste des petits ruminants virus (PPRV) from East Africa. This isolate was derived from the intestine of a goat suffering from severe clinical disease during the 2010 outbreak in Ethiopia. The full genome sequence of this isolate, PPRV Ethiopia/2010, clusters genetically with other lineage IV isolates of PPRV, sharing high levels of sequence identity across the genome. Further, we have carried out a phylogenetic analysis of all of the available African partial N gene and F gene PPRV sequences to investigate the epidemiology of PPRV with a focus on the emergence of different lineages of PPRV in Africa.

Introduction

Different morbilliviruses are the cause of numerous clinically devastating diseases including measles in humans, canine distemper in dogs, rinderpest in large ruminants and peste des petits ruminants (PPR) in small ruminants and camelids. With the eradication of rinderpest focus has shifted onto PPR and it is increasingly regarded as an important viral disease of domestic ruminants that is a significant burden to the agricultural sectors in endemic areas (Munir, 2013). Currently, PPRV is endemic in Africa, the Middle East and across much of Asia (Banyard et al., 2010). The detection of PPRV or antibodies that denote natural infection has been reported from almost all African countries with the exception of vast territories across Southern Africa (Libeau et al., 2014). For some time, it has been clear that at least one lineage of PPR virus, lineage III, is present within East Africa having been reported from Ethiopia, Tanzania, Uganda and Sudan. Interestingly, lineage IV PPRV has also been reported in Sudan (Banyard et al., 2010; Kwiatek et al., 2011), in Egypt (Sharawi and Abd El-Rahim, 2010) and in Eritrea (Cosseddu et al., 2013). However, circulation of lineage IV PPRV in Ethiopia is not known.

Ethiopia has over 62 million PPR-susceptible livestock (sheep and goats) and has the third largest agricultural sector across Africa. Ethiopia also ranks eighth in the world in terms of small ruminant populations, playing an economically important role in the livelihood of resource-poor farmers. PPR was first suspected in Ethiopia in 1977 following clinical observations consistent with infection with PPRV (Pegram and Tereke, 1981) and was later diagnosed...
Emergence of Lineage IV PPR Virus in Ethiopia

M. Muniraju et al.

as the causative agent of disease in goats in the country (Roeder et al., 1994). The virus detected in 1994, alongside a further isolate reported in 1996 was genetically determined to cluster within lineage III (Kwiatek et al., 2007; Banyard et al., 2010). Although PPR is endemic in Ethiopia, the circulation of lineage IV PPRV has not previously been reported. Therefore, the main aim of this study was to identify whether lineage IV virus is circulating in Ethiopia. Further, as there is no PPRV full genome sequence available from East African regions, we have sequenced the full genome of the Ethiopian virus and have made comparisons with existing available PPRV genetic data. The isolate reported here (KJ867541; Ethiopia/2010) is the first report of lineage IV in Ethiopia and is the first complete PPRV genome to be characterized from an outbreak in East Africa. Further, we have performed a full phylogenetic assessment of African PPRV isolates using both full genome sequences and partial genes (N and F) sequences available in the GenBank database.

Materials and Methods

Outbreak and virus

A PPR outbreak was reported at the National Veterinary Institute (NVI) at Addis Ababa, Ethiopia following the procurement of 50 male goats aged 6–8 months from Debre Zeit market showing no signs of ill health. Within 2 weeks of animal purchase, the goats developed clinical disease consistent with PPRV infection. Numerous clinical samples were collected at post-mortem for confirmatory diagnosis. The RNA for this PPRV was recovered from an intestinal tissue collected on 13th of August 2010 and sent to The Pirbright Institute and as such the virus had not been subjected to selective pressures that may cause cell culture adaptation.

Complete genome sequencing

Viral RNA was extracted using Qiagen Viral RNA mini kit (Hilden, Germany), and the SuperScript™ III One-Step RT-PCR System with Platinum® Taq High Fidelity polymerase (Life Technologies, Paisley, UK) was used for the reverse transcription of viral RNA into cDNA and further amplification in a single steps. PPRV specific primers were used to generate twelve overlapping amplicons covering the entire genome of the isolate. Amplicons were gel purified and sequenced using an ABI-3730 automated sequencer (Applied-Biosystems, Foster City, CA, USA) as described previously (Muniraju et al., 2013). The genome termini were determined using 3'/5' RACE (Li et al., 2010). A total of 525 sequences were assembled into overlapping contigs that represented the full genome (Lasergene v. 10.1, Madison, WI, USA), with an average of 12.6-fold coverage at each nucleotide position.

Sequence analysis

The complete genome sequence of PPRV Ethiopia 2010 (GenBank accession no. KJ867541) generated in this study was compared and phylogenetically analysed with other eight PPRV complete genomes available in the NCBI database. Similarly, the partial N and F gene sequences extracted from the PPRV Ethiopia 2010 in this study were compared and phylogenetically analysed with other available PPRV partial N/F gene sequences from African isolates.

The PPRV partial N gene sequence data of 255 nucleotides (genome position 1360–1614) or partial F gene sequence data of 322 nucleotide (genome position 5779–6100) sequences that have a detailed history of collection date and place were retrieved from the NCBI database (including submission to July 2014). These sequences were aligned using the ClustalW algorithm incorporated in Bioedit software v7.2.0 (Hall, 1999) and edited to remove unreliable sequences/regions. Further, the identical sequences originating from the same geographical location, host and year were excluded to avoid redundancy in phylogenetic analysis. The partial N gene data set contained 83 sequences sampled in Africa over a period of 46 years (1968–2013), and the partial F gene data set contained 38 sequences sampled in Africa over a period of 40 years (1971–2010).

Fig. 1. Neighbour-joining tree based on the complete genome sequences showing the relationships between the African and Asian PPRV isolates. The scale bar indicates nucleotide substitutions per site. The Kimura 2-parameter model with percentage of replicate trees in which the associated taxa clustered together in the 1000 bootstrap replicates is shown next to the branches. The isolate sequenced in this study is indicated by arrow.
Phylogenetic analyses were performed using MEGA6 (Tamura et al., 2013). The neighbour-joining tree was computed using Kimura 2-parameter model. The test of phylogeny was carried out through bootstrap method with 1000 number of replications and the gaps/missing data treated with pairwise deletion.

**Results and Discussion**

The total genome size of Ethiopia/2010 was identical to that of previously published PPRV isolates, 15 948 nucleotides. As expected, the genome was organized as described for all other isolates of PPRV and included: the nucleocapsid (N), phospho (P/C/V), matrix (M), fusion (F), haemagglutinin (H) and the large polymerase (L) proteins. All untranslated regions including the genome and anti-genome promoter regions, gene start and stop sequences and intergenic trinucleotides were present as expected. Interestingly, this isolate clusters with lineage IV isolates of PPRV (Fig. 1) whilst all previous isolates from Ethiopia (1994 and 1996) have been phylogenetically typed as lineage III viruses Fig. 2 (Kwiatek et al., 2007; Banyard et al., 2010). We have

| Table 1. PPRV lineages circulating in different countries of Africa based on partial N/F gene sequence analysis |
|---|---|---|---|---|
| Country | Lineage* | Year of outbreak | NCBI submission | Publication |
|---|---|---|---|---|
| Ivory Coast | I | 1989 | YES | Chard et al. (2008) |
| Guinea | I | 1988, 1991 | YES | Kwiatek et al. (2007), Banyard et al. (2010) |
| Guinea Bissau | I | 1989 | YES | Kwiatek et al. (2007) |
| Burkina Faso | I | 1988 | YES | Kwiatek et al. (2007) |
| Senegal | II | 1999 | NO | Banyard et al. (2010) |
| Mauritania | II | 2012 | YES | El Arbi et al. (2014) |
| Mali | II | 1999 | YES | Kwiatek et al. (2007) |
| Sierra Leone | II | 2009 | YES | Munir et al. (2012) |
| Ghana | II | 1976, 1978, 2010 | YES | Kwiatek et al. (2007), Banyard et al. (2010) |
| Nigeria | II | 1975, 1976, 2010, 2012, 2013 | YES | Diallo et al. (1994), Chard et al. (2008), 2010, 2012 and 2013 sequences are unpublished |
| Ethiopia | III | 1994, 1996 | YES | Kwiatek et al. (2007); Banyard et al. (2010) |
| Sudan | IV | 2010 | YES | This study |
| Tanzania | III | 1971, 1972, 2000 | YES | Banyard et al. (2010); Kwiatek et al. (2011) |
| Uganda | IV | 2000, 2005, 2008, 2009 | YES | Banyard et al. (2010); Kwiatek et al. (2011) |
| Egypt | IV | 2008, 2009, 2010, 2012, 2013 | YES | Luka et al. (2011), 2008, 2010, 2012, 2013 sequences are unpublished |
| Eritrea | IV | 2010, 2009, 2012 | YES | Banyard et al. (2010), Sharawi and Abd El-Rahim (2010) 2010 and 2012 sequences are unpublished |
| Morocco | IV | 2002, 2003, 2005, 2011 | YES | Cosseddu et al. (2013) |
| Gabon | IV | 2008 | YES | Kwiatek et al. (2011); Muniraju et al. (2013) |
| CAR | IV | 2011 | YES | Maganga et al. (2013) |
| Cameroon | IV | 2004 | YES | Banyard et al. (2010) |
| Algeria | IV | 1997 | YES | Banyard et al. (2010) |
| Congo | IV | 2010 | YES | De Nardi et al. (2012) |
| Congo | IV | 2006 | NO | This study, sequence yet to be submitted |
| Western Sahara | IV | 2010 | NO | Libeau et al. (2014) |
| Tunisia | IV | 2012, 2013 | NO | Soufien et al. (2014) |
| DRC | IV | 2012 | NO | Libeau et al. (2014) |
| Angola | IV | 2014 | NO | Libeau et al. (2014) |
| Niger | II | 2012 | NO | Farougou et al. (2013); Libeau et al. (2014) |
| Chad | II | 1993 | NO | Bidjeh et al. (1995); Libeau et al. (2014) |
| Kenya | III | 2006 | NO | Libeau et al. (2014) |
| Somalia | III | – | NO | Libeau et al. (2014) |
| Libya | Serology | – | NO | Libeau et al. (2014) |
| Comoros | Serology | 2010 | NO | FAO (2013); Libeau et al. (2014) |

*Lineages of PPRV isolates were named by following the partial N gene sequence phylogenetic analysis.*
Fig. 2. Neighbour-joining tree based on the partial PPRV N gene sequences showing the relationships between the African PPRV isolates. Scale bar indicates nucleotide substitutions per site. The Kimura 2-parameter model with percentage of replicate trees in which the associated taxa clustered together in the 1000 bootstrap replicates is shown next to the branches. The isolates sequenced in this study are indicated by arrows.
recently reported a full genome sequence of lineage IV PPRV from Morocco (Muniraju et al., 2013), and these two isolates are closely related with 97.9% homology. At the nucleotide level, Ethiopia/2010 shared 97% homology with Turkey/2000, 96.8–96.9% homology with Tibet/30/2007, Tibet/33/2007 and Tibet/bharal/2008, 97.6% with Sungri 1996, 92.4% with Nigeria/1976/1 and Nigeria/1975/1 and 89.3% homology with Côte d’lvoire/1989. Previously, across both East and North Africa, the circulation of lineage IV PPRV has only been reported in Sudan, Eritrea, Uganda, Egypt and Morocco (Banyard et al., 2010; Kwiatek et al., 2011; Libeau et al., 2014). Lineage IV PPRV had also been recorded from Cameroon in 1997, the Central African Republic (CAR) in 2004 and in Nigeria in 2008 (Fig. 2). Multiple lineages have also been reported as being present on several other African countries such as Burkina Faso, Senegal and Nigeria (Table 1). In Ethiopia lineage III has been detected since 1994 along with lineage IV PPRV that has been record in 2010 in this study. As both lineage III (1971 isolate) and Lineage IV (2000 isolate) have been reported since 1971 and 2000, respectively in neighbouring Sudan, the presence of lineage IV virus in Ethiopia earlier than 2010 cannot be ruled out.

A neighbour-joining tree was constructed using 83 partial N gene sequences (255 nucleotide) from PPRV isolates reported across Africa (Fig. 2). The sequences available from 1968 to 2013 divided phylogenetically into four lineages. Lineage I was limited to Western Africa and has not been reported since 1994 (Batten et al., 2011). Lineage II is circulating in Western Africa (Nigeria, Ghana, Sierra Leone and Mali) having been reported sporadically in Nigeria, Ghana, Senegal, Sierra Leone, Mali and Mauritania between 1975 and 2012. Lineage III appears to be restricted to East Africa since initially being reported in Sudan in 1971, Ethiopia in 1994 and most recently in Uganda (2007) and Tanzania (2010 and 2013, Fig. 2) (Banyard et al., 2010). Within Africa, lineage IV was first reported in Cameroon in

Fig. 3. Neighbour-joining tree based on the partial PPRV F gene sequences showing the relationships between the African PPRV isolates. Scale bar indicates nucleotide substitutions per site. The Kimura 2-parameter model with percentage of replicate trees in which the associated taxa clustered together in the 1000 bootstrap replicates is shown next to the branches. The isolate sequenced in this study is indicated by arrow and those from Luka et al., 2012 by stars.
Emergence of Lineage IV PPR Virus in Ethiopia

M. Muniraju et al.

1997, in Sudan in 2000 and in the CAR and Eritrea in 2004 and 2002, respectively. Since then, lineage IV PPRV has been reported in Sudan up to 2009 and Eritrea in 2011. Here, we have demonstrated lineage IV virus reported from Ethiopia. During 2008, a devastating outbreak of PPRV was seen in northern Africa in Morocco caused by lineage IV PPRV (Muniraju et al., 2013) and later on in Egypt in 2010 (Sharawi and Abd El-Rahim, 2010), Algeria in 2010 (De Nardi et al., 2013) and in Tunisia (Soufen et al., 2014). Recent reports (2010–2013) of lineage IV PPRV circulation in Nigeria demonstrated virus circulation in Western Africa. Lineage IV PPRV has also been reported in 2011 from the Gabon, on the west coast of Central Africa.

As Lineage IV virus has been reported from East-, North-, West- and Central Africa, it may also be the cause of disease in other areas where recent outbreaks have been reported (Table 1) without genetic characterization. Further, it is clear that more than one lineage is circulating in some of the African countries, particularly in East Africa in Sudan (Lineage III and IV), Ethiopia (Lineage III and IV) and Uganda (Lineage III and IV) and in West Africa in Nigeria lineage II and IV (Table 1). Although Lineage I and II both were reported in Senegal and Burkina Faso, currently only lineage II appears to be present in Senegal. Lineage I PPRV (Ivory Coast/1989) has not been seen in Africa or elsewhere since 1994 (Batten et al., 2011). However, by analysing partial F gene sequence data, Luka et al. (2012) reported the circulation of Lineage I, II and IV in Uganda during outbreaks in 2007 and 2008. Using the existing data and that reported here a reanalysis of these data have shown that the sequences reported in 2007 and 2008 belong to lineage III and IV (Fig. 3). This also corroborates well with the one virus sequence reported in 2007 from Uganda as lineage III (Banyard et al., 2010). Two viruses (Ugn-LF1-07 HQ407501 and Ugn-LF3-07 HQ407502) reported as lineage II by Luka et al. (2012) grouped with viruses from lineage III in the present analysis. Similarly, three viruses (Ugn-14-08 HQ407497, Ugn-18-09 HQ407499 and Ugn-16-09 HQ407498) phylogenetically classified as belonging to lineage I by Luka et al. (2012) clustered with isolates from lineage IV in the present analysis. From the neighbour-joining tree of the available N gene sequence data for PPRV (Fig. 2), it appears that lineage IV PPRV has become the dominant strain circulating in many regions. From the phylogenetic analysis, lineage IV appears to differentiate into four clusters. Ethiopia 2010 (reported in this study) belongs to cluster one (C1) along with East and North African viruses from Sudan (2004–2009), Egypt (2010–2012), Morocco (2008), and Eritrea (2002–2011). Viruses from Morocco (2008), the CAR (2004) and some of the Eritrea viruses were distinct from the reported virus Ethiopia 2010 whilst viruses from closely neighbouring countries (many viruses from Sudan and 2005 virus from Eritrea) were genetically closer. The lineage IV PPRV circulating in Nigeria between 2010 and 2013, Gabon (2011) and Sudan (2008) form a second cluster (C2). Cluster three was formed by viruses present in the Sudan (2000), and cluster four includes isolates from Cameroon (1997) and a single isolate from the Sudan (2000).

Comparing between the nucleotide sequence similarity of partial N gene sequence data of isolates from C1 viruses with Ethiopia 2010, 99.2% similarity was found with many viruses from Sudan and only one virus (2005) from Eritrea whereas 93.7–98.8% nucleotide similarity was seen for other C1 viruses (Table 2). From this analysis, it suggests that the Ethiopia 2010 outbreak may be due to the trans-

| Country | Isolates                                                        | Sequence similarity (%) |
|---------|----------------------------------------------------------------|-------------------------|
| Sudan   | HQ131933 Sudan Kuku Khartoum                                   | 99.2                    |
|         | KHSUD1 Sheep 2000                                               |                         |
|         | HQ131935 Sudan Kassala KSUD1 Camel 2004                         | 99.2                    |
|         | HQ131934 Sudan Atbara NSUD1 Camel 2005                         | 99.2                    |
|         | HQ131937 Sudan Tambool BNSUD1 Camel 2006                        | 99.2                    |
|         | HQ131939 Sudan Kassala KSUD Camel 2007                         | 99.2                    |
|         | HQ131942 Sudan Atbara NSUD Camel 2008                           | 99.2                    |
|         | HQ131922 Sudan Abudaelaq KSUD Sheep 2008                        | 99.2                    |
|         | HQ131945 Sudan Rabak BNSUD Sheep 2009                           | 99.2                    |
|         | HQ131932 Sudan Dongola NSUD Goat 2009                           | 99.2                    |
|         | HQ131931 Sudan Ed Damar NSUD Sheep 2008                         | 98.4                    |
| Egypt   | JN202924 Egypt Goat Nov 2010                                    | 98.8                    |
|         | JN202926 Egypt Goat Nov 2010                                    | 98.8                    |
|         | JN202925 Egypt Goat 2010                                         | 98.4                    |
|         | JX312807 Egypt Ismailia2 Sheep 2012                             | 98.4                    |
|         | JN202925 Egypt Goat Nov 2010                                    | 98.4                    |
| Eritrea | JX398130 Eritrea Guliee Goat Jan 2005                            | 99.2                    |
|         | JX398127 Eritrea May Harish Goat Jan 2011                        | 98.6                    |
|         | JX398128 Eritrea Hakum Goat Mar 2005                            | 95.6                    |
|         | JX398129 Eritrea Goat Jan 2011                                   | 94.5                    |
|         | JX398126 Eritrea Gahtelay Sheep Dec 2003                         | 94.1                    |
|         | JX398131 Eritrea Torat Goat Jan 2005                            | 94.1                    |
|         | JX398132 Eritrea Goat May 2002                                   | 93.7                    |
| Morocco | HQ131927 Morocco 9 Sheep 2008                                    | 98.8                    |
|         | HQ131923 Morocco 1 Sheep 2008                                   | 97.6                    |
|         | KCS94074 Morocco Goat 2008                                      | 96.8                    |
|         | HQ131924 Morocco 2 Sheep 2008                                   | 96.8                    |
| Congo   | Congo Brazaville 2006                                           | 96.8                    |
| CAR     | HQ131962 CAR Goat 2004                                           | 96.4                    |
boundary spread of PPRV between the neighbouring countries. This can occur readily in areas where land borders are ill defined, and the movement of animals between countries is unrestricted.

In conclusion, from the available gene sequences including the data generated in this study, only lineage III and IV are circulating in East Africa, lineage I (Ivory Coast/1998) appears restricted to western Africa and currently not in circulation, lineage II (Nigeria/1975) appears limited to western and central Africa with recent incursions into the western part of North Africa. Further, lineage IV PPRV appears to have extended its distribution following initial detection in Africa in 1997. Furthermore, in the absence of reports of the presence of other lineages it appears to have emerged as a dominant lineage of the virus, being detected in numerous regions where historically other lineages have predominated. However, limitations exist with our understanding of the epidemiology of PPRV. Further, genetic analyses are required to understand the epidemiology of the virus across Africa as although the eradication of rinderpest has led to a renewed interest in PPRV, the ability to genetically type viruses is still lacking in many regions where the virus remains endemic. As such where genetic data do exist, such information is likely to merely represent a snapshot of the true situation regarding this virus and its distribution across the developing world.

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