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Short communication

Emergence of peste des petits ruminants virus lineage IV in Ismailia Province, Egypt

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ABSTRACT

Peste des petits ruminants (PPR) is an acute, highly contagious fatal disease of small ruminants characterized by high fever, ocular and nasal discharge, pneumonia, erosive stomatitis and severe enteritis that ultimately results in high mortalities. Peste des petits ruminants virus (PPRV) is widely distributed and endemic in several African, middle eastern and south Asian countries and it poses a threat to European countries. Egyptian veterinary medical authorities stated that Egypt is free from PPRV and the only measures for disease control are test and slaughter of infected population to maintain the free status. The aim of our investigation was to detect PPRV in Ismailia province as an indicator of the infection status in Egypt and perform molecular characterization of the emerging virus to gain insight into the origin of circulating virus. A total of 40 representative clinical samples, from a single goat case and goat flock in 2010 and sheep flock in 2012, were tested for PPRV by RT-PCR. About 21 (52.5%) samples were positive. The phylogenetic analysis of the detected viruses revealed circulation of PPRV lineage IV. The circulating viruses are closely related to Sudanese and Saudia Arabian strains with nucleotide identity ranged from 99.2% to 99.6%, respectively. Also, it is closely related to Moroccan 2008 viruses with identities ranged from 97.6% to 98%. Epidemiological investigation at the national level is recommended for monitoring PPRV spread and implementing an appropriate control program.

1. Introduction

Peste des petits ruminants virus (PPRV), a member of the genus Morbillivirus in the family Paramyxoviridae (Gibbs et al., 1979), is the causative agent of peste des petits ruminants (PPR) which is an acute highly contagious fatal disease of small ruminants. The disease is characterized by high fever, ocular and nasal discharge, pneumonia, erosive stomatitis and severe enteritis that ultimately results in high mortalities (Abu-Elzein et al., 1990). The viral genome is a linear, single stranded, non-segmented, negative sense RNA composed of approximately 15,948 nucleotides encoding six structural (N, P, M, F, H and L) and two nonstructural (C and V) proteins (Bailey et al., 2005).

PPR is a notifiable transboundary disease that threatens small ruminant production in many developing countries (Banyard et al., 2010). The disease was first described in sheep and goats of West Africa in 1940 (Gargadennec and Lalanne, 1942). Since that time, it has continued to spread in Africa, Asia and European part of Turkey (Banyard et al., 2010).

Although PPRV has been occurring as one strain or serotype (Shaila et al., 1996), genetic classification, based on partial sequencing of the fusion (F) protein gene (Ozkul et al., 2002) and/or the 3′ end of the nucleoprotein (N) gene (Kwiatek et al., 2007), has identified 4 distinct lineages (lineages I, II, III and IV) and is considered an effective tool to survey virus spread worldwide. Lineage I is geographically distributed in old 1970s west African strains and recently central African strains; Lineage II in west Africa in Ivory coast, Guinea and Burkina Faso strains; Lineage III in east African, Sudan, Yemen and Oman strains; and lineage IV in south east Asian, The Arabian Peninsula, middle east, northern and central African strains (Banyard et al., 2010). Lineage classification of PPRV based on N gene sequence analysis is more suitable for phylogenetic distinction between closely related circulating viruses and provides a better picture of PPRV molecular epidemiology because this region is comparatively more divergent. (Kwiatek et al., 2007; Kerur et al., 2008).

In Egypt, PPRV was isolated for the first time in 1987 and 1990 (Ismail and House, 1990). Since that time, all the PPRV reports have

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been based on the detection of virus antibodies (Mouaz et al., 1995; Abd El-Hakim, 2006; Abd El-Rahim et al., 2010). Egypt was officially free of PPR according to the OIE until the outbreaks at sheep farms in Ismailia and Cairo provinces in 2012 (http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=13505). Samples from Ismailia outbreak are included in the current study.

Therefore, the aim of this study was to detect PPRV in Ismailia province, as an indicator of the infection status in Egypt and lineage classification of the emerging virus to determine its origin.

2. Material and methods

2.1. History of the outbreaks and clinical examination

In November 2010, cases consistent with PPR were noted in Ismailia province, Egypt during routine examination of a single diseased goat and unrelated fifteen head goat flock with a history of respiratory disease, diarrhea and high mortalities. Furthermore, in May 2012, we examined seven hundred head sheep flock with the same history.

2.2. Sample collection, processing and RNA extraction

A total of 40 representative clinical samples including whole blood, oral necrotic epithelium and fecal material were collected, from a single goat case and goat flock in 2010 and sheep flock in 2012, and sent to faculty of Veterinary Medicine, Suez Canal University, Egypt. Total RNA as extracted from 200 mg of oral necrotic tissue, 300 μl whole blood and 300 μl of 10% fecal suspension using RNA pure high purity total RNA extraction kit (Bioteke Corporation, China) according to the manufacturer's instructions.

2.3. Reverse transcription and polymerase chain reaction (PCR)

Five microliter of extracted RNA was reverse transcribed to cDNA using RevertidAid First Strand cDNA Synthesis Kit (Thermo scientific) according to the manufacturer's instructions. The reverse primer of N gene (NP4) was used in the cDNA synthesis reaction. A 350 bp fragment of N gene was amplified by PCR using NP3 (Forward 5′-TCTCGGAAATCGCC TCACAGACTG-3′) and NP4 (Reverse: 5′-CCTCCTCCTGGTCCTCCAGAATCT-3′) according to Couacy-Hymann et al. (2002).

2.4. Sequencing

Ten representative N gene specific PCR products were gel purified using PCR product/gel purification kit (BioTeke Corporation, China) according to the manufacturer and then sequenced using an automated DNA sequencer ABI3730XL (Solgent Co. Ltd. Korea).

2.5. Nucleotide sequences alignment, percentage identity level and phylogenetic analysis

The nucleotide sequences of N gene were aligned with other representative PPRV sequences available in GenBank. Comparative alignment was performed using Clustal W of MegAlign software (Lasergene version 7.2). Furthermore, sequence identity and divergences were calculated using MegAlign software. A phylogenetic tree was constructed by the neighbor-joining method employing the Kimura 2-parameter model in MEGA 6 software (www.megasoftware.net) by aligning 255 bp of representative lineage specific nucleotide sequences. The tree topology was evaluated by 1000 bootstrap replicates.

3. Results

3.1. Clinical picture

The clinical examination of diseased animals revealed high fever (41–42 °C), depression, anorexia, mucopurulent ocular and nasal discharges, conjunctivitis, erosive and necrotic stomatitis, coughing and diarrhea/or constipation. The morbidity, mortality and case fatalities are shown in (Table 1).

3.2. Detection of PPRV by RT-PCR

Of 40 samples tested by RT-PCR for the N gene, a total of 21 (52%) samples were positive (Table 2). A 350 bp fragment was amplified from positive samples.

3.3. Percentage identity and phylogenetic analysis

Nucleotide sequence analysis of 10 N gene PCR products revealed 3 different sequences. The obtained sequences were submitted to GenBank (accession numbers: JN202923, JN202924 and JX312807).

The overall nucleotide similarity between the three PPRV N genes detected in Ismailia province was 98.8–99.2%. The percentage of nucleotide identity to Sudanese and Saudi Arabian viruses ranged from 99.2% to 99.6%. Furthermore, the nucleotide identity to Moroccan viruses was ranged from 97.6% to 98%.

Based on the phylogenetic tree topology, our strains were clustered within the lineage IV with close relation to Sudan and Saudi Arabia strains (Fig. 1).

Table 1
Morbidity, mortality and case fatality rates of PPR.

Flock	Year	Total No. of animals	Morbidity rate (%)	Mortality rate (%)	Case fatality rate (%)
Goat flock	2010	15	93.3% (14/15)	93.3% (14/15)	100% (14/14)
Sheep flock	2012	700	21.4% (150/700)	21.4% (150/700)	100% 150/150

Table 2
Detection of PPR on clinical samples by RT-PCR.

Animal species	Year	Total number of tested animals	Total number of collected samples	Clinical samples		
				Necrotic oral epithelium	Whole blood	Fecal samples
Goat case	2010	1	2	1/1	1/1	–
Goat flock	2010	12	18	4/6	2/6	3/6
Sheep flock	2012	15	20	5/10	2/5	3/5
Total		28	40	10 (58.8%)	5 (41.7%)	6 (54.5%)