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NUCLEOTIED SEQUENCING AND PHYLOGENIC ANALYSIS OF FUSION (F) EPITOPE FOR EGYPTIAN PESTES DES PETIT RUMINANTS VIRUS (PPRV) PREDICTING UNIQUE CRITERIA STATED AS EGYPT 2009

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Sharawi[1], S.S.A. and Abd El-Rahim[2], I.H.A.*

ABSTRACT

Peste des petit ruminants virus (Abd El-Rahim[2]) was previously isolated and serologically identified from suspected outbreaks of PPR among sheep and goats in Qalyubia Province, Egypt at 2006. In this study six of this PPRV isolates were confirmed by reverse transcriptase-PCR (RT-PCR) using primer set for fusion protein (F) epitope, then cDNA were send to Institute of Animal Health Pirbright, England to analyze for their nucleotide sequences of this F protein gene and phylogenic analysis properties, by matching with other reference world recorded isolates. The gene sequenced a 322 nucleotide cDNA fragment of the fusion protein gene was obtained. The isolates showed unique phylogenic analysis had not previously been identified internationally and defined as Egypt 2009. In addition; by using the obtained sequences generated from this gene coding protein; Egyptian PPRVs were grouped phylogenetically belongs to lineage IV. To the author knowledge, this is the first report describing the fusion protein (F) gene sequence, phylogenic analysis and lineage typing of Egyptian isolates of PPRVs.

Keywords: Peste des petit ruminants virus; PCR; Epitope; F gene; Sequencing; Phylogeny.

[1] Department of Virology, Faculty of Veterinary Medicine, Banha University, 13736 Moshtoher, Banha, Egypt.
[2] Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt.

INTRODUCTION

The morbillivirus genus consists of a group of single stranded, negative-sense RNA viruses belonging to the family Paramyxoviridae of order Mononegavirales **(Murphy *et al.*, 1999 and Westover and Hughes 2000)**. The genus was thought to consist of four major members; measles virus (MV), rinderpest virus (RPV), peste-des-petits ruminants virus (PPRV) and canine distemper virus (CDV). Virus members of this group have six structural proteins: the nucleocapsid protein (Np), which encapsulates the virus genomic RNA, the phosphoprotein (P), which associates with the polymerase protein (L for large protein), the matrix (M) protein, the fusion (F) protein and the haemagglutinin (H) protein. The matrix protein, intimately associated with the internal face of the viral envelope, makes a link between the nucleocapsid and the virus external glycoproteins: H and F, which are responsible, respectively, for the attachment and the penetration of the virus into the cell to be infected **(Gibbs *et al.*, 1979 ; Diallo, 2003 and Diallo *et al.*, 2007)**. Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants. This disease is characterized by high fever, oculonasal discharge, pneumonia, necrosis and ulceration of mucous membrane and inflammation of gastrointestinal tract, leading to severe diarrhea. PPR occurs in an endemic form, it may have morbidity of 80-90% and mortality between 50 and 80 % **(Lefevre and Diallo, 1990)**. PPR was first described in Côte d'Ivoire **(Gargadennec and Lalanne, 1942)** and thereafter, it has been recognized in many of the sub-saharan countries that lie between the Atlantic ocean and the red sea **(Lefevre and Diallo, 1990)**. The affected area extends north to Egypt and south to Kenya, in Eastern-Africa, and to Gabon in Western-Africa. PPR has not been recognized in most of Northern and Southern-Africa. PPR is present in nearly all middle eastern countries up to Turkey **(Furley *et al.*, 1987; Lefevre *et al.*, 1991; Taylor, 1990 and Ozkul *et al.*, 2002)**. It is also widespread in India and southwest Asia **(Shaila *et al.*, 1989)**. At present; PPR occurs in most African countries situated in a wide belt between the Sahara and equator, the middle east (Arabian

peninsula, Syria and Jordan) and the Indian subcontinent. Outbreaks of PPR are now known to be common in India, Nepal, Bangladesh, Pakistan and Afghanistan (**Abdollahpour *et al.*, 2006**). The Existence of PPR has been recognized in Egypt in Qalyubia province since 2006, increased to an alarming level involving newer areas (**Abd El-Rahim *et al.*, 2010**). PPRV is genetically grouped into four distinct lineages (I, II, III and IV) on the basis of partial sequence analysis of fusion protein (F) gene; despite the fact that only a single serotype of PPRV has been reported (**Shaila *et al.*, 1996; Dhar *et al.*, 2002 and Ozkul *et al.*, 2002**). This classification of PPRV into lineages has broadened the understanding of the molecular epidemiology and worldwide movement of PPR viruses.

The objectives of our research were to confirm the detection of PPRVs isolated previously from suspected outbreaks of PPR among sheep and goats in Qalyubia Provence, Egypt by reverse transcription polymerase chain reaction (RT-PCR) assay, employing F gene based primer described by **Dhar *et al.* (2002)**. Furthermore, the genetic relationships between these PPRVs were investigated comparing the nucleotide sequences of the PCR products amplified from F gene with other PPRVs sequences available at Institute of Animal Health Pirbright (IAH-P), England.

MATERIALS & METHODS

Extraction of RNA from PPRV sample suspensions:

RNA of six field isolates of PPRVs; from sheep and goat of different sex and ages, previously isolated and serologically identified from PPR suspected animals in Qalyubia province, Egypt, during 2006 (**Abd El-Rahim *et al.*, 2010**); were extracted using QIAamp® Viral RNA Mini Kit (Qiagen®, Germany), following the manufacturer's instructions.

Reverse transcription (RT) :

Synthesis of cDNA was performed in 20 µl reaction using transcriptor first strand cDNA synthesis kit (Roche®, cat no. 04 374 012 001), according to the manufacturer procedures. Reverse transcription was carried out on dry block at 55 °C for 30 minutes followed by 85 °C for 5 minutes. RT products were cooled on ice and stored at -20 °C until use.

Polymerase Chain Reaction:

Based on sequences from highly conserved regions within the PPR virus genome **(Dhar *et al.*, 2002);** two specific PPR virus oligonucleotide primers F1b (F) (5\ - AGT ACA AAA GAT TGC TGA TCA CAG T - 3\) and F2d (R) (5\ - GGG TCT CGA AGG CTA GGC CCG AAT A - 3\ ) were used (TIB-MOL BIOL syntheselabor GumbHm Berlin, Germany). PCR was carried out in a final reaction volume of 25 µl using 200 µl capacity thin wall PCR tube with the following reagents: 3.00 µl cDNA template, 12.5 µl of PCR Master mix (MBI Fermentas) containing 0.05U/µl Taq DNA polymerase (recombinant) in reaction buffer, MgCl2 (4 mM) and dNTPS (0.4 mM of each), 1µl of forward and reverse primers (10 pmoles of each primer) and 7.5 µl of deionized water. Thirty five cycles of amplification was carried out using PCR reaction conditions as described by **Dhar *et al.* (2002)** as follows: 95 °C for 5 min., followed by 35 cycles at 94 °C for 30 sec., 50 °C for 60 sec. and 72 °C for 2 min., and a final extension step at 72 °C for 10 min. Ten microliters of the PCR products were resolved on a 2% agarose gel stained with 0.5 lg/ ml ethidium bromide at constant 80 V for 30 min. in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light.

Nucleotide sequencing of RT-PCR products and phylogenetic analysis:

Six RT products of the positive PPRVs were sent to the Institute of Animal Health Pirbright, England, to analyze the nucleotide sequences of this F protein gene and phylogenic analysis properties by matching with other reference world recorded isolates.

RESULTS

The F gene PPRV primers (F1b-F2d) amplify the product size of 448 bp amplicons **(Figure 1).** The amplicons from all the six positive samples migrated similarly in the gel at respective locations. The predicted consensus sequence, 322 bp of the F gene of RT-PCR products for all examined PPRVs were:

ATG CTC TAT CAG TGA TAA CCA AGA ATG TAA GAC CAA TTC AAA CTC TGA CAC CTG GGC GTA GAA CTC GCC GTT TTG CTG GAG CTG TTC TAG CCG GAG TAG CAC TTG GAG TTG CGA CAG CCG CTC AGA

TAA CTG CAG GAG TCG CCC AGC TTG TCC CTT CCG TTC
TTC ATC AAT CAT TGA TGA ATA GAA TGT CAT GCG A.
ACT CCC AAG CAA TTG AGA
GTT TAA AAA CCA GTC TTG
AGA AGT CGA ATC AGG CAA
TAG AAG AAA TCA GAC TTG
CAA ATA AGG AGA CCA TAC
TAG CAG TAC AGG GCG TCC
AGG ATT ATA TCA ACA ATG

The inferred phylogenetic relationship between the isolates recovered in this research and other PPRVs was showed **(Figure 2).** The Egyptian PPRVs isolates showed unique phylogenic analysis belonged to PPRV lineage IV.

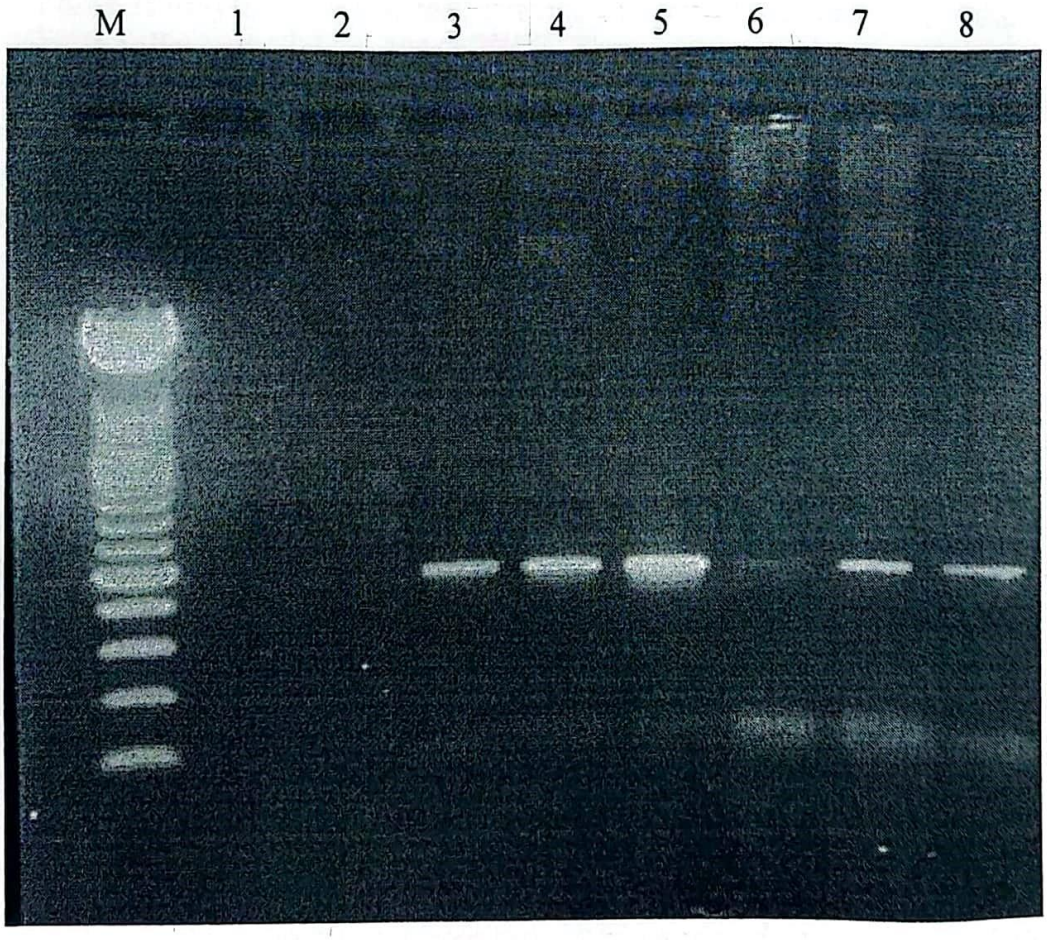

Figure 1. From left: Lane: 1-2 –ve control & 3-8 electrophoresis pattern of RT- PCR products for F-gene PPRVs, approximately at 448 bp, M : DNA ladder (100 bp).

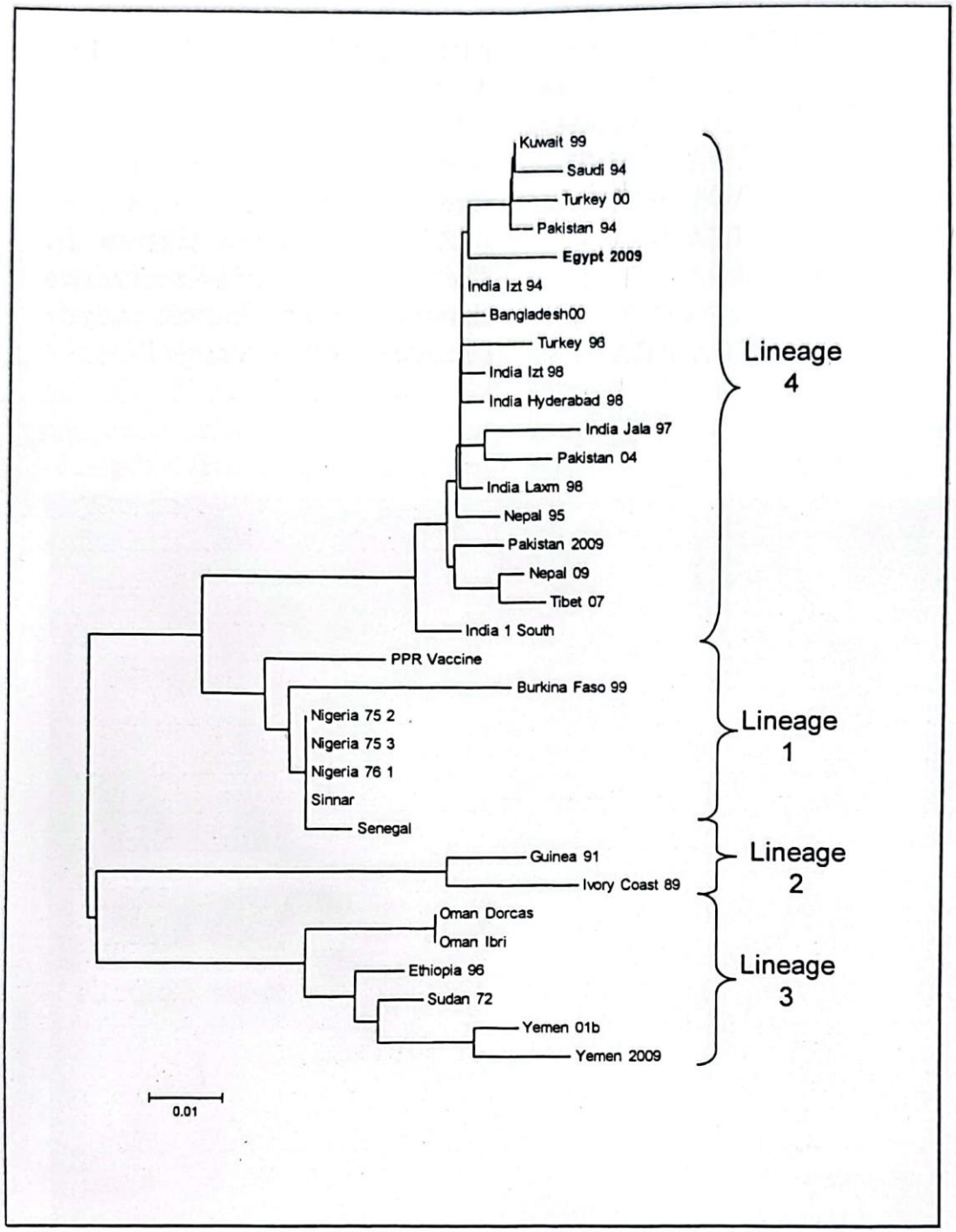

Figure 2. Phylogenetic analysis of nucleotide sequences from the amplified products of PPRVS fusion protein (F) gene with different lineage world widely, Egyptian PPRV was revealed as "Egypt 2009" that has unique criteria in lineage IV.

DISCUSSION

A highly sensitive PCR using F-gene primers (F1-F2) for the detection of PPRV was developed, give an amplification of a 372 bp region between positions 777 to 1148 nucleotides of F gene. It has become the most popular tool, so far for diagnosis, as well as molecular epidemiological studies of PPRVs **(Forsyth and Barrett, 1995; Shaila *et al.*, 1996)**. However, **Dhar *et al.* (2002)** noticed that in some samples, primer pair F1-F2 failed to amplify the target sequence and they described another F-gene primer pair (F1b-F2d), which amplifies a 448 bp fragment of the F-gene, encompassing the sequence amplified by primer pair F1-F2. Previous clinical symptoms of PPR was recorded, as well as isolation and serological identification of PPRV, were confirmed among migratory sheep and goat flock in Egypt during 2006 **(Abd El-Rahim *et al.*, 2010)**. In our study, six of this PPRV isolates were confirmed by reverse transcriptase-PCR using primer set for fusion protein (F) epitope, based on sequences from highly conserved regions within the PPRV genome; F1b and F2d according to **Dhar *et al.* (2002);** where an amplification of a 448 bp was showed. The obtained result confirmed the incidence of PPR in that outbreak at different age, sex and locations in Egypt in Qalyubia province,. The prevalence and distribution of PPRV in Turkey were recorded by RT-PCR using the same primer pair (F1b-F2d) **(Ozkul *et al.*, 2002)**. RT-PCR assay preclude the need for virus isolation and, because of the rapidity with which completely specific results could be obtained, the assay appeared to be the test of choice for PPRV detection **(Nanda *et al.*, 1996)**.

Although, there is only one serotype of the virus (Barrett et al., 1993), PCR in combination with nucleotide sequencing has made it the method of choice for molecular characterization of PPRVs **(Couacy-hymann *et al.*, 2002)**. There are four groups of PPRV phylogeny (lineages) on the basis of partial sequence analysis of the fusion (F) protein gene, in accordance of distinct geographical origin **(Shaila *et al.*, 1996)**. Lineage I and II are found exclusively in West Africa, whereas lineage III has been found in eastern Africa and Arabia. The fourth lineage is confined exclusively in the Middle East,

Arabia and Indian subcontinent **(Shaila *et al.*, 1996).** Except one isolate (TN92/1) from southern India, which belonged to lineage III, all Indian PPRV isolates identified so far, belonged to lineage IV only **(Nanda *et al.*, 1996; Dhar *et al.*, 2002).** To assess the genetic similarity and divergence among our field PPRVs of Kalubia province, as well as their relatedness to the previously described isolates of other PPRV isolates around the world, the F gene sequence data were analyzed. The isolates showed unique phylogenic analysis had not previously been identified at the international level and defined as Egypt 2009. In addition; by using the obtained sequences generated from this gene coding protein; Egyptian PPRVs were grouped phylogenetically belongs to lineage IV. The obtained sequence from our PPRVs isolates were similar to PPRV lineage 4, which originated in the Middle East, Arabia, and southern Asia **(Ozkul *et al.*, 2002).** Apparently, no reports regarding specific application of phylogenic analysis for characterization of Egyptian PPRVs was found in literature, so the obtained results may be spot light on the nature of circulating PPRVs among sheep and goat flocks in Egypt and most suitable viral seed for PPRV vaccine candidates. To the author knowledge, this is the first report describing the fusion protein (F) gene sequence, phylogenic analysis and lineage typing of PPRVs Egyptian isolates.

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