Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 5, 895-898

Turkey pox virus characterization from recurring infection in Egypt

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ARTICLE INFO

Recieved: 16 February 2024

Accepted: 25 April 2024

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Keywords:

Turkey pox Virus
PCR
Phylogenetic analysis
Embryonated Chicken Egg

ABSTRACT

Poxvirus infections of avian species worldwide are caused by viruses of a single genus (Avipoxvirus), they belong family *Poxviridae*. The Turkey pox virus (TKPV) is one of the most serious illnesses and a major source of financial losses for the developing turkey industry. Even though the fowl pox virus vaccine is used to prevent the disease, TKPV still creates significant economic difficulties, therefore isolating and adapting the virus is crucial for the development of homologous vaccines that will manage the illness. In the summer of 2021, the TKPV disease spread to numerous farms inside the Giza Governorate. Ten samples of skin lesions from various farms were gathered and given labels. In accordance with Koch's postulates, the collected samples were ground, homogenized, and centrifuged to extract supernatant, which was then injected into healthy birds to confirm the disease symptoms and susceptibility. For more confirmation the virus was isolated on embryonated chicken egg (ECE) through chorio-allantoic membrane (CAM) route at 10 day old after 5 to 6 days of inoculation the pock lesion appeared A fragment of the P4b gene coding for DNA polymerase core protein of TKPV was amplified by PCR then sequenced. A newly discovered isolate of field-isolated TKPV was revealed by phylogenetic analysis to have high similarity (between 96% and 100%) with the sequences that have been published. The new isolate of Turkey pox virus strain MKP 334 core protein (P4b) gene (with Accession No. MZ983434.1 in GenBank) has 100% to MG787222.1 Turkey pox virus isolate TurPVIR12 p4b gene and differ from the only isolated strain of TKPV that isolated at 2020 in Mansoura (MT219997.1 Turkey pox virus strain mans17T P4b 2020 EGYPT) but they have 99.6 identity.

Introduction

Turkey farming was previously done in small, haphazard numbers, but in recent years, it has started to grow in size, drawing significant attention to the diseases that affect turkeys in Egypt. Canary pox virus (CNPV), fowl pox virus (FPV), ostrich pox virus (OSPV), penguin pox virus (PEPV), pigeon pox virus (PGPV), quail pox virus (QLPV), and Turkey pox virus (TKPV) are examples of highly contagious virus diseases that affect all birds. Each of these viruses is a member of the genus Avian pox viruses, which is a subfamily of the *Poxviridae* family called *Chordopoxvirinae* (ChPV) (Lebdah *et al*., 2019).

The double-stranded (ds) DNA genome of APVs is between 260 and 365 kilobases in length and contains more than 250 putative genes. APVs and ChPV share common regulatory elements for gene expression during replication in the cytoplasm of the cell. Compared to other ChPV members, the genomes of APVs show extensive gene families, novel host range genes, and large-scale genomic rearrangements (Mosad *et al*., 2020).

This disease in Turkey has garnered international attention. Brunett (1934) reported the first instance of the Turkey pox virus in a flock of turkey at the Veterinary College in New York. The illness has been documented in India and other nations, and despite appropriate management and medical care, it has caused significant financial losses (Singh *et al*., 2003). In contrast to the diphtheritic or combined form, mortality in the cutaneous form is typically low; however, this depends on the agent's virulence, the host's susceptibility, and environmental factors. (Haydar *et al*., 2017).

Avipoxvirus is still active in backyard and commercial management systems in Egypt and throughout Africa. A possible hazard and source of infection for domesticated poultry species is the free-roaming and wild birds. The molecular analysis gives us important details about the taxonomy and evolution of APV that is found throughout Africa (Lebdah *et al*., 2019).

The Turkey pox virus and the fowl-pox virus have different genomic makeups, according to the RE analysis so the need of homologous vaccine using TKPV isolate is very important (Singh *et al*., 2007). Due to many economic losses caused by TKPV nowadays updating of the molecular knowledge of the virus is very important to facilitate the control of the disease.

Materials and methods

Samples

The virus samples were extracted from the pox virus that was circulating in Turkey, where both the cutaneous and diphtheritic forms of the infection were observed. In the summer of 2021, the TKPV disease spread to numerous farms within the Giza Governorate. Ten samples of skin lesions from various farms were gathered and given labels. Using sterile sand as an abrasive, the scab-covered lesions from the afflicted flocks were collected aseptically and ground in PBS (pH 7.2). Homogenization of the triturated sample resulted in a 10% (w/v) suspension in PBS (Kabir *et al*., 2015).

Isolation on embryonated chicken eggs (ECE)

10-day-old ECEs were used to isolate TKPV using the chorio-allantoic membrane (CAM) method. The embryos were incubated for 5 to 6 days, and then they were refrigerated for 1 to 2 hours at 4 to 8 degrees Celsius. The harvested CAM was utilized to prepare the inoculum.

ISSN: 2090-6277/2090-6269/

Experimental injection in turkey

In accordance with Koch's postulates, the sample was centrifuged, the supernatant was collected, and injected into a healthy turkey. Using the Wing Web Route, 1 ml (Walker *et al*. 2006).

DNA extraction and PCR

The sample's entire genomic makeup was extracted and purified in accordance with the manufacturer's instructions using Thermo Scientific's GeneJet Genomic DNA Purification Mini Kit. GeneAmp Polymerase Chain Reaction (Creacon, Thermo cycler, Holand) system cycler was used to amplify specific DNA. P4b primers were used in the PCR for the amplified genomic DNA in accordance with Puro *et al.* (2012), as listed in Table 1.

The cycling conditions for the 50 µl PCR reactions were as follows: 5 minutes of initial denaturation at 94°C, 35 cycles of denaturation at 94°C for 45 seconds, 1.5 minutes of annealing at 48°C, 2 minutes of elongation at 60°C, and a final extension at 60°C for 10 minutes. An agarose gel was used to elute particular DNA bands. Using the E.Z.N.A.®Gel Extraction Kit (D2500-01, Omega BIO-TEK, USA), the PCR products that were produced were purified. The ABI PRISM® 3100 Genetic Analyzer (Micron-Corp. Korea) was used for sequence analysis.

Data analysis

The PCR products were sent straight to be sequenced, and the output was aligned for its homology Gel documentation system (Geldoc-it, UVP, England). Totallab analysis software (Ver.1.0.1) was used to apply the results for data analysis. In order to verify the identity of aligned sequences, BLAST analysis was performed on the NCBI website (http://www.ncbi.nlm.nih.gov/webcite). By using the Pairwise Distance method and the Clusteral W software analysis (www.ClusteralW.com). The nucleotide sequences were also compared with TKPV virus isolates sequences available in the GenBank.

Results

Isolation of TKPV on ECE through viral inoculation via CAM injection, resulting in the development of a Pock lesion on CAM. After three blind passages, the pock lesion appears at the injection site in all collected samples, and after five passages, the lesion diffuses throughout the CAM as tiny, diffused pocks with a diameter of roughly 2-3 mm, as shown in (Fig. 1).

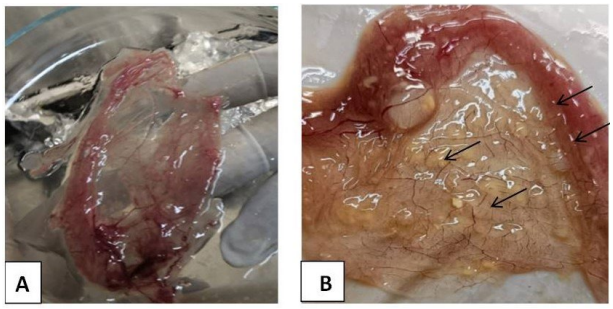

Fig. 1 A. Control ECE the CAM appears clear without any changes. B. Pock lesion appearance on CAM (small, diffused pocks about 2-3 mm diameter).

After one week, the healthy turkey that had been injected showed tiny nodules at the injection site that grew larger and spread throughout the body. Three weeks later, a scab appeared, and after five to six weeks, the skin lesion fell off.

The phases of TKPV lesion progression following virus injection into healthy birds are displayed in Fig. 2.

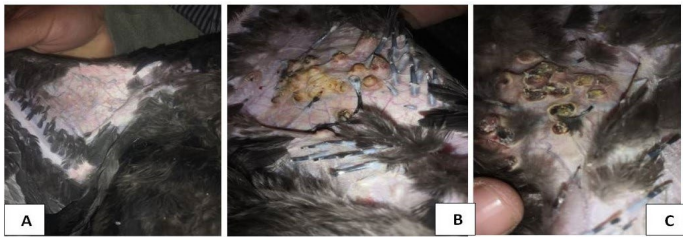

Fig. 2. Stages of lesion progression of TKPV. A) Small nodule appears at the site of the injection after the 1st week. B) The nodule proliferated after three weeks. C) The scab and enlarged after 5-6 weeks.

PCR was used to confirm the existence of TKPV in the isolated material, and a 578 bp P4b gene product was amplified, matching the description provided by (Luschow *et al*., 2004). The result of amplification of the P4b gene appears clearly in Fig. 3.

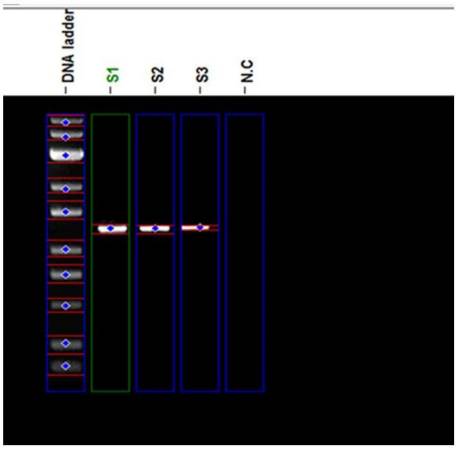

Fig. 3. Computerized detection for P4b gene (~578 bp) fragments of the samples collected from infected birds. S1: Egg Isolate virus…S2: Isolated virus from experimental turkey. S3 Standard TKPV.

The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Maximum Composite Likelihood model. This analysis involved 31 nucleotide sequences. Codon positions included the $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 496 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 as shown in Table 2, and Fig. 4.

Discussion

TKPV is a serious disease that affects Egyptian turkey flocks, resulting in significant financial losses due to skin destruction, weight loss, and decreased egg production (Tripathy, 1984). The Turkey pox virus and the fowl-pox virus have different genomic makeups, according to the RE analysis (Singh *et al*., 2007).As well as variation in the physico-chemical properties (Singh *et al*., 2003) and phylogenetic analysis (Jarmin *et al*., 2006) It has been established that TKPV and FPV are not the same. However, cross-protection studies have now demonstrated that TKPV is distinct from other avipox viruses (Villegas, 1998) and this helps the identification of the Turkey pox virus that was isolated from a wild outbreak in farming birds. (Singh *et al*., 2007).

Isolation of TKPV on ECE through viral inoculation via CAM injection, resulting in the development of a Pock lesion on CAM. tiny, diffused

Table 1. specific Primer sequence of P4b gene.

Gene	Sequences	Target fragment size	Reference
P4b	Forward: 5'-CAGCAGGTGCTAAACAACAA-3 Reverse: 5'-CGGTAGCTTAACGCCGAATA-3'	578 bp	*Puro et al. (*2012)

Table 2. Estimates of Evolutionary Divergence between Sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
[1]																															
[2]	0.01																														
[3]	0.00	0.00																													
[4]	0.01	0.00	0.00																												
[5]	0.01	0.00	0.00	0.00																											
[6]	0.01	0.00	0.00	0.00	0.00																										
[7]	0.01	0.00	0.00	0.00	0.00	0.00																									
[8]	0.01	0.00	0.00	0.00	0.00	0.00	0.00																								
[9]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00																							
[10]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																						
[11]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																					
[12]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																				
[13]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																			
[14]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																		
[15]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																	
[16]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																
[17]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10															
[18]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10														
[19]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10												
[20]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.00	0.00	0.10											
[21]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10										
[22]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.00	0.00	0.10	0.00	0.10										
[23]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.00	0.00	0.10	0.00	0.10	0.00									
[24]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.00	0.00	0.10	0.00	0.10	0.00	0.00								
[25]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10							
[26]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10	0.00						
[27]	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10	0.00	0.00					
[28]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10	0.00	0.00	0.00				
[29]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10	0.00	0.00	0.00	0.00			
[30]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10	0.00	0.00	0.00	0.00	0.00		
[31]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.10	0.00	0.10	0.10	0.10	0.00	0.00	0.00	0.00	0.00	0.00	

[1]#MZ983435.1:1485Turkey poxvirus_strain_MKP1230core_protein (P4b)_EGYPT_2021
[2] #MT219997.1_Turkey pox_virus_strain_mans17T_P4b_2020_EGYPT
[3]#MG787223.1:4484_Turkey pox_virus_isolate_TurPVIR13_P4b_(p4b)_gene_IRAN_2019
[4]#MN542415.1_Fowlpox_virus_isolate_Sharkia2017/VSVRI_core_protein_(P4B)_gene_partial_cds
[5] #MN708968.1_Fowlpox_virus_strain_VSVRI_EGYPT
[6] #MN542415.1_Fowlpox_virus_isolate_Sharkia2017/VSVRI_EGYPT
[7]#MW147745.1_Fowlpox_virus_isolate_FWPVH/Egypt/2018_4b_core_protein_(P4b)_gene_partial_cds
[8]#GU108504.1_Fowlpox_virus_strain_AT_Altenmarkt_Tr./68/5/2008_4b_core_protein_(4b)_gene_partial_cds
[9]#MW147745.1_Fowlpox_virus_isolate_FWPVH/Egypt/2018_4b_core_protein_(P4b)_gene_partial_cds
[10]#KU947037.1_Fowlpox_virus_isolate_FWPV_KFS_major_core_protein_(fpv167)_gene_partial_cds
[11] #OR027039.1_Fowlpox_virus_isolate_FP/EG/ISMAILIA-2_P4B_gene_partial_cds
[12] #OR027040.1_Fowlpox_virus_isolate_FP/EG/Sharqia-3_P4B_gene_partial_cds
[13] #OR027040.1_Fowlpox_virus_isolate_FP/EG/Sharqia-3_P4B_gene_partial_cds
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[17]#MW602950.1_Pigeonpox_virus_isolate_PPVNV1_P4b_(4b)_gene_partial_cds
[18]#MW602950.1_Pigeonpox_virus_isolate_PPVNV1_P4b_(4b)_gene_2011_EGYPT
[19]#MN708968.1_Fowlpox_virus_strain_VSVRI_core_protein_(P4b)_gene_partial_cds
[20]#MW602950.1_Pigeonpox_virus_isolate_PPVNV1_P4b_(4b)_gene_partial_cds
[21]#MW147745.1_Fowlpox_virus_isolate_FWPVH/Egypt/2018_4b_core_protein_(P4b)_gene_partial_cds
[22] #MN892361.1:15-456_Pigeonpox_virus_isolate_PPLH_4b_gene_partial_cds
[23]#MG787222.1:6447_Turkey pox_virus_isolate_TurPVIR12_P4b_(p4b)_gene_partial_cds
[24]#LN795891.1:1417_Fowlpox_virus_partial_FPV167/CNPV240_gene_France_2017_strain_FWPV/1965
[25]#MW686010.1:1578_Turkey pox_virus_strain_TKPV/BAU/Dinajpur/2017_P4b_core_protein_(P4b)_gene_partial_cds
[26]#MW686009.1:7584_Turkey pox_virus_strain_TKPV/BAU/Mymensingh/2017_P4b_core_protein_(P4b)_gene_partial_cds
[27]#DQ873808.1:Turkey pox_virus_4b_core_protein_gene_partial_cds_INDIA.2016.
[28]#MG787214.1:7487_Fowlpox_virus_isolate_FPVIR4_P4b_(p4b)_gene_IRAN_2018
[29] #MK651854.1:25-505_Fowlpox_virus_strain_v852-5_4b_core_protein_gene_BRASIL_2019
[30]#MK651852.1:22502_Fowlpox_virus_strain_USP852_4b_core_protein_gene_BRASIL_2019
[31] #KY464130.1:19-499_Fowlpox_virus_isolate_SG/EHI-SC02/Feb2016_virion_core_protein_Singapore

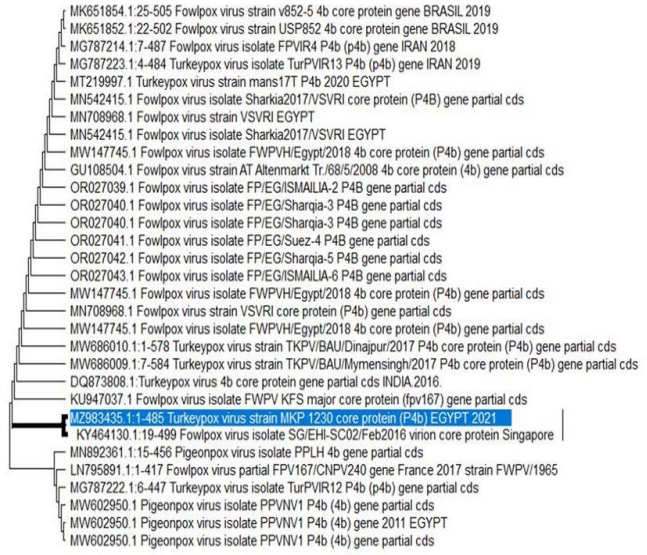

Fig. 4. Phylogenetic tree for P4b gene Sequence for Isolated virus. Turkey pox virus strain MKP 1230 core protein P4b EGYPT 2021 (accession number Z983435.1 in GenBank) is 100% similar to Turkey pox virus isolate TurPVIR13 P4b gene IRAN 2019 and differs slightly from the TKPV isolate that was isolated in Mansoura in 2020 (MT219997.1 Turkey pox virus strain mans17T P4b 2020 EGYPT), the first Egyptian TKPV isolate to be submitted to GenBank. The TKPV isolate described in this paper is the second one from Egypt that has been added to GenBank.

pocks with a diameter of two to three millimeters (Nakhla *et al*., 2007) describing the same findings. According to Koch's postulates, a healthy turkey that had been injected one week earlier showed tiny nodules at the injection site that grew larger and spread throughout the body after three weeks. A scab then developed, and after five to six weeks, the skin lesion fell off. The phases of TKPV lesion progression following virus injection into healthy birds. These results agreed with Radwan and Mikhael (2020). PCR was used to confirm the existence of TKPV in the isolated material, and a 578 bp P4b gene product was amplified, matching the description provided by (Luschow *et al*., 2004). The result of amplification of the P4b gene appears clearly. The sequencing of the P4b gene from turkey reveals that The new isolate of Turkey pox virus strain MKP 1230 core protein P4b EGYPT 2021 (with Accession No.MZ983435.1 in GenBank) has 100% to MG787223 Turkey pox virus isolate TurPVIR13 P4b gene IRAN 2019 and slightly differ from isolate of TKPV that isolated at 2020 in Mansoura (MT219997.1 Turkey pox virus strain mans17T P4b 2020 EGYPT) the first Egyptian TKPV isolate submitted to GenBank. So isolated TKPV in this paper is the second Egyptian TKPV isolate submitted to GenBank. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model. This analysis involved 31 nucleotide sequences. Codon positions were the $1^{st}+2^{nd}+3^{rd}+$ Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 496 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

Conclusion

Thus, the experiment's final conclusion confirms that the virus affects

Turkey, as all molecular analyses show a nearly perfect match between the isolates of the Turkey pox and Fowl pox viruses. However, the use of the FPV vaccine causes a number of issues, necessitating the preparation of a homologous vaccine from the Egyptian TKPV isolate in order to achieve perfect immunity.

Conflict of interest

The authors declare that they have no conflict of interest.

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