J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
50(1): 11-21, March 2025
DOI: 10.14710/jitaa.50.1. 11-21

Analysis of G-Protein coupled receptor gene of Capripoxvirus from sheep in Saudi Arabia

M. Magzob Abdellatif[1*], A. Anwar Alsharari[2], Y. Hassan Ali[1], S. Intisar Kamil[1], and M. Helal El-Sayed[1]

[1]Department of Biology, College of Sciences and Arts-Rafha, Northern Border University, Arar, Saudi Arabia
[2]National Center for the Prevention and Control of Plants Pests and Animal Diseases, Department of Biological Sciences, College of Science, Arar, Northern Border University, Arar, Saudi Arabia
Corresponding E-mail: muazm20@gmail.com

Received September 13, 2024; Accepted January 24, 2025

ABSTRACT

Capripox is a highly infectious, often fatal, illness that results in severe losses; it is caused by Capripoxvirus (CaPVs) of the Poxviridae. It is challenging to distinguish between members of the CaPVs, even if the infection may be diagnosed based on the clinical picture. The objective of the study was to analyze G-protein Coupled Receptor gene identified from Sheep Capripoxvirus in Rafha. Saudi Arabia. Sequence alignment revealed multiple substitutions in the nucleotides and their deduced amino acids. Identity of the *GPRC gene* sequence among local strains was estimated to be 96.7-100%, whereas homology among selected CaPVs was found to be 88.0-100%. Evolutionary divergence analysis showed a discrepancy of 0.0 to 0.03% among local viruses and 0.0 up to 0.94% related to homologous CaPVs included. Phylogeny displayed CaPVs into three groups; local strains scattered into four branches together with other SPPVs from Saudi Arabia, while the second clustered SPPVs from Saudi Arabia, Iraq and Nigeria and the third includes GTPVs and LSDVs. Analysis showed that at least four variants of CaPVs are circulating among sheep in the region. Molecular analysis will help to identify the involved strains to adopt effective preventive measures.

Keywords: Capripoxvirus, GPRC gene, Sheep. Saudi Arabia

INTRODUCTION

Capripoxvirus (CaPV) caused a highly infectious often fatal disease in ruminants, manifested by local or systemic cutaneous eruption, induced by a double-stranded DNA virus classified in the Chordopoxvirinae of the Poxviridae (Kitching, 2007). Losses are due to increased mortality rates, reduced production and weight, damage to wool and hides, immunization campaigns, and limitations on the exportation of livestock and their products, along with restrictions on animal movements (Garner *et al.*, 2000; Yeruham *et al.*, 2007; Tuppurainen *et al.*, 2017; Hamdi *et al.*, 2021). It is a notifiable disease in Africa, the Middle East, and several countries in Asia (OIE, 2017). The disease was reported in different parts of the Kingdom of Saudi Arabia (Abu-Elzein *et al.*, 2003; Boshra *et al.*, 2015; Ibrahim *et al.*, 2020). Although tentative diagnosis is mainly based on the clinical picture, it's difficult to differentiate members of the CaPVs,

despite previous literature indicating host-virus specificity (Tulman *et al.*, 2002; Babiuk *et al.*, 2009). Certain sheeppox and goatpox strains have been reported
to infect sheep and cause fatal diseases (Davies, 1976; Kitching *et al.*, 1989). Identification of CaPVs cannot be reached by antigen-antibody reactions as a result of the antigenic similarity and the presence of one serotype (Kitching *et al.*, 1986; Kitching *et al.*, 1989). Since literature on mixed infection and natural susceptibility of sheep to goatpox and vice versa are contradictory; molecular methods emerge as a proper tool to identify the viruses implicated in epidemics (Kitching *et al.*, 1986; Gershon and Black, 1988; Kitchin *et al.*, 1989; Cao *et al.*, 1995; Tulman *et al.*, 2002). G-Protein Coupled Chemokine Receptor gene (GPRC), have been involved in virulence, cell stimulation, replication, antibody neutralization reaction as well as responses to antiviral medications of viruses, is thought to be a reliable approach for differentiating between sheeppox virus (SPPV), goatpox virus (GTPV), and lumpy skin disease virus (LSDV) (Chensue, 2001; Seet and McFadden, 2002; Le Goff, *et al.*, 2009; Lamien *et al.*, 2011). A very scary work on the characterization of sheeppox virus in Eastern Saudi Arabia was reported (Seet and McFadden, 2002; Hamouda *et al.*, 2017).

No data were so far available on the molecular biology of SPPVs in the Northern border of the country. Molecular characterization of the circulating strains will help to implement effective measures to manage disease outbreaks. This work was designed to analyze the nucleotide sequence of *GPRC gene* extracted from field CaPVs from sheep in Rafha. Saudi Arabia.

MATERIALS AND METHODS

Collection of Samples

Skin scabs (n=10) were collected from infected sheep in Rafha, Saudi Arabia. Samples were suspended in 0.01M phosphate buffer saline (PBS), pH 7.4, homogenized, are preserved at -20ºC till examined.

Extraction of DNA

Tissue suspension was centrifuged for 10min at 1000rpm, then the nucleic acid was purified using a PUREGENE®DNA extraction kit (Gentra System, Minneapolis, USA) following the manufacturer's procedure, kept at –20°C till used.

PCR

The GPCR gene-based PCR was completed as described by (Su *et al.*, 2015), using forward (GPCR-F: 5'-TTT ATC AGC ACT AGG TCA TTA TCT-3'), and reverse primers (GPCR-R: 5'-TATCAC TCC CTT CCA TTT TTA T-3'); The reaction mix includes 1µl purified DNA, 5µl 10X PCR buffer, 0.3µl Taq DNA polymerase (5U/mL) (Invitrogen), 4µl 2.5mM dNTPs, and 0.75µl of each primer (10mM), 38.2 µl nuclease-free water, with the following reaction condition: denaturation for 4min at 98°C, then 35 cycles of denaturation for 30s at 95°C, annealing for 30s at 49.5°C, extension for 120s at 72°C, and a final elongation for 10min at 72°C. Amplicons were visualized on ethidium bromide-stained agarose gel (1%).

Sequencing of GPCR Gene

The PCR products (5µl) were added to 2µl of ExoASP-IT® (USB). After 15min at 37°C, ExoSAP-IT was deactivated by 15min at 80°C. Purified amplicons were sequenced using a BigDye terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3730XL Analyzer. Nucleotide sequences were determined on both strands of the gene at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea). Sequences were deposited in the GenBank under the following accession numbers MT025822, MT025823, MT025824, MT025825, MT025826, MN934941, MN934942, MN934943 and MN967001 (Table 1).

Sequence Alignment

Homologous sequences (n=15) were obtained via Blastn search using a nucleotide query on the National Center for Biotechnology Information (NCBI) database (Table 1). Nucleotides as well as their deduced amino acid sequence alignments were performed using ClustalW in SeqMan within Lasergene (version 7.1.0, DNASTAR, Inc, Madison, WI, USA).

Evolutionary Analysis

The nucleotide substitutions per site among sequences were computed. All ambiguous posi-

Table 1. Dataset used for the analysis. Accession number, virus species, virus strain, host, country, percent of identity and reference for each sequence

No	Accession No	Virus species	Virus Strain	Host	Country	Reference
1	MT025823	SPPV	Rafhapox030	Sheep	Saudi Arabia	This study
2	MT025822	SPPV	Rafhapox030	Sheep	Saudi Arabia	This study
3	MT025824	SPPV	Rafhapox032	Sheep	Saudi Arabia	This study
4	MN934942	SPPV	Rafhapox002	Sheep	Saudi Arabia	This study
5	MN934941	SPPV	Rafhapox001	Sheep	Saudi Arabia	This study
6	MT025826	SPPV	Rafhapox034	Sheep	Saudi Arabia	This study
7	MN967001	SPPV	Rafhapox303	Sheep	Saudi Arabia	This study
8	MN934943	SPPV	Rafhapox003	Sheep	Saudi Arabia	This study
9	MT025825	SPPV	Rafhapox033	Sheep	Saudi Arabia	This study
10	MN072630	SPPV	Saudi Arabia	Sheep	Saudi Arabia	(Biswas *et al.* 2020)
11	MN072628	SPPV	Nigeria	Sheep	Nigeria	(Biswas *et al.* 2020)
12	MN072626	SPPV	Abu Gharib	Sheep	Iraq	(Biswas *et al.* 2020)
13	MG232393	SPPV	SPPV/SA6/2016	Sheep	Saudi Arabia	(Ibrahim *et al.* 2020)
14	MG232391	SPPV	SPPV/SA4/2017	Sheep	Saudi Arabia	(Ibrahim *et al.* 2020)
15	MN072620	GTPV	India	Goat	India	(Biswas *et al.* 2020)
16	MH381810	GTPV	AV41	Goat	China	Unpublished
17	MN072621	GTPV	Vietnam	Goat	Vietnam	(Biswas *et al.* 2020)
18	MN072622	GTPV	Goatpox virus isolate Turkey	Goat	Turkey	(Biswas *et al.* 2020)
19	KX576657	GTPV	Gorgan	Goat	Iran	(Mathijs *et al.* 2016)
20	MT134042	LSDV	LSDV/Russia/Udmurtiya/2019	Cattle	Russia	(Sprygin *et al.* 2020)
21	OM793604	LSDV	LSDV_33-KZN_RSA_1977	Cattle	South Africa	(van Schalkwyk *et al.* 2022)
22	MW732649	LSDV	LSDV/HongKong/2020	Cattle	China	(Flannery *et al.* 2022)
23	OP688128	LSDV	V392.1	Cattle	Bangladesh	(Parvin *et al.* 2022)
24	OK318001	LSDV	V281	Cattle	Nigeria	(Wolff *et al.* 2021)

tions were removed for each sequence pair (pairwise deletion option). The evolution was predicted using a matrix of pairwise similarities calculated by the Maximum Composite Likelihood approach (MCL) (Tamura and Nei 1993). The investigation was carried out using Molecular Evolutionary Genetics Analysis (MEGA X).

Phylogenetic Analysis

The phylogenetic tree was built utilizing the Neighbor-joining method in MEGAX. Bootstrap analysis was used to test the topology of the trees with 1000 replicates (Sanderson and Wojciechowski, 2000).

RESULTS AND DISCUSSION

Nine out of ten samples tested gave positive results for GPCR gene-based PCR (Image not shown). Capripoxvirus infections are endemic in several countries throughout the world. Since they restrict the expansion of the intense pa sture system in small ruminant sectors and lower theirproduc-
tive capacity, they are classified as category A livestock diseases (Yune and Abdela, 2017). *G-Protein Coupled Receptors (GPRC) gene* which plays a role in defending during the immune response's overwhelming attack (Seet and McFadden, 2002; Yune and Abdela, 2017), has been selected for differentiation between members of CaPVs (Le Goff *et al.*, 2009; Lamien *et al.*, 2011). Nine CaPVs were identified by PCR from sheep displaying pox lesions. Sequence alignment revealed multiple substitutions in the nucleotides and their deduced amino acids. Mutations may provide a survival advantage and are more likely to be passed on. These beneficial mutations can help the virus evade the host's immune system or adapt to the environment. Previous reports found that several field LSDV strains included alterations in the GPCR gene, either nucleotide sequence changes or amino acid mutations, such as deletion or insertion (El-tholoth and El-kenawy, 2016). Based on the nucleotide and amino acid sequence of the GPCR gene protein, investigations conducted in Russia in 2017 identified LSDV strain as a novel vaccine-like LSDV strain in cows (Sprygin *et al.*, 2020).

Nucleotide sequence alignment of the *GPRC gene* amplified from nine field sheep poxviruses revealed multiple substitutions. Likewise, deduced amino acid alignment displayed multiple substitutions in the translated peptide (Figure

Table 2. Base substitutions per site of *GPCR gene* identified from CaPVs sequences (local viruses are underlined).

SN	Virus strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	SPPV_MT025826																							
2	SPPV_MT025825	0.01																						
3	SPPV_MT025824	0.02	0.03																					
4	SPPV_MT025823	0.02	0.03	0.00																				
5	SPPV_MT025822	0.02	0.03	0.00	0.00																			
6	SPPV_MN967001	0.01	0.01	0.02	0.02	0.02																		
7	SPPV_MN934943	0.01	0.02	0.02	0.02	0.02	0.01																	
8	SPPV_MN934942	0.01	0.02	0.01	0.01	0.01	0.01	0.01																
9	SPPV_MN934941	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00															
10	SPPV_MN072630	0.82	0.83	0.77	0.79	0.79	0.81	0.77	0.76	0.76														
11	SPPV_MN072628	0.82	0.83	0.77	0.79	0.79	0.81	0.77	0.76	0.76	0.00													
12	SPPV_MN072626	0.82	0.83	0.77	0.79	0.79	0.81	0.77	0.76	0.76	0.00	0.00												
13	SPPV_MG232393	0.01	0.03	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.91	0.91	0.91											
14	SPPV_MG232391	0.02	0.03	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.91	0.91	0.91	0.00										
15	LSDV_OP688128	0.84	0.85	0.78	0.80	0.80	0.83	0.78	0.77	0.77	0.03	0.03	0.03	0.93	0.93									
16	LSDV_OM793604	0.82	0.84	0.75	0.77	0.77	0.81	0.76	0.75	0.75	0.03	0.03	0.03	0.93	0.94	0.02								
17	LSDV_OK318001	0.84	0.85	0.78	0.80	0.80	0.83	0.78	0.77	0.77	0.03	0.03	0.03	0.92	0.93	0.00	0.02							
18	LSDV_MW732649	0.82	0.84	0.76	0.78	0.78	0.81	0.76	0.75	0.75	0.03	0.03	0.03	0.92	0.93	0.01	0.01	0.01						
19	LSDV_MT134042	0.82	0.84	0.75	0.77	0.77	0.81	0.76	0.75	0.75	0.03	0.03	0.03	0.93	0.94	0.02	0.00	0.02	0.01					
20	GTPV_MN072622	0.85	0.86	0.79	0.81	0.81	0.84	0.79	0.78	0.78	0.04	0.03	0.04	0.94	0.94	0.03	0.03	0.03	0.03	0.03				
21	GTPV_MN072621	0.85	0.86	0.79	0.81	0.81	0.84	0.80	0.78	0.78	0.04	0.03	0.04	0.93	0.93	0.03	0.03	0.03	0.03	0.03	0.00			
22	GTPV_MN072620	0.85	0.86	0.79	0.81	0.81	0.84	0.80	0.78	0.78	0.04	0.03	0.04	0.93	0.93	0.03	0.03	0.03	0.03	0.03	0.00	0.00		
23	GTPV_MH381810	0.85	0.86	0.79	0.81	0.81	0.84	0.80	0.78	0.78	0.04	0.03	0.04	0.93	0.93	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	
24	GTPV_KX576657	0.85	0.86	0.79	0.81	0.81	0.84	0.80	0.78	0.78	0.03	0.03	0.03	0.94	0.94	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00

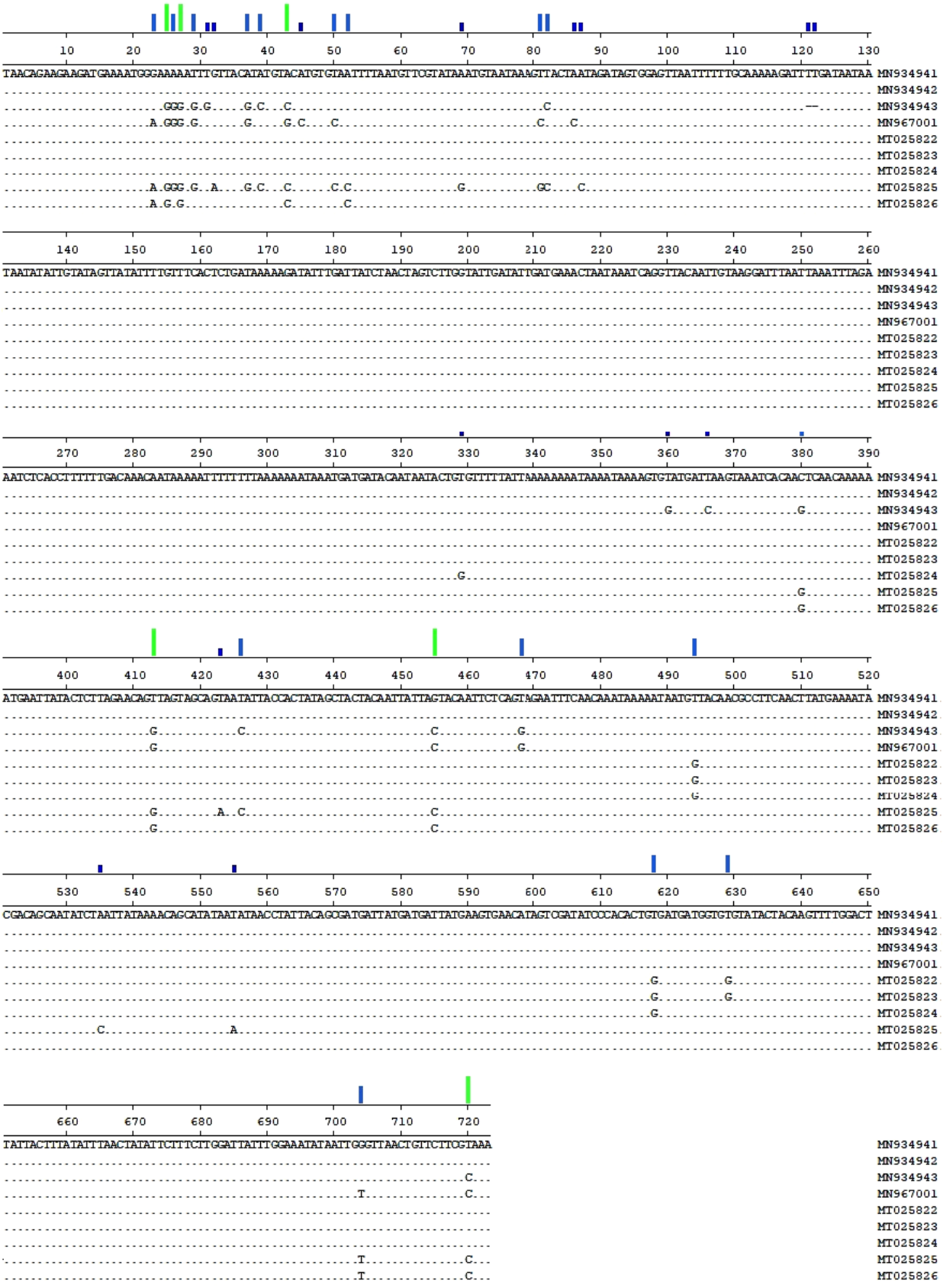

Figure 1. Sequence alignments of partial *GPRC gene* extracted from field sheep poxviruses of this study

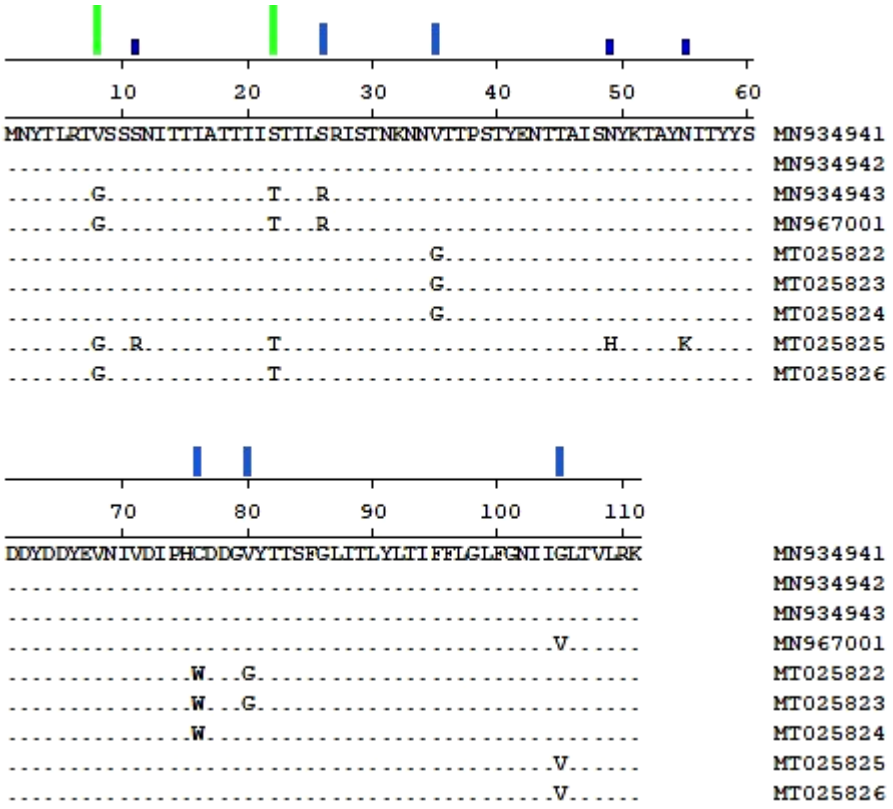

Figure 2. Deduced amino acid sequence alignments in partial G-Protein Coupled Receptors (GPRC) protein

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
1		100.0	97.5	97.8	99.6	99.6	99.5	96.7	98.4	95.7	95.4	91.5	99.0	95.2	95.2	95.4	95.4	99.7	99.4	99.9	96.4	96.6	95.6	96.5	1	MN934941.
2	0.0		97.5	97.8	99.6	99.6	99.5	96.7	98.4	95.7	95.4	91.5	99.0	95.2	95.2	95.4	95.4	99.7	99.4	99.9	96.4	96.6	95.6	96.5	2	MN934942.
3	2.5	2.5		97.6	97.2	97.2	97.1	97.6	98.2	93.7	93.4	90.0	97.0	93.2	93.2	93.1	93.2	97.4	97.4	97.5	94.4	94.2	93.7	94.6	3	MN934943.
4	2.3	2.3	2.4		97.4	97.4	97.3	97.8	98.5	94.2	93.9	91.4	97.5	93.7	93.7	93.5	93.8	97.8	97.9	97.9	94.9	94.6	94.2	95.1	4	MN967001.
5	0.4	0.4	2.8	2.7		100.0	99.7	96.3	98.2	95.6	95.2	90.5	98.9	95.0	95.0	95.0	95.0	99.3	99.3	99.4	96.3	96.1	95.5	96.4	5	MT025822.
6	0.4	0.4	2.8	2.7	0.0		99.7	96.3	98.2	95.6	95.2	90.5	98.9	95.0	95.0	95.0	95.0	99.3	99.3	99.4	96.3	96.1	95.5	96.4	6	MT025823.
7	0.5	0.5	3.0	2.8	0.3	0.3		96.2	97.9	95.2	94.9	91.0	98.5	94.7	94.7	95.0	95.0	99.3	98.9	99.4	95.9	96.1	95.1	96.0	7	MT025824.
8	3.4	3.4	2.4	2.3	3.8	3.8	3.9		98.2	93.1	92.8	89.9	96.4	92.6	92.6	92.4	92.7	96.7	96.8	96.8	93.8	93.5	93.1	93.9	8	MT025825.
9	1.6	1.6	1.8	1.5	1.8	1.8	2.1	1.8		94.8	94.5	91.7	98.1	94.3	94.3	94.3	94.5	98.6	98.5	98.8	95.6	95.5	94.8	95.7	9	MT025826.
10	4.5	4.5	6.6	6.1	4.6	4.6	5.0	7.3	5.4		95.3	88.4	95.7	95.4	95.5	95.4	95.1	96.0	96.3	96.1	98.3	98.9	100.0	98.2	10	OP688128.
11	4.7	4.7	6.9	6.4	5.0	5.0	5.3	7.6	5.7	4.9		88.0	95.4	99.5	99.4	99.6	100.0	95.6	95.8	95.7	96.3	96.2	95.3	96.4	11	KX576657.
12	9.0	9.0	10.7	9.2	10.2	10.2	9.6	10.9	8.8	12.7	13.1		92.1	87.7	87.7	86.7	86.6	90.9	91.9	91.1	89.0	87.9	88.2	89.1	12	MG232391.
13	1.0	1.0	3.0	2.5	1.1	1.1	1.5	3.7	1.9	4.5	4.8	8.4		95.2	95.2	95.0	95.0	99.3	99.4	99.4	96.4	96.1	95.6	96.5	13	MG232393.
14	5.0	5.0	7.2	6.6	5.1	5.1	5.5	7.8	5.9	4.8	0.5	13.4	5.0		99.9	100.0	99.6	95.4	95.5	95.6	96.3	96.4	95.5	96.4	14	MH381810.
15	5.0	5.0	7.1	6.6	5.1	5.1	5.5	7.8	5.9	4.6	0.6	13.4	5.0	0.1		99.9	99.4	95.4	95.5	95.6	96.2	96.3	95.6	96.3	15	MN072620.
16	4.7	4.7	7.3	6.8	5.1	5.1	5.1	8.0	5.9	4.7	0.4	14.6	5.1	0.0	0.1		99.6	95.4	95.4	95.6	96.3	96.4	95.5	96.4	16	MN072621.
17	4.7	4.7	7.1	6.5	5.2	5.2	5.2	7.7	5.7	5.1	0.0	14.7	5.2	0.4	0.6	0.4		95.4	95.4	95.5	96.0	96.1	95.2	96.1	17	MN072622.
18	0.3	0.3	2.7	2.2	0.7	0.7	0.7	3.4	1.4	4.1	4.6	9.7	0.7	4.7	4.7	4.7	4.8		99.7	99.9	96.7	96.6	95.9	96.8	18	MN072626.
19	0.6	0.6	2.6	2.1	0.7	0.7	1.1	3.3	1.5	3.8	4.3	8.6	0.6	4.6	4.6	4.7	4.7	0.3		99.9	96.8	96.6	96.2	96.9	19	MN072628.
20	0.1	0.1	2.5	2.1	0.6	0.6	0.6	3.2	1.2	4.0	4.4	9.5	0.6	4.6	4.6	4.6	4.6	0.1	0.1		96.8	96.7	96.1	97.0	20	MN072630.
21	3.7	3.7	5.8	5.3	3.8	3.8	4.2	6.5	4.6	1.7	3.9	11.9	3.7	3.8	3.9	3.8	4.2	3.4	3.3	3.2		99.3	98.4	99.9	21	MT134042.
22	3.5	3.5	6.0	5.6	4.0	4.0	4.0	6.8	4.7	1.1	3.9	13.2	4.0	3.7	3.8	3.7	4.0	3.5	3.5	3.4	0.7		99.1	99.5	22	MW732649
23	4.5	4.5	6.6	6.0	4.6	4.6	5.1	7.3	5.4	0.0	4.8	12.9	4.6	4.7	4.6	4.7	5.0	4.2	3.9	4.0	1.6	1.0		98.3	23	OK318001.
24	3.5	3.5	5.7	5.1	3.7	3.7	4.1	6.3	4.5	1.8	3.7	11.8	3.6	3.7	3.8	3.7	4.0	3.2	3.2	3.1	0.1	0.5	1.7		24	OM793604.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		

Figure 3. Percent identity of GPRC gene sequence among local strains (underlined) and CaPVs downloaded from GenBank

Figure 4. Phylogeny of CaPVs based on partial GPCR gene sequence

1 and 2). Analysis of the GPRC gene sequence showed that the identity among field strains is 100-98.9% and 90.51 to 98.21% when compared to homologous strains downloaded from the GenBank databases. Higher similarity percentages may represent closer evolutionary relationships, furthermore it might indicate conserved functional domains essential for the virus's survival and replication. Similarity among Huining and Zhangye strains exhibited 95.0-99.7 and 93.0 -100% (Su *et al.*, 2015). Chinese sheeppox varied from 99.4% to 99.6% and 95.0 ~ 99.8% with other strains of CaPVs (Zhou *et al.*, 2012). Nucleotide sequence homology of the *GPRC gene* among Rafha strains was estimated to be 96.7-100%, whereas identity between selected CaPVs was found to be 88.0-100% (Figure 3). Previous work showed that all LSDV isolates are closely related to each other with a nucleotide and AA identity ranging from 99% to 100% (Rouby *et al.*, 2019). Tran *et al.*, (2024) found that the sequence of the GPCR of the Vietnam strain of LSDV was 99.91% similar to the Xinjiang/2019 strain, 99.65% comparable to the two vaccination strains, and 98.87% related to the Saratov/2017 strain. Evolutionary Divergence analysis revealed a low amount of divergence between CaPVs included in the study. Analysis showed ~~a~~ discrepancy of 0.0 to 0.03 among local sheep pox strains and 0.00 up to 0.94 related to homologous viruses included in the study (Table 2).

The tree based on the GPRC gene showed that the CaPVs strains studied were divided into three groups. One grouped local strains scattered into four branches, together with other SPPVs from Saudi Arabia (MG232393 and MG232391), whereas the second clustered SPPVs from Saudi Arabia, Iraq and Nigeria and the third includes GTPV from India, China, Vietnam, Turkey and Iran and LSDV from Russia, South Africa, China, Bangladesh and Nigeria (Fig 4). Phylogenetic analysis revealed that the selected CaPV strains clustered together in the same group could be divided into four subgroups. One grouped local viruses scattered into four branches together with other SPPVs from Saudi Arabia, whereas the second clustered SPPVs from Saudi Arabia, Iraq and Nigeria and the third includes GTPV from India, China, Vietnam, Turkey and Iran and LSDV from Russia, South Africa, China, Bangladesh and Nigeria. Likewise, previous research presented a tree comprised of three closely similar genetic clusters including the strains causing lumpy skin disease, sheep pox, and goat pox (Le Goff *et al.*, 2009; Rouby *et al.*, 2019). There is inconsistency in the viral-host nomenclature; for instance, a virus identified in sheep is gathered with the goat poxvirus strains, and vice versa (Le Goff *et al.*, 2009). Notably, GTPV/Saudi Arabia/1993 and the GTPVs originating from Sudan and Oman are grouped separately, in alignment with their original host (Le Goff *et al.*, 2009). However, it disagrees with reports that presented six CaPVs from Saudi Arabia into two separate clades: SPPVs and GTPVs. While SPPV/Saudi Arabia 4/2017, SPPV/Saudi Arabia 5/2016, in addition to SPPV/Saudi Arabia 6/2016 are closely related to one another, SPPV/Saudi Arabia 3/2013 and SPPV/Saudi Arabia 1/2014 are not (Abu-Elzein *et al.*, 2003; Boshra *et al.*, 2015; Hamouda *et al.*, 2017; Ibrahim *et al.*, 2020). GTPV Saudi Arabia 2/2017 is clustered with GTPVs from India, China and Bangladesh in the GTPV group. Other data displayed five strains organized into two groups, Chinese SPPVs as they are grouped. Turkey/98 Van2 (SPPV Turkey) is relatively similar to Chinese strains compared to other Turkish SPPV TU-V02127 and other SPPVs from African countries (Zhou *et al.*, 2012). The tree revealed that at least four distinct strains of CaPVs have been detected among sheep in the northern region of Saudi Arabia, it may suggest that these strains share a closer evolutionary relationship compared to strains outside these subgroups, suggesting that sequencing of GPCR gene is a promising approach for subgrouping. However, Tran *et al.* (2024) identify two different LSDV strains that exist in Vietnam.

CONCLUSION

Sequence alignment and phylogenetic analysis based on the GPRC gene revealed a genetic discrepancy with multiple substitutions in the nucleotides and their deduced amino acids among local SPPV strains. Analysis showed that at least four variants of CaPVs are circulating among sheep in the region. The results of this research will help in identifying SPPVs, especially in the Northern Border of the country, to implement effective preventive measures to con-

trol the virus.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA, for funding this research work through the project number "NBU –FFR–2025–377–01".

CONFLICT OF INTEREST

We are declaring that they have no conflict of interest.

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