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The Sheep Pox Virus's Antiapoptotic Gene Sequence Analysis as a Host Immunity Evading Gene

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Abstract Sheep pox is a highly contagious infectious disease that primarily affects small ruminants such as sheep. The causative agent is a member of the Poxviridae family, and causes severe skin rashes, fever, diarrhea, deaths in young litters and abortions in pregnant ewes. In recent years, many single and multiple cases of SPV have been reported with failure of control and treatment strategies. This indicates that the SPV virus may undergo genetic changes that reduce host immunity, so it had to be studied to diagnose the gene encoded by the virus and identify its sequences, such as the apoptosis gene. one hundred and twenty-five scab samples were collected from suspected in the Diwaniyah Province throughout September 2023 and January 2024, the samples were subjected to a PCR examination to identify the presence of the anti-apoptosis gene. Result revealed that the expected amplicons size 515 bp of SPV was detected in forty samples (32%) of skin lesions. Number of base substitutions per site between sequences was shown. The identity score of apoptosis related gene of ten positive local isolates was 100% with Abu Gharib _Iraq vaccines Registered breeds in global sequences. Phylogenetic tree analysis based on the partial apoptosis related gene showed that all samples tested were closely related in sequence alignment with NCBI-BLAST capripoxviruses: sheeppox virus envelope protein apoptosis related gene. This study provided sequence information of anti-apoptotic gene for several SPV isolates, whichpositively affects the epidemiological study of Capripoxvirus.
Keywords: apoptosis related genes, Capripoxvirus, PCR, SPV.

Introduction Sheeppox virus (SPV) is an international viral disease that primarily affects sheep and goats, causing skin lesions and fever (1,2,3). Causing large losses in countries with extensive small ruminant breeding programs (4). This disease imposes significant economic costs and risks on the country, neighbors and trading partners, Trade bans can be mentioned due to the presence of these major cross-border diseases (5,6). The virus responsible for sheep pox and goat pox is Capri poxvirus, a large double-stranded DNA virus with dimensions of 170-260 nm by 300-450 nm from the poxviridae family (7,8). This enormous genome encodes every gene required for their specific intracellular replication (9). Clinical indicators can include fever, nodules and papules, internal sores in the lungs, respiratory, and gastrointestinal mucosa, and cause the animals' mortality (10,11,12). Other symptoms include decreased milk yield and weight gain, higher rates of miscarriage, greater susceptibility to pneumonia, and high mortality (13,14). The geographical distribution of sheep pox has been rather steady. Sheep pox and goat pox are widespread in many countries, including Iraq, Iran, Turkey, Pakistan, India, Afghanistan, China, Nepal, Bangladesh, and Africa. Sporadic outbreaks were observed in a number of nations in Southern Europe and other parts of the world as a result of considerable trade with other foreign countries. (15,16). Poxviruses exploit genetic recombination to acquire host genes and escape immunity (17). One pathway is apoptosis regulator genesis, which is a controlled process of cellular death that occurs in response to external (extrinsic apoptosis) or internal (intrinsic) stimuli. Important for development, tissue homeostasis, and the elimination of damaged or pathogen-infected cells (18, 19). They are listed in group A of contagious disease by the World Organization for Animal Health (OIE) (20). This paper describes the genetic identification of sheep pox virus from field strains using phylogenetic analysis to target partial sequences of apoptosis-related genes and compare them to other global sequences, including vaccination strains.

Materials and Methods

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Ethical Approval

The study was approved by the Committee for Research Ethics at the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Samples collection

Six hundred sheep in Diwaniyah, Iraq (Al Sannih, Daghara, and Al Badir) were evaluated, with one hundred and twenty-five of them suspected of having SPPV. This is the study that has been conducted. From September 2023 to January 2024. Skin lesion samples were collected aseptically, transported to the laboratory in a cool bag, and stored at -20°C until used for molecular testing.

Genomic DNA Extraction

Skin lesions were placed in a sterilized Petri plate and then cut into smaller pieces utilizing sterile scissors. Transfer to a sterile 1.5ml microcentrifuge tube, homogenize with tissue lysis buffer, and purify using a silica-based column per the manufacturer's instructions (ADDBio, South Korea). Eluted DNA was stored at -20°C until further examination.

Polymerase Chain Reaction (PCR)

The targeted genes were amplified using primers (Table 1) developed specifically for SPPV in this work. The reaction comprises of 20 µl of PCR master mix (ADDBio, South Korea), 1 µl of each primer (for apoptosis associated gene), and 2 µl of template viral DNA. The thermal conditions included one cycle of initial denaturation at 95 C for 10 minutes, followed by 30 cycles of denaturation at 95 C for 30 seconds, annealing at 60 C for 30 seconds, extension at 72 C for 1 minute, and one cycle of ultimate extension at 72 C for 5 minutes. PCR products were electrophoresed on an agarose gel with ethidium bromide staining and photographed using a gel documentation system (Syngene, Taiwan).

Table (1) Primers used for detection of Antiapoptotic gene

Target gene	Sequence '5-------3'		Amplicon size	NCBI accession number	Start	End
Pox-apopto-F	Forward	GCTCGTTTAGTGCTAAATCATCATC	253 bp	MN072629	174	198
	Reverse	ATACGCGAATGCTGTGAGGT			426	407

DNA Sequencing Method

Macrogen (South Korea) selected ten samples from each gene from the positive PCR samples for DNA Sanger sequencing. They were slightly trimmed from noise signals, phylogenetically examined, and compared to other strains from around the world. These sequences were submitted to NCBI to gain accession numbers (see Table 2). Phylogenetic analysis A phylogenetic tree and several sequence alignments were created using partial sequences from the gene of local SPPV isolates (Mega X program).

Statistical Analysis

Chi-square (X2) was used to identify substantial variations in sickness prevalence data and the impact of other factors. Variations were deemed statistically significant ($P < 0.05$) (21).

Results

The Endpoint PCR results were detected in 40 samples. 125 sheep of both sexes and ages were selected. The results demonstrated this (32%). Whereas 85 samples (68%) proved negative for sheeppox viral DNA (Table 2). The statistical test showed a significant difference among positive and negative cases ($P<0.01$).

Table 2: Percentages of positive sheep pox cases by PCR

Number of sheep with skin lesions	Number of sheep	Percentage
Infected sheep	40	32
Non-Infected sheep	85	68
Total	125	100
Chi-square value	-------	32.4
P- value	-------	<0.0001(HS)

HS: Highly significant difference at P<0.01

Detection of Sheep pox genes by Conventional PCR Assay

The PCR results were detectable in 40 of the 125 suspicious sheep. Ten samples having positive PCR results were for apoptosis-related genes. After electrophoresis, the results Out of 125 skin lesions, 40 samples generated bands with expected diameters of 253 bp (Fig. 1), which corresponded to the universal ladder (50-1500 bp). Whereas 85 skin lesion samples (68%) provied negative for sheep pox virus DNA using PCR (Table 2). The statistical analysis revealed a substantial difference between positive and negative cases ($P < 0.01$).

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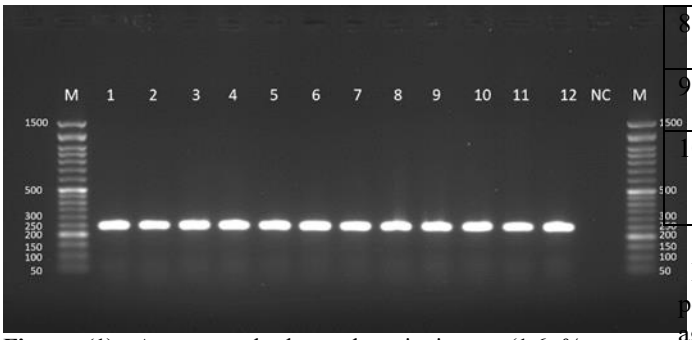

Figure (1): Agarose gel electrophoresis image (1.6 % agarose) shows positive amplicons (1-12) of sheep pox targeting partial region of antiapoptotic gene (size= 253 bp). NC is negative control in which similar PCR conditions were used except the H2O was added instead of DNA. M is molecular marker from Genedirx (South Korea).

Gene sequence and phylogenetic analysis

Ten local isolates with antiapoptotic gene sequences were deposited in the NCBI database: (OR535203), (OR535204), (OR535202), (OR535201), (OR535200), (OR535199), (OR535198), (OR535197), (OR535196), and (OR535195). These were evaluated and compared to NCBI Gen Bank sequences, which revealed some genetic differences between the detected strains and those obtained from NCBI, Table (3).

Table (3) Local SPPV isolates with their accession numbers

No.	Obtained acession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical to	Genbank Accession number	Country	Identity (%)
1	OR535195	Sheeppox	MN072631	Turkey	100
2	OR535196	Sheeppox	MN072630	Saudi Arabia	100
3	OR535197	Sheeppox	MN072629	Canda	100
4	OR535198	Sheeppox	MN072628	Nigeria	100
5	OR535199	Sheeppox	MG000157	India	100
6	OR535200	Sheeppox	MN072626	Abu Ghareb _ Iraq	100
7	OR535201	Sheeppox	MN072630	Saudi Arabia	100
8	OR535202	Sheeppox	MN072629	Canda	100
9	OR535203	Sheeppox	MN072628	Nigeria	100
10	OR535204	Sheeppox	MG000157	India	100

Phylogenetic tree and homology sequence of sheep pox virus antiapoptotic gene demonstrated a 100% agreement between the locally identified strain and the global stain (Fig. 4). ten sequences (accession no. (OR535203), (OR535204), (OR535202), (OR535201), (OR535200), (OR535199), (OR535198), (OR535197), and (OR535196) had the same identity 100% in comparison with homologues global sequence from Turkey. Table 3: Saudi Arabia, Canda, Nigeria, India, Abu Gharib _ Iraq, Nigeria, and India

Multiple sequence alignment

There are also visible conserved motives between all ten sequences motive in nucleated no. (25, 26, 27,28) - The motive in the region between nucleated NO. 56-61.

Figure (2): Multiple sequence alignment of the sheeppox virus within partial region of antiapoptotic gene. This shows similarity and differences carried out by MegaX.

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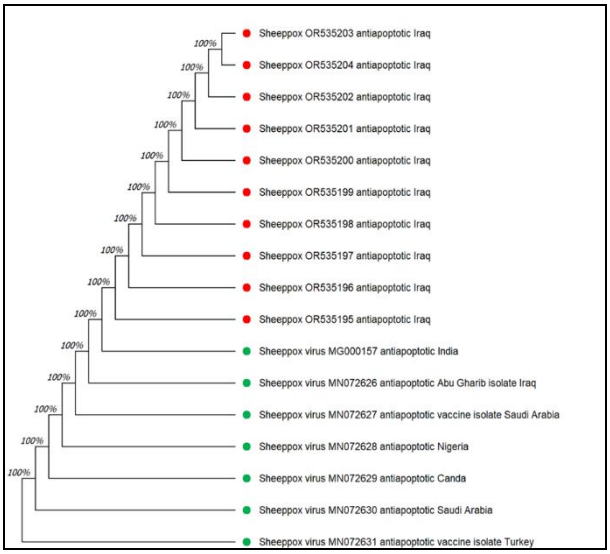

Figure (3): Evolutionary analysis by Maximum Likelihood method of sheeppox virus targeting partial region within the antiapoptotic gene.

Discussion

Capripoxvirus was isolated for the very first time in Kurdistan, northern Iraq, and identified as the GPV Sersenik strain (22). The collected DNA from skin lesions was subjected to PCR using primers specialized for antiapoptotic gene, yielding an expected amplicon of size 253 bp. There was not any amplifier in negative situations. This suggests that the primers were highly specific. To identify the sheeppox virus.

The percentage of positive sheep cases in different areas of Al-Diwaniya reached 32%. This results are consistent with other investigations, such (12) That resulted in 31.3%. Similar findings were observed by (23), who documented a morbidity rate of 36% in different ages from AL-Diwaniyah (24) In Duhok Provinces, the morbidity rate reached 30% in lambs aged 2-4 months. The infection percentage agreement could be caused by There are multiple causes such as the sickness being endemic in this location, the environment, overpopulation, management system, immune status of herds, and vaccination programmes. The conclusion was supported by offering a description of (23, 24).

In contrast, the pox viruses possess several immune escape mechanisms and encode a wide range of immune-modulating proteins (25). This contains antiapoptotic gene,as a highly effective technique to limit infections (26,27,28). This was another motive to carry out this molecular method. to see if these genes are permanent or changeable. The study employed BLAST and BioEdit software to look at the gene for similarities to the indicated strain from GenBank. The results showed a high degree of similarity, indicating that the sequences were identical thus offering an additional Lots of confidence.

Previous studies have dealt with molecular examinations of an inhibitor of apoptosis of this gene, including a study in freshwater pearl mussel, Hyriopsis schlegelii in China (29), as well as the Sheep Ovary During the Reproductive Cycle, which may be involved in controlling the estrus cycle in sheep (30).

Poxviruses and herpesviruses encode secreted copies of cytokine receptors as a unique strategy for evading the host's immune response (31).

Conclusion

To the best of our knowledge, this is first study targeting apoptosis evasive gene in Iraq. It was showed that identified sequence highly similar to that in Alhaliya and Al-Ilmiyyah strains which means this gene is highly conserved. Thus, could be useful as a diagnostic gene for viral diagnosis or for vaccine development.

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Conflict of interest

No conflict of interest is disclosed by the authors.

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