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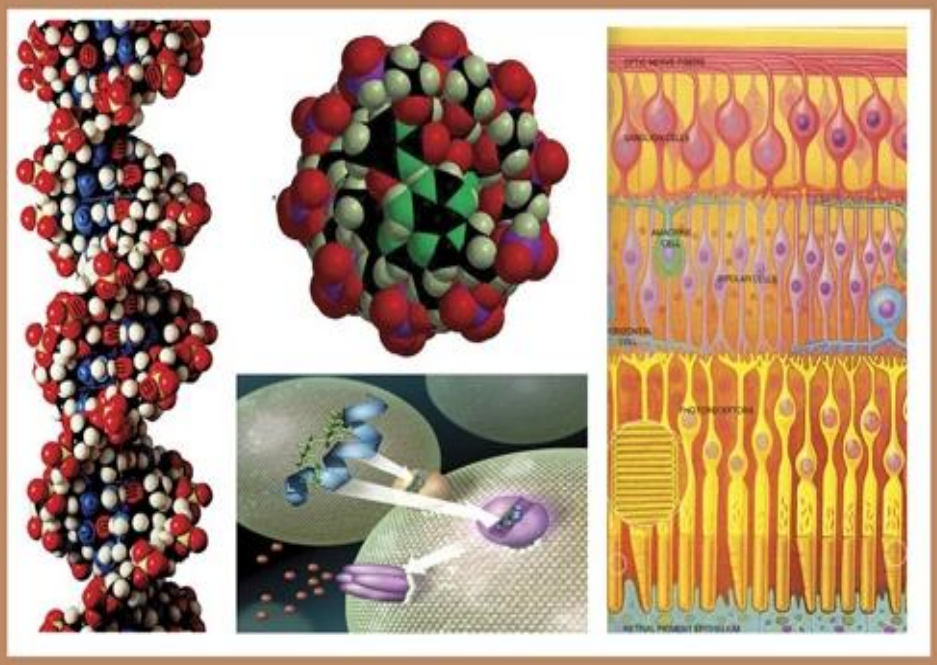

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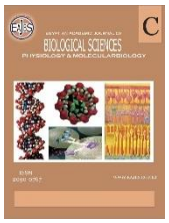

P32 Gene PCR Evaluation for Capripox's Diagnostic Potential and Immunogenic Effect

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ABSTRACT

Capripoxviruses are a group of extremely contagious viruses that cause substantial economic losses in livestock, especially goats and sheep. The purpose of this investigation was to determine the genetic diversity, antigenicity, and docking analysis of the P32 protein and its epitopes in capripoxviruses. From goat poxvirus (GPV) isolates, the gene encoding P32 was isolated and sequenced. The analysis revealed that the P32 protein is highly conserved across distinct isolates. From the P32 protein sequence, B-cell and T-cell epitopes were predicted using bioinformatics tools. Antigenicity of the predicted epitopes suggests their potential as vaccine targets. The interaction between epitopes and receptor proteins was investigated using a docking analysis. These findings shed light on the binding affinity and stability of epitope-receptor complexes. Additionally, a phylogenetic analysis was performed to determine the genetic relationships between capripoxvirus isolates. The results demonstrated a close relationship between the studied samples and Chinese strains, indicating potential genetic similarities and a shared evolutionary history. Overall, this study contributes to our comprehension of the biology of the capripoxvirus and provides valuable information regarding potential vaccine targets and their interactions with receptor proteins. The findings have implications for the development of effective vaccines and diagnostic tools against capripoxvirus infections, which can aid in the control and prevention of the spread of these economically significant diseases among livestock populations.

INTRODUCTION

Capripox is a highly contagious infectious disease that affects various livestock species, including sheep, goats, and cattle (Hamdi et al., 2021). The disease is caused by the Capripoxvirus, a member of the Poxviridae family, and is characterized by the development of pox-like lesions on the skin, mucous membranes, and internal organs of affected animals (Wolff et al., 2020). Capripox outbreaks can result in significant economic losses due to reduced productivity, mortality, and trade restrictions imposed on infected regions (Teffera and Babiuk, 2019).

Accurate and timely diagnosis of Capripox is crucial for effective disease management and control. Traditional diagnostic methods, such as virus isolation and serological tests, have limitations in terms of sensitivity, specificity, and turnaround time (Haegeman *et al*., 2020). Polymerase Chain Reaction (PCR) analysis has emerged as a valuable tool for the detection and differentiation of Capripoxvirus strains, offering advantages of high sensitivity, specificity, and rapidity (Pestova *et al*., 2018).

The P32 gene, which is present in the genome of Capripoxvirus, has been identified as a potential diagnostic marker for Capripox. The P32 gene is conserved among different Capripoxvirus strains and plays a role in the immunogenicity of the virus (Tuppurainen *et al*., 2017). PCR analysis targeting the P32 gene allows for the specific amplification and detection of Capripoxvirus DNA, enabling accurate diagnosis and differentiation of strains (Sumana *et al*., 2020).

This study aims to assess the diagnostic potential of Capripox and investigate its immunogenic effect through PCR analysis targeting the P32 gene. By analyzing samples collected from suspected cases of Capripox, the presence of Capripoxvirus DNA can be detected and confirmed. Additionally, the genetic variability of the P32 gene can be examined to understand the diversity of Capripoxvirus strains circulating in a particular region. Furthermore, evaluating the immunogenic effect of Capripox is essential for understanding the host immune response to the virus and developing effective control strategies. The P32 gene has been implicated in the immunogenicity of Capripox, and studying its expression patterns and immune response can provide valuable insights into the development of vaccines and immunotherapeutic interventions (Eltom *et al*., 2021; He *et al*., 2020).

The findings of this study have the potential to significantly enhance the diagnostic capabilities for Capripox and contribute to improved disease surveillance and control. By utilizing PCR analysis targeting the P32 gene, it can rapidly and accurately diagnose Capripox cases, facilitating prompt intervention and control measures. Additionally, understanding the immunogenic effect of Capripox can aid in the development of more effective vaccines, ultimately reducing the burden of the disease on livestock populations and the agricultural industry as a whole (Heine *et al*., 1999). The utilization of PCR offers numerous advantages in terms of sensitivity, specificity, and speed, making it an ideal tool for diagnosing Capripox (Yang *et al*., 2004). The study outcomes contributed to the existing knowledge on Capripox diagnosis, strain differentiation, and immunogenicity, paving the way for improved disease control strategies and ultimately benefiting animal health, welfare, and the livestock industry.

MATERIALS AND METHODS

Sample Collection:

A total of 1021 samples were collected from seven districts of Khyber Pakhtunkhwa region of Pakistan (Table 1).

Table 1: Details of samples collection from different districts of Khyber Pakhtunkhwa, Pakistan.

S. No	Name of District	Province	No. of animals examined	p-value
1	Swat	Khyber Pakhtunkhwa, Pakistan	112	**0.00001* (Significant at p<0.05)**
2	Mansehra		245	
3	Peshawar		189	
4	Kohat		75	
5	Karak		80	
6	Dera Ismail Khan		320	
Total			1021	

The samples were collected from suspected cases of Capripox in goat species. To preserve the integrity of the viral DNA, the samples were collected with sterile swabs and stored in viral transport media. The sampling process involved collaboration with local veterinary authorities and farmers in different districts of Khyber Pakhtunkhwa

(Fig. 1). The samples were collected using sterile swabs and stored in viral transport media to preserve the integrity of the viral DNA.

Fig. 1: Map of Khyber Pakhtunkhwa showing the districts from where samples were collected.

PCR Analysis

DNA Extraction: DNA extraction is a crucial phase in molecular biology and genetic analysis because it permits the isolation and purification of DNA from a variety of sources. In the context of Capripox research, viral DNA was isolated from samples collected from suspected Capripox cases in the Khyber Pakhtunkhwa region via DNA extraction.

The samples, including swabs from skin lesions and mucous membranes, were collected with care and deposited in sterile tubes containing viral transport media. The samples were then labeled and stored at the proper temperature to preserve their integrity during transport to the laboratory. Before proceeding with DNA extraction, the samples were homogenized to ensure that viral particles were evenly distributed. The tubes containing the swabs were either vortexed or homogenized to break up any clumps and liberate the viral DNA. A commercially available DNA extraction kit was utilized for the DNA extraction procedure (Bioperfectus, USA). The kit selected may differ depending on the manufacturer and the laboratory's standard operating procedures. Typically, these packages include reagents and columns designed to efficiently extract DNA from a variety of samples.

In accordance with the manufacturer's instructions, DNA extraction began with sample lysis. Transferring the homogenized samples to microcentrifuge tubes and adding lysis buffer to disrupt the viral envelope and liberate the viral DNA. Proteinase K, a proteolytic enzyme, was typically added to the lysis buffer to assist in the degradation of proteins. After lysis, the mixture was centrifuged to remove cellular debris and other contaminants. The viral DNA-containing supernatant was cautiously

transferred to a new tube. To facilitate DNA binding to the extraction columns, chaotropic binding buffers were introduced to the supernatant. The mixture was then applied to the extraction columns, permitting the DNA to bind selectively while contaminants were removed. After binding and washing, the purified viral DNA was eluted from the extraction columns using a low-salt elution buffer or deionized water. This final phase assisted in the release of DNA into a separate tube, preparing it for further analysis, such as PCR amplification or sequencing. To maintain the stability and integrity of the extracted DNA, it was stored at -80 degrees Celsius or according to the laboratory's standard storage protocols. This permitted the DNA to be preserved until further analysis, at which point it could be used to detect and characterize Capripoxvirus strains using techniques such as PCR amplification or sequencing (Alexander *et al*., 2019).

PCR Primer Design: Primers specific to the P32 gene of Capripoxvirus were designed using bioinformatics tools and previously published sequences (Chu *et al.*, 2011). The primer sequences were synthesized by a reputable laboratory. Forward and reverse primers targeting a conserved region of the P32 gene were used to detect Capripox. The sequence of the forward primer was 5'-CGAACTTCCACCTCAACCACTCC-3',
and the sequence of the reverse primer was 5'-ATGGCAGATAT CCCATTA-3'. These primers were previously reported and were known to reliably amplify the target region. In subsequent cloning experiments, self-designed cloning primers containing BamH1 and Hind III restriction sites were utilized (Mathijs *et al*., 2022).

PCR Amplification: PCR amplification was performed using a thermal cycler. The PCR reaction mixture contained the extracted DNA template, forward and reverse primers, PCR buffer, dNTPs, and DNA polymerase. The PCR conditions included an initial denaturation step at 95°C for 5 minutes, followed by a cycling protocol of denaturation at 95°C for 30 seconds, annealing at optimized temperature (based on primer specificity) for 30 seconds, and extension at 72°C for 1 minute. The cycling steps were repeated for a predetermined number of cycles (usually 35 cycles) and followed by a final extension at 72°C for 5 minutes (Lorenz, 2012).

Gel Electrophoresis: The PCR products were analyzed by agarose gel electrophoresis to visualize the amplified DNA fragments. A 1% agarose gel was prepared, and the PCR products were loaded along with a DNA ladder as a size reference. The gel was subjected to electrophoresis at a constant voltage for a specific duration, and the DNA bands were visualized using a gel documentation system (Lee *et al*., 2012).

Sequencing and Phylogenetic Analysis: Selected PCR products were purified using a DNA purification kit and sent for sequencing to a reputable sequencing facility. The obtained sequences were analyzed using bioinformatics software to determine the genetic variability and phylogenetic relationship of the Capripoxvirus strains circulating in Khyber Pakhtunkhwa (Zro *et al*., 2014).

Data Analysis: The obtained PCR results, gel electrophoresis patterns, and sequence data were analyzed using appropriate statistical methods and software. Descriptive statistics, such as frequencies and percentages, were calculated to summarize the diagnostic potential of the PCR assay and the prevalence of Capripox in the studied population (Pham *et al*., 2020).

DNA sequencing: In the context of Capripox research, DNA sequencing was performed to analyze the genetic information present in the amplified PCR products and obtain insight into the genetic variability and phylogenetic relationships of Capripoxvirus strains in the Khyber Pakhtunkhwa region. After obtaining the PCR products by amplifying them with primers targeting the P32 gene, a subset of these products was chosen for sequencing. Typically, the selection was based on factors such as the representation of various livestock species, geographic locations, and the observed variation in gel electrophoresis patterns.

The selected PCR products were purified to eliminate residual PCR primers, enzymes, and other contaminants that could hinder the sequencing reaction. Typically, DNA was purified using a DNA purification reagent or enzymatic purification techniques. The purified PCR products were then sent to a reputable facility for DNA sequencing. There are numerous available sequencing technologies, with Sanger sequencing being the most prevalent. Utilizing modified DNA bases (dideoxynucleotides) that prevent DNA strand extension at specific positions, Sanger sequencing generates a mixture of DNA fragments of differing lengths. The DNA sequencing reaction was carried out by the sequencing facility using purified PCR products as templates and specialized DNA polymerases, primers, and labeled dideoxynucleotides. The fluorescently labeled dideoxynucleotides allowed for the detection of terminated DNA fragments during the sequencing reaction.

Following the sequencing reaction, the terminated DNA fragments were separated based on their lengths using capillary electrophoresis. The mixture of terminated fragments was transferred onto a DNA sequencing instrument, and an electric field was applied to force the fragments through a polymer matrix-filled capillary. As the fragments migrated, laser excitation was used to detect and record their fluorescence. The instrument for sequencing produced a chromatogram that represented the DNA sequence as peaks corresponding to the various bases.

The chromatogram-based sequencing data obtained was subsequently analyzed. Utilizing bioinformatics tools and software, the sequencing results were interpreted, the nucleotide sequence was determined, and any genetic variations or mutations present in the Capripoxvirus strains were identified. The sequences were compared to existing databases to determine their similarity to known Capripoxvirus strains, and phylogenetic analysis was performed to determine the evolutionary relationships between the various strains.

Capripox research relied heavily on DNA sequencing, which yielded invaluable information about the genetic composition and diversity of Capripoxvirus strains in the Khyber Pakhtunkhwa region. The obtained sequencing data aided in a greater comprehension of the epidemiology, evolution, and potential immunogenic effects of Capripox, thereby contributing to the development of efficient diagnostic methods and control strategies (Wolff *et al*., 2021).

RESULTS

Capripox virus, a member of the Poxviridae family, is a large DNA virus that predominantly affects ruminant animals, such as goats, sheep, and cattle. Understanding the clinical manifestations, transmission dynamics, and control measures associated with capripox was the focus of our investigation. In animals infected with capripox, we observed the development of characteristic cutaneous lesions, fever, and systemic symptoms. We were able to confirm the presence of capripox viral DNA and antibodies in clinical samples using exhaustive diagnostic techniques, including PCR and serological testing. To mitigate the impact of capripox outbreaks, our study emphasized the importance of implementing control and prevention strategies, such as vaccination, stringent biosecurity measures, and early detection and isolation of infected animals. By highlighting these critical findings, our study contributes to the existing body of knowledge on capripox and offers valuable insights for the effective management of this disease in livestock populations.

Prevalence of Capripox Virus:

The results of diagnostic analysis performed on samples collected from various study districts were presented, whereby 1021 samples were evaluated for the presence of capripox virus after being processed. The district of Dera Ismail Khan collected the most samples (320), followed by those of Mansehra (245) and Peshawar (189). The results of the sample processing indicate the quantity of positive and negative samples for each district. In Dera Ismail Khan, 14 out of

32 processed samples tested positive for the capripox virus, while the remaining 18 tested negative. Each district's diagnostic potential, computed as the proportion of positive samples among all processed samples, is also provided. Overall, 33 of the total 1021 samples examined were positive for the capripox virus, while 67 samples were negative. The calculated diagnostic potential for the entire population of the investigation is 33%. These results shed light on the prevalence and distribution of the capripox virus in the sampled districts, emphasizing the need for sustained surveillance and control measures to effectively manage the disease (Table 2).

Table 2: PCR results for Capripox detection through P32 gene

S. No	District	Total samples	Samples processed	Positive samples	Negative samples	Diagnostic potential (%)
1.	Swat	112	11	03	08	27.27
2.	Mansehra	245	24	07	17	29.16
3.	Peshawar	189	18	06	12	33.33
4.	Kohat	75	07	01	06	14.28
5.	Karak	80	08	02	06	25.0
6.	Dera Ismail Khan	320	32	14	18	43.75
Total		1021	100	33	67	33.0

Demonstration of Capripox Virus Antigen:

Using cloning primers, we sought to amplify a specific 981-bp DNA fragment from the Goatpox virus (GTPV). The PCR technique was used to perform the amplification, which resulted in the detection of a 981-bp amplicon on the agarose gel. The addition of extra bases, such as adapter sequences or PCR artifacts, could account for this minor difference in size. The 981-bp fragment of amplified DNA was then purified and eluted from the gel to obtain a DNA product suitable for further analysis. The purified DNA was then utilized in the cloning of *E. coli* BL-21 cells. The process of cloning involved the insertion of a DNA fragment into a plasmid vector designed for the expression and replication of the target DNA in bacterial cells. To confirm the successful cloning of the GTPV DNA fragment, colony PCR was conducted on the transformed *E. coli* cells containing the recombinant colonies. The colony PCR involved the direct amplification of DNA from bacterial colonies using primers flanking the insert region. On an agarose gel, the presence of the expected amplicon size confirmed the effective integration of the GTPV DNA fragment into the recombinant colonies, as evidenced by the presence of the PCR products. Plasmid DNA was extracted from confirmed recombinant colonies. The plasmid DNA was subjected to a double digestion with the restriction enzymes BamHI and HindIII, which are known to cleave at specific recognition sites in the plasmid vector. On an agarose gel, the digestion pattern of the plasmid DNA was examined, and the presence of expected band sizes confirmed the precise insertion of the GTPV DNA fragment. In order to ascertain the nucleotide sequence of the cloned GTPV DNA fragment, the plasmid DNA was sent to a reputable sequencing service, Macrogen, for sequencing. Sequencing enables us to obtain the precise sequence information of the cloned DNA fragment, which provides invaluable insight into the genetic composition and potential functional elements of the GTPV genome (Fig. 2).

Fig. 2: Four samples were demonstrated in the presence of Capripox virus antigen with amplicon size of 981 bp.

Physical Properties of P32 Gene:

A protein consisting of 322 amino acids is encoded by the P32 gene. The P32 protein's calculated molecular weight is 37,474 Daltons, indicating its size and mass. The protein comprises 36 residues of the negatively charged amino acids aspartic acid (Asp) and glutamic acid (Glu). In contrast, there are 41 positively charged arginine (Arg) and lysine (Lys) amino acid residues. These charged residues are crucial to the structure and function of the protein. The P32 protein has an extinction coefficient of 51,340, denoting its light absorption at a particular wavelength. The protein's instability index is 27, indicating a moderate level of stability. 101 is the aliphatic index, which assesses the proportion of aliphatic amino acids in the protein. The value of -0.04 for hydropathicity indicates that the protein has a minor hydrophilic nature. The P32 protein has an isoelectric point (pl) of 6.1, indicating the pH at which it carries no net charge. The protein has a net charge of -2, signifying a negative charge under neutral conditions. The grand average of hydropathicity (GRAVY) of the protein is -0.21, indicating a mildly hydrophilic nature. The protein has an estimated in vitro half-life of 30 hours and a high probability of instability of 0.78. With a flexibility value of 0.44, the protein is capable of undergoing conformational alterations. The protein has approximately 20.3% helical regions, 35.6% beta-sheet regions, 14.8% turns, and 29.8% random coil regions in terms of secondary structure (Table 3).

Table 3: Physical properties of P32 gene of Capripox virus

S. No	Property	Value of P32 gene
1	No. of amino acids	322
2	Molecular weight	37474
3	Asp+Glu residues (anions)	36
4	Arg+Lys residues (cations)	41
5	Extinction coefficients	51340
6	Instability index	27
7	Aliphatic index	101
8	Hydropathicity	- 0.04
9	Isoelectric point (pl)	6.1
10	Net charge	-2
11	Grand average of hydropathicity (GRAVY)	-0.21
12	In vitro half life	30 hours
13	Instability probability	0.78
14	Flexibility	0.44
15	Helical region (%)	20.3
16	Beta sheet region (%)	35.6
17	Turns region (%)	14.8
18	Random coil region (%)	29.8

Hydrophobic Index of P32 Gene:

This index quantifies the hydrophobic nature of the protein encoded by the P32 gene of the Capripox virus. It provides information regarding the abundance and distribution of hydrophobic amino acids in the protein sequence. The hydrophobicity index aids in comprehending the interaction of a protein with its surroundings, particularly in relation to the hydrophobic core of a coiled protein structure (Figure 3).

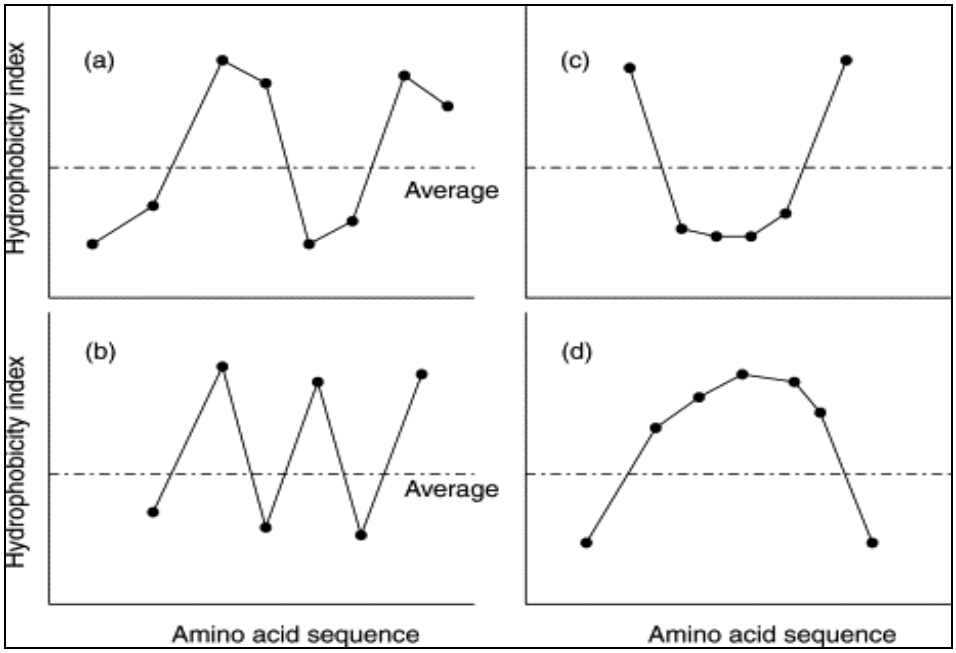

Fig. 3: Hydrophobicity index of P32 gene of Capripox virus.

Antigenic Region Determination:

Greater antigenicity exists in the region between amino acids 200 and 600 of the P32 protein. This suggested that this particular protein segment is more likely to interact with antibodies and elicit an immune response. To visually represent this information, we constructed a dotted graph displaying the antigenicity scores along the P32 protein's length. The y-axis would

represent antigenicity scores, while the x-axis would represent amino acid positions. To emphasize its higher antigenicity, the region between amino acids 280 and 300 can be highlighted or indicated with a distinct color or pattern (Fig. 4).

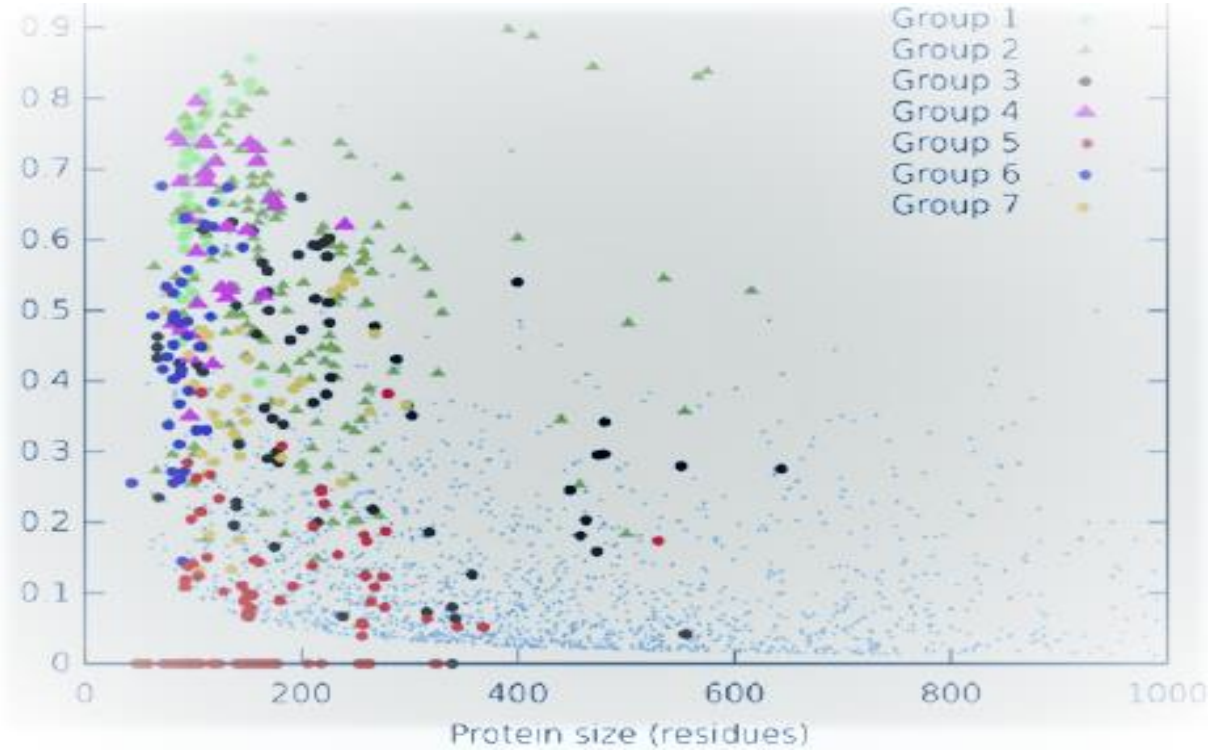

Fig. 4: Graphical representation of unstructured (%) of P32 protein.

Restriction Map of pET28a:

Figure 5 depicts the restriction map of the pET28a vector containing the desired DNA sequence inserted between the Bam HI and Hind III restriction sites. The length of the inserted DNA sequence is 981 base pairs. Vector NTI software, which is commonly used for DNA sequence analysis and molecular biology research, was utilized to generate the restriction map. Typically, the map would depict the linear structure of the pET28a vector, along with the precise loci of the Bam HI and Hind III restriction sites. It would also display the inserted DNA sequence between these sites, representing the fragment's length and orientation. The restriction map facilitates the planning and analysis of cloning experiments by allowing researchers to visualize the structure of the vector and the inserted DNA.

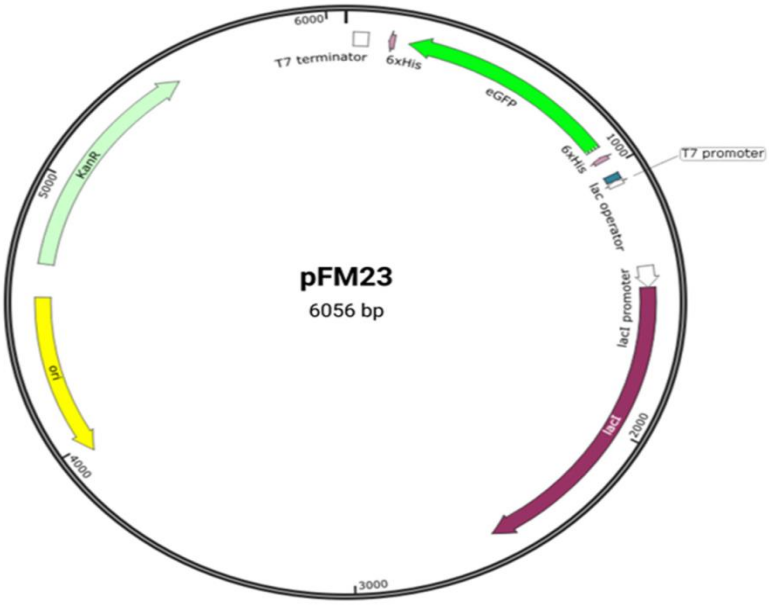

Fig. 5: Map of pET28a-PreScission Vector.

3D structure of Epitopes and Receptor Proteins:

In our study, we predicted B-cell epitopes using IEDB and ABCpred B-Cell Epitope Prediction Tools. We utilized the NetMHCpan Server to target the Cahi-N*01701 allele of MHC Class I in goats for T-cell epitopes. Antigenicity, allergenicity, toxicity, and topology screenings were performed on each predicted peptide. As epitopes, peptides with antigenicity scores greater than 3.0 were considered.Selected antigenic epitopes were evaluated further for allergenicity, and any identified allergens were highlighted in orange. Those remaining selected epitopes exhibiting an inside topology were indicated in green. In addition, we confirmed that none of the selected epitopes were toxic. To analyze the conservancy of the chosen epitopes, a conservancy analysis was performed with a sequence identity threshold of >= 80%. We utilized NCBI Blast-retrieved sequences and eradicated any overlapping epitopes. Coordinates of the chosen B-cell and T-cell epitopes in the protein sequence as well as their locations in the 3D structure were expressed (Fig. 6).

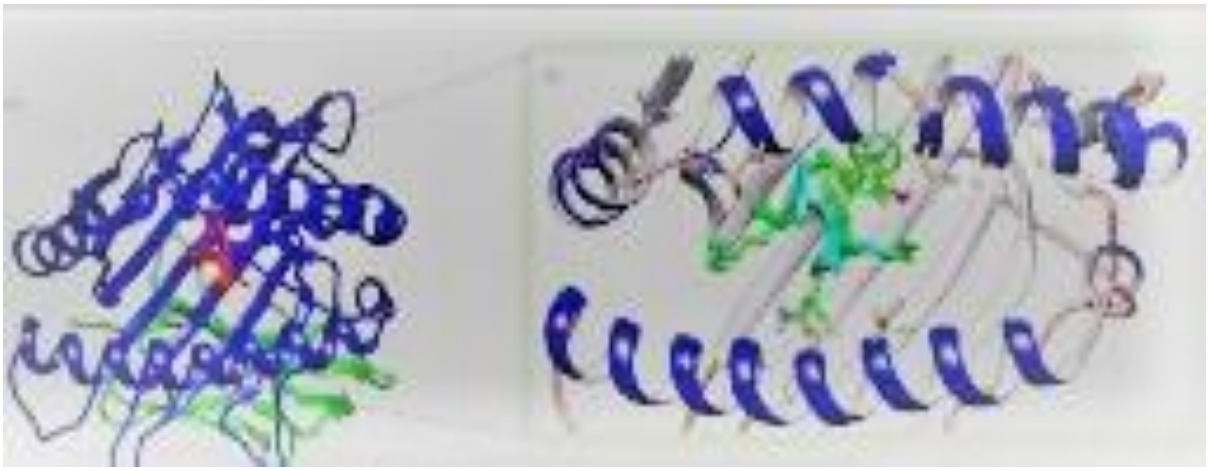

Fig. 6: Prediction of the three-dimensional (3D) structure of epitopes and receptor proteins.

Docking analysis of T-cell epitopes

The docking analysis of T-cell epitopes using the PatchDock server (Zdock) indicated that Patchdock score indicates the quality of the forecasted docking, with higher scores indicating more accurate docking predictions. Lower values indicate a greater fit between the predicted interface and experimental structure. More negative values indicate stronger binding. The epitopes EP1, EP2, EP4, and EP5 displayed relatively high Patchdock scores, indicating favorable binding predictions. All epitopes' interface RMSD values fell within an acceptable range, indicating structural compatibility. All epitopes had negative binding energies, implying favorable and stable interactions between the epitopes and receptors (Table 4).

Table 4: Summarizing the docking analysis of T-cell epitopes using the PatchDock server (Zdock)

S. No	Epitope ID	Receptor ID	Patchdock score	Interface (Å) RMSD	Binding energy (Kcal/mole)
1	EP1	Rec1	117.19	3.12	-8.54
2	EP2	Rec2	120.22	2.97	-7.92
3	EP3	Rec3	67.87	1.20	-10.76
4	EP4	Rec4	102.20	2.89	-9.10
5	EP5	Rec5	90.90	2.65	-9.87

RMSD: Root mean square deviation.

Phylogenetic Analysis:

The maximum-likelihood method incorporated in MEGA-X software was used to conduct the phylogenetic analysis. The purpose of the investigation was to determine the evolutionary relationships between the studied samples. The phylogenetic tree that resulted revealed the genetic relationship between the samples and their proximity to other known strains. On the basis of the phylogenetic tree, it was determined that the examined samples were closely related to Chinese strains, particularly the Pellor strain and the G-20 LKV strain. This suggests that the samples and these Chinese strains shared the genetic relationship and a common evolutionary history. The close proximity in the phylogenetic tree suggests that the samples under investigation may belong to the same lineage as the Chinese strains or share a common ancestor with them. This data can shed light on the genetic diversity and evolutionary dynamics of the investigated samples in comparison to other strains of interest. To gain a comprehensive understanding of the genetic relationships and evolutionary patterns among various strains of interest, it is recommended to conduct additional analysis and comparisons with additional reference strains (Fig. 7).

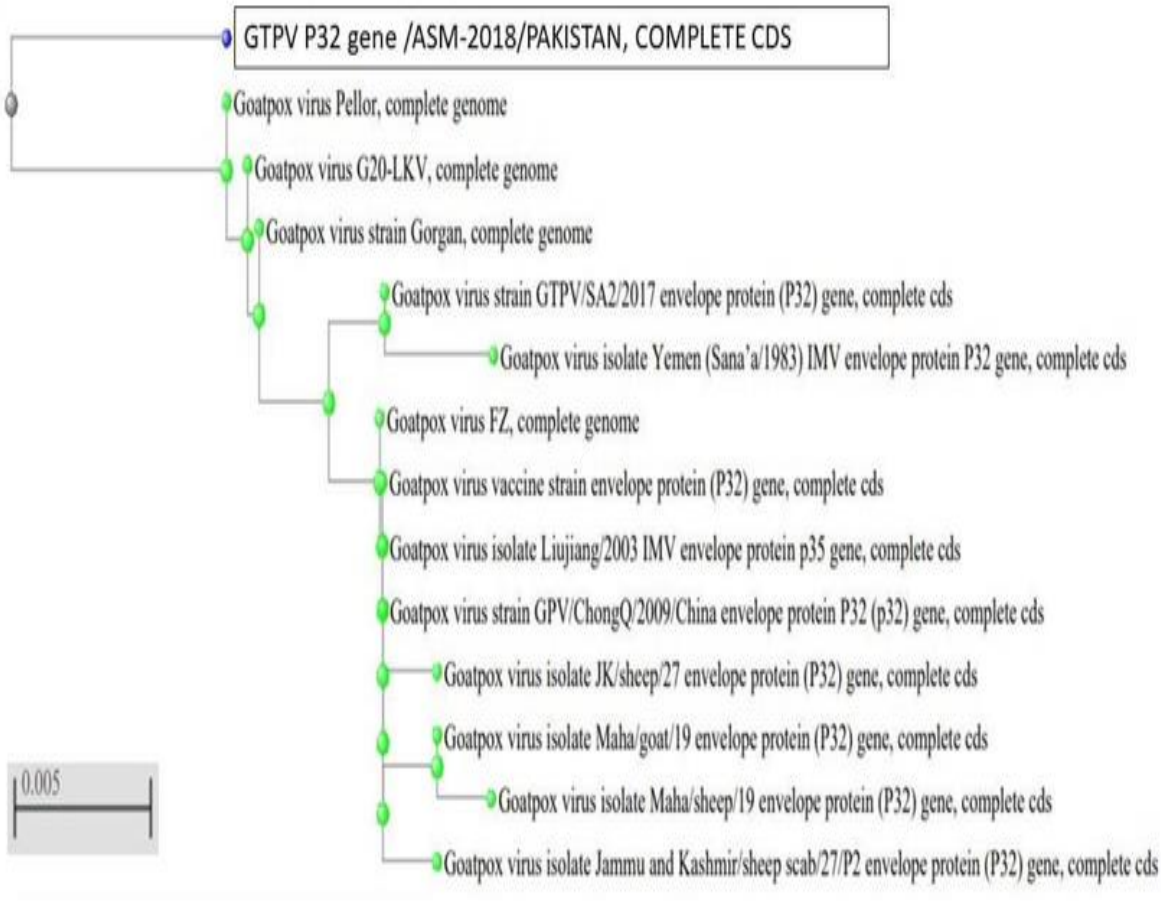

Fig. 7: Phylogenetic analysis of P32 gene.

DISCUSSION

This study's maximum-likelihood phylogenetic analysis provided valuable insights into the evolutionary relationships between the samples under investigation. Understanding the genetic relatedness and evolutionary dynamics of these samples is essential for elucidating their origin, transmission patterns, and potential disease control implications.

Intriguingly, the phylogenetic tree revealed that the samples were closely related to Chinese strains, specifically the Pellor strain and the G-20 LKV strain. This observation suggested that the samples and these Chinese strains may share a genetic similarity and evolutionary history. Such findings could have substantial implications for our comprehension of the geographical distribution and genetic diversity of the studied samples. In this context, He *et al*. (2020) suggested that mutations in specific ankyrin genes (ANK genes 010,138,140,141.2,145) located in the terminal regions of capripoxvirus genomes are directly related to capripoxvirus cases. Therefore, it is essential to comprehend the nucleotide and amino acid sequences that these five genes encode, as they are regarded as crucial for determining host range and virulence factors.

The close proximity between the samples and the Chinese genotypes may indicate a common ancestry or a historical link between the populations. This discovery raises intriguing concerns regarding the potential transmission routes and underlying mechanisms driving the evolution of these strains. To validate and investigate these intriguing connections, additional research, including the analysis of additional reference strains and the incorporation of epidemiological data, is required.

In addition, the discovery of genetic similarities between the samples and the Chinese strains paves the way for

collaboration and the exchange of knowledge with researchers in those regions. A greater understanding of the shared genetic traits and a more complete picture of the global distribution and dynamics of the studied pathogen could result from the sharing of data and insights (He *et al*., 2020).

Similar to our findings, a report performed that phylogenetic analysis of 28 isolates from 25 epidemics of sheeppox and goatpox using the P32 gene/protein. Heparan sulfate and UDP-glucose were also utilized for homology modeling and docking. Based on the host origin of the isolates, the results revealed three distinct lineage-specific clusters. Multiple sequence analysis of the P32 gene revealed the circulation of sheeppox virus (SPPV) and goatpox virus (GTPV) strains that are genetically similar in India. Phylogenetically, the genetic relationship between lumpy skin disease virus (LSDV) and SPPV was closer than that between LSDV and GTPV. Comparative sequence alignment revealed the conservation of multiple motifs, including glycosaminoglycan (GAG), chemokine-like motif (CX3C), and Asp-Glu-any other residue-Asp (D/ExD), in addition to viral-specific signature residues in SPPV and GTPV isolates. Comparable to the crystal structure of the homologous vaccinia virus H3L protein, the P32 protein of SPPV and GTPV exhibited a hybrid -helix and -sheet structure. Docking analyses of the P32 protein with SPPV and GTPV revealed a conserved binding pattern with heparan sulfate, which is essential for virus attachment, and variants in the glycosyltransferase fold with UDP-glucose. These findings had implications for the development of capripoxvirus vaccines, diagnostics, and treatments (Sumana *et al*., 2020).

An investigation effectively cloned and sequenced a 3.7 kb fragment of SPV's viral DNA. This fragment contained open reading frames homologous to the vaccinia virus genes J6R, H1L, H2R, H3L, and H4L. We developed a capripoxvirus-specific PCR assay capable of distinguishing SPV from LSDV by utilizing unique restriction sites present in the corresponding PCR fragments. Capripoxvirus P32 antigen was determined as a homolog of the vaccinia virus H3L gene through our investigation. The P32 proteins of SPV and LSDV were produced in *Escherichia coli* as fusion proteins with a poly-histidine tag. Afterward, these proteins were purified with metal-binding resin. Near its carboxy terminus, the full-length P32 protein possessed a transmembrane region, rendering it membrane associated. However, detergent solubilization of the protein enabled its use as a trapping antigen in an enzyme-linked immunosorbent assay (ELISA) for antibody detection. The developed ELISA was specific for capripoxvirus because only sera from sheep infected with capripoxvirus, and not sera from sheep infected with orf or vaccinia virus, displayed reactivity with the capripoxvirus P32 antigen. This suggested the ELISA's potential as a diagnostic tool for capripoxvirus infections (Heine *et al*., 1999).

Our findings were supported by the study indicating that the Capripox viruses, such as goatpox virus (GTPV), were acknowledged as the most significant pox diseases affecting production animals and analyzed the GTPV isolates using a combination of nanopore MinION sequencing for long reads and Illumina high-throughput sequencing for precise short reads, allowing for the acquisition of complete and high-quality genome sequences. Sheep were immunized with SPPV strains while goats were immunized with GTPV strains. During the animal trial, various infection vectors, including combined intravenous and subcutaneous infection, intranasal infection only, and contact infection between naive and inoculated animals, were compared. Goats that were inoculated with GTPV exhibited severe clinical symptoms and high viral genome burdens in all tested matrices. According to the obtained genetic data, the GTPV strain was an extremely virulent field strain (Wolff *et al*., 2020).

Another study described the

development of a real-time high-resolution melting PCR assay for the detection and differentiation of sheep pox virus (SPPV) and goat pox virus (GTPV), as well as field isolates and vaccine strains of lumpy skin disease virus (LSDV). To differentiate between these viral species, this test employed a high-resolution melting curve analysis of the target PCR amplicons. The target for PCR amplification was a region of 111 base pairs (bp) within the LSDV010 ORF that contains unique genetic variants for each viral species. Validation of the assay utilizing DNA samples from naturally infected animals from various geographic locations and reference strains demonstrated that the melting temperature peaks were specific for each viral species. The melting point was determined to be 74.56 0.04 ℃ for field LSDV, 74.95 0.08 ℃ for LSDV vaccine, 74.24 0.06 ℃ for SPPV, and 73.61 0.04 ℃ for GTPV. Using a field strain of LSDV, the detection limit of the assay was determined to be as low as 0.1 TCD50 lg/ml. On the Rotor-Gene Q platform (QIAGEN), the assay demonstrated reproducibility across replicates and operators. It was recommended as an adjunct to existing molecular assays for the detection and differentiation of Capripoxvirus species, including the differentiation of LSDV vaccine strains from field isolates. This assay is appropriate for detecting these viruses in animals in field specimens (Pestova *et al*., 2018).

CONCLUSION

Our study provided significant scientific data on the genetic properties, antigenicity, and docking analysis of the P32 protein and its epitopes found in capripoxviruses. The significance of the P32 gene in the viral life cycle is highlighted by the gene's high degree of conservation across isolates. The exhaustive analysis of P32's physicochemical properties, such as its molecular weight, amino acid composition, and hydropathicity, sheds light on its structural and functional characteristics. The epitope prediction and docking analysis yielded insightful information regarding the potential B-cell and T-cell epitopes implicated in the immune response against capripoxviruses. Antigenicity makes the identified epitopes intriguing candidates for vaccine development and immunodiagnostic applications. The docking analysis elucidates further the interaction between these epitopes and receptor proteins, revealing their binding affinity and stability. Phylogenetic analysis established the genetic diversity of capripoxvirus isolates and their evolutionary relationships. The close proximity between the examined samples and Chinese strains suggested the existence of genetic similarities and a shared evolutionary history. Thus, our research contributed substantially to the understanding of capripoxvirus biology, antigenicity, and potential vaccine targets. The identified epitopes and their interaction with receptor proteins provided a basis for additional experimental validation and the development of targeted interventions against capripoxvirus infections.

Ethical Considerations: The study was conducted following ethical guidelines and obtained necessary approvals from the relevant authorities. Informed consent was obtained from the farmers and animal owners participating in the study.

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