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Molecular Detection of Avian Poxvirus in Chickens and Pigeons of Diyala Province in Iraq

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ABSTRACT

Fowlpox is an infectious viral disease affecting domestic and wild birds. The current study was conducted using PCR-based molecular detection to determine the avian pox virus (APV) and its genetic sequence. A total of 40 pigeons and 40 chickens suspected of APV were obtained from different bird markets. Following euthanasia, tissue samples were taken from the vents, eyes, wings, and beaks for molecular analysis. DNA extraction was focused on the core protein gene region (pb4). PCR results confirmed the presence of APV in all samples, generating specific cDNA bands of 329 bp. Four GenBank accession codes (OR670580, OR670581, OR670582, and OR670583) represented pigeon pox samples, whereas two GenBank accession numbers (OR619724 and OR619725) represented samples of fowlpox. A significant similarity of 99% was found by genetic analysis between the reference target sequences and the sequenced samples. In viral samples of fowlpox and avipoxvirus, nucleic acid variants (205 C > G and 204 T > C) were found with missense and silent effects on particular proteins (p.101Leu>Val and p.108Pro). Phylogenetic analysis organized the samples into clades representing fowlpox and pigeon pox viruses, showing close relationships with strains from different geographical regions. This study unequivocally demonstrates the susceptibility of both domestic and wild birds to avian pox, highlighting the pivotal role of phylogenetic analysis and molecular detection in elucidating novel perspectives on the genetic landscape of these viruses.

Keywords: Fowl pox, Gene, Pigeon pox, Polymerase Chain Reaction

INTRODUCTION

Fowlpox, a viral disease that affects both domestic and wild birds (Williams et al., 2021), is caused by a wide range of DNA viruses within the Poxviridae family. It has significant rates of morbidity and mortality and causes mild to severe lesions (Zhao et al., 2014). The Poxviridae family consists of two subfamilies, namely Chordopoxvirinae, and Entomopoxvirinae (Weli and Tryland, 2011). The fowlpox virus (FPV) is a distinctive, large, oval-shaped enveloped virus with linear dsDNA genomes ranging from 250 to 365 kb. Characterized by a viral particle diameter of 270-350 nm (Weli and Tryland, 2011), FPV demonstrates rapid growth in cell cultures and on embryonated eggs' chorioallantoic membranes (Diallo et al., 2010). The core structure harbors genes shared by all poxviruses, crucial for fundamental replication processes, while terminally positioned genes encode proteins influencing host range limiting (Tulman et al., 2004). Nearly all types and varieties of chicken are prone to contracting FPV (Cui et al., 2023). Birds with large wattles and large combs seem more likely to acquire pox lesions due to the greater surface area of these parts (Delhon, 2022). According to Pattison et al. (2008), there are two forms of the illness, the more frequent being cutaneous (dry pox) and diphtheric/or pharyngeal (wet pox). On featherless parts of the body (comb, the area around the beak, the wattles, the eyelids, or even the legs and wings), the cutaneous form manifests as papules, nodules, or scabs. These lesions have the potential to progress into ulcers, leading to worsening conditions that can hinder the bird's mobility, ability to eat, and vision (Sultana et al., 2019). If the cutaneous form is predominant, mortality rates are generally low. This is attributed to the fact that the lesions induced by the virus in the cutaneous form are primarily confined to the skin. Consequently, there is a limited impact on vital organs or essential physiological functions crucial for survival.

(MacLachlan and Dubovi, 2017; Sultana et al., 2019). The development of white opaque nodules or yellowish patches on the mucous membranes of the oral cavity, tongue, esophagus, or upper trachea of the birds distinguishes the diphtheritic form, which is more severe and causes significant mortality and economic losses in affected flocks (Singh et al., 2003). According to Yeo et al. (2019), several vectors can spread the APV, such as biting arthropods like mites and mosquitoes, aerosols generated by infected birds, and intake of contaminated food or water. Due to its characteristic dry, crusty skin lesions, which are typically seen on the face, comb and wattle, and other unfeathered regions of the bird, fowl pox is recognized as a prevalent, chicken enzootic disease (Skinner and Laidlaw, 2009; Delhon, 2022). Pigeon pox, on the other hand, is a slower-spreading disease that affects both sexes, has a global distribution, and can be fatal. Vaccination with live chicken poxvirus or pigeon poxvirus is commonly used to prevent the disease (Tripathy and Reed, 2013). Despite the effectiveness and regular use of vaccines to minimize morbidity and mortality, there have been instances of infections observed in certain flocks that have received immunizations (Odoya et al., 2006). Sequencing and PCR-based amplification of conserved genomic areas are frequently used in confirmatory diagnosis (Manaroll et al., 2010). The objective of this study was to analyze specific traits of the virus from a virological perspective. The study aimed to detect FPV using molecular detection methods and analyze the identified strain through phylogenetic analysis in both chickens and pigeons. Additionally, the study seeks to determine the similarity between the identified strains in Diyala Governorate and reference strains available in the NCBI GenBank database.

MATERIALS AND METHODS

Ethical approval

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval No. Vet Medicine (188); September 2023 (A and A).

Samples collection

From October 2022 to March 2023, the study was carried out in the College of Veterinary Medicine at Diyala University, Iraq. A total of 40 suspected cases of fowl pox in Gallus gallus and forty Columba Livia Domestica of varying ages were gathered from bird shops around the Diyala governorate. Trained experts examined the pigeons and chickens, documenting clinical signs, such as erosions, crusts, or nodules on the vent area, and head skin (particularly the beak and eyelids). Additionally, individual case histories for each bird were meticulously collected (Figure 1). Samples were obtained immediately after euthanizing the pigeons and chickens, and tissue samples from affected regions (vents, eyes, wings, and base of the beak) were processed for molecular detection.

Figure 1. Naturally infected chicken and pigeon with avian poxvirus. **A**: Chicken, pock lesions appeared on the head and around the eye. **B**, **C**, and **D**: An adult pigeon infected with poxvirus in different areas (Nodular Lesion).

Tissue preparation

Tissue samples were taken from the vent, eyes, wings, cere, and base of the beak after being euthanized. Viral DNA was isolated from the tissue samples using the gSYNC™/ DNA extraction Kit (Canada), following the manufacturer's instructions. For the PCR procedure, a classical PCR premix solution containing Taq DNA polymerase, MgCl2, dNTP mixture, buffer, and 1.5 µl of the template was used. The PCR components were thoroughly mixed and subsequently centrifuged. Following this, a fresh reaction mixture was prepared while maintaining a low temperature on ice. The RT-PCR tubes were then transferred to a thermal cycler for subsequent processing.

The commercially available Bioin Gentech Vet PCRTM Avian Pox Virus kit (Chile) was employed for the amplification of the APV (P4b) core protein gene. Oligonucleotide primers designed specifically for APV detection, sourced from the micro-gene company (Korea), were utilized to generate a target band of 329 base pairs (refer to Table 1). The amplification process comprised 32 cycles, involving denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step at 72°C for 7 minutes was incorporated into the protocol (Manarolla et al., 2010). The resulting PCR products were subjected to electrophoresis on a 1.5% agarose gel stained with Red safe Nucleic acid staining. Fragment sizes were determined using a 100 bp ladder (Intron, Korea).

To investigate genetic polymorphism in comparison to samples in the NCBI database, a comprehensive tree was constructed to determine the serotyping and phylogenetic distribution of the observed variants. A commercial forward sequencing method was employed to amplify the PCR products, and the resulting DNA sequences were compared with known sequences in the reference database. BioEdit Sequence Alignment Editor Software (Version 7.1) was utilized for the editing, alignment, and analysis of the sequencing data. Numerical positions were assigned to nucleic acid variations within the reference genome, and these were deposited in the NCBI-bankit database.

The amino acid sequences of the targeted P4b-encoded core proteins were extracted from the protein data library to convert the nucleic acid variants into amino acid residues. Using the Expasy online software, nucleic acid sequences were transformed into corresponding amino acid sequences. Amino acid sequence alignment was carried out to compare the altered sequences with their reference counterparts. For a comprehensive phylogenetic analysis, a specific tree was generated.

The NCBI-BLASTn server was utilized to compare the detected variations with homologous reference sequences. The neighbor-joining method was applied to construct an all-inclusive tree, visualized as a circular cladogram using the iTOL suite (Letunic and Bork, 2019).

Table 1. Gene name and oligonucleotide primers sequence designed

Gene name and SNP		Primers sequence 5′→3′	Guanine to cytosine (%)	Product size (bp)	Annealing temperature
SNP of selected gene(4b)	F	AATCTTAGAAAAGACGCAGATGCT	55%	329	58
	R	AAGTTTGTTGATTGAAACCTAGTCG	50%		

SNP: Single nucleotide polymorphisms, GC:

RESULTS

All samples from forty pigeons and poultry tested positive for APV. All the amplified cDNA showed identical mobility on 1.5% agarose gel. Positive samples generated a specific DNA band of 329bp (Figures 2, 3).

Sequencing results

Within this genomic locus, six positive samples were analyzed, comprising two samples for fowl pox virus (S2 and S3) and four samples for Pigeon pox virus (S1, S4, S5, and S6). The amplification targeted the P4b gene sequences of both fowl and pigeon pox viruses. Consequently, the variation observed in the P4b gene can serve as a marker for polymorphism in both viruses, suggesting its potential adaptability to genetic diversity.

To validate the identity of these PCR amplicons, sequencing reactions were conducted and subjected to an NCBI BLASTn analysis, revealing an exact match (Ye et al., 2012). The NCBI BLASTn engine revealed that there were approximately 99% sequence similarities between the sequenced sample and the targeted reference target sequences for the 329 bp amplicons of the fowlpox viruses. The precise locations and other features of the

retrieved PCR fragments were determined by comparing the observed nucleic acid sequences of the studied sample with the retrieved nucleic acid sequences (GenBank accession NoOR099892.1; Figure 4A). The NCBI BLASTn engine also revealed that there were almost 99% sequence similarities between the sequenced sample and the desired reference target sequences for the 329 bp amplicons of the pigeon pox viruses. Accurate locations and other details of the retrieved PCR fragments were detected (Figure 4B) by comparing the observed nucleic acid sequences of the studied sample with the retrieved nucleic acid sequences (GenBank acc. OP131515.1).

The features of the 329 bp amplicons' sequences were emphasized after placing them within the genomic sequences of the pigeon and fowlpox viruses. The overall length of the amplified amplicons was also ascertained (Table 2 A and B).

Regarding fowlpox viruses, two of the examined samples had a single nucleic acid variation (more precisely, a nucleic acid substitution) according to the alignment of the 329 bp local samples.

Upon comparison of the local samples with the most closely related reference nucleic acid sequences obtained from NCBI, notable variations were observed (Figure 5A). Similarly, in the case of pigeonpox viruses, two of the examined samples exhibited a single nucleic acid variation, specifically represented by a nucleic acid substitution during the alignment of the 329 bp sample. Distinctions were identified when contrasting the local samples with the most similar reference nucleic acid sequences acquired from NCBI (Figure 5B).

Concerning fowlpox viruses, the identified nucleic acid variant was 205 C>G. This difference observed in the currently observed nucleic acid sequences in the analyzed local samples was not positioned in the corresponding reference sequences of the viral P4b gene (GenBank acc. no. OR099892.1). Subsequent investigation demonstrated that the main protein was missense-impacted by this nucleic acid alteration. In particular, it caused the core protein sequence's position 101 to contain valine (Val) instead of leucine (Leu, Figure 6A). The identified nucleic acid variation for pigeons' pox viruses was 204 T > C. The matched reference sequences of the viral P4b gene (GenBank accession number: OP131515.1) did not contain this variation. It was discovered through analysis of its effects on the core protein that this variant is quiet and has no effect on the amino acid sequence. Proline (Pro) at position 108 of the core protein sequence served as an example of this synonymous variant (Figure 6. B). All the sequences were translated to proteins by using the Expasy translate suite.

However, these changes might have been triggered by the invader as a response to medications that target its core protein (Topalis et al., 2016). To get a distinct accession number in the NCBI database, the detected alterations were correspondingly put in the NCBI-banks database. Two GenBank accession numbers (OR619724 and OR619725) were acquired for this investigation to denote the S2 and S3 fowlpox virus samples, respectively. Additionally, four GenBank accession numbers representing the pigeon pox virus samples of S1, S4, S5, and S6 were obtained (OR670580, OR670581, OR670582, and OR670583, respectively).

Phylogenetic analysis

A phylogenetic tree was created in this study using nucleic acid variations from the amplified 329 bp P4b gene amplicons of the investigated viral particles. This provided insights into the phylogenetic distances between the local samples from chickens and pigeons and other avian pox virus sequences from NCBI. In this study, a phylogenetic tree was created by analyzing nucleic acid variations within the amplified 329 bp region of the P4b gene amplicons obtained from fowlpox and pigeonpox viruses. The tree included samples namely (S1-S6) and other relevant sequences from fowlpox and pigeonpox viruses (Figure 7). This tree provided visual representations of the viral sequences and their phylogenetic relationships. The tree included a total of 53 aligned nucleic acid sequences. It organized the sequences into two main clades representing fowlpox viruses and pigeonpox viruses, with two out-group clades consisting of other viral sequences. The inclusion of out-groups helps assess the variations among fowlpox viruses, pigeonpox viruses, and related sequences from other viral strains. A strong correlation was observed between the samples obtained from fowls (S2 and S3) and adjacent sequences within the fowlpox virus clade, which were obtained from different regions worldwide. The viral isolates were evenly distributed within the fowlpox virus clade, which contained the highest number of viral sequences (18 strains) at varying phylogenetic distances (Figure 7).

Within this main clade, both S1 and S2 samples have shown an extremely slight tilt with respect to the other neighbor positions. This pattern of the tiny altered positioning was due to the presence of only one genetic variation (205 C>G) in both samples. The generated phylogenetic tree provides confirmation of the sequencing reactions as it accurately reflects the neighbor-joining-

based positioning of the investigated sequences. Interestingly, the local samples S2 (OR619724) and S3 (OR619725) were found to be closely related to variable strains that have been isolated from different geographical locations worldwide. Most of these strains of fowl pox virus have been deposited from Iraq (Tikrit, Salah Al Deen), (NCBI MN915017.1, Egypt (NCBI MN708968.1), Iran (NCBI MG787229.1), Brazil (NCBI MK651856.1), India (NCBI MK370901.1), USA (NCBI MH175260.1) Turkey (KF722862.1), Tanzania (NCBI KF722863.1) and from China (NCBI KF875986.1).

On the other hand, the examined viral local isolates, namely S1, S4, S5, and S6 are closely related to several neighboring sequences within the avipox (pigeons) virus clade. These neighboring sequences have originated from distinct regions. The studied viral isolates occupy similar positions within the avipox virus clade. This clade comprises 14 sequences of varying strains. Within this major clade, the altered S4 and S6 local samples exhibit a very subtle deviation compared to the positions of their neighboring sequences.

However, it is worth noting that the examined S1, S4, S5, and S6 samples are closely associated with variable strains isolated from Australia (NCBI OP131512.1, OP131513.1, OP131514.1, and OP131515.1).

Apart from the fowlpox and avipox (pigeons) clades, two out-group clades were incorporated namely (S1 and S5). These clades were attributed to the canarypox virus with NCBI, LN 795883.1, LK 021649.1, LN795887.1, LN795890.1, LK021655.1 and LK021658., LK021648.1 from France GQ487567.1, MF102266.1 and MF102267.1 from Iran and the pigeon pox virus with NCBI OR099893.1 and OR099894.1 from Libya, MF102269.1, MF102270.1 and MF102271.1 from Iran and MF496043.1, MH365477.1, MH721412.1 and MH721417.1 from India, MH175237.1 from Canada.

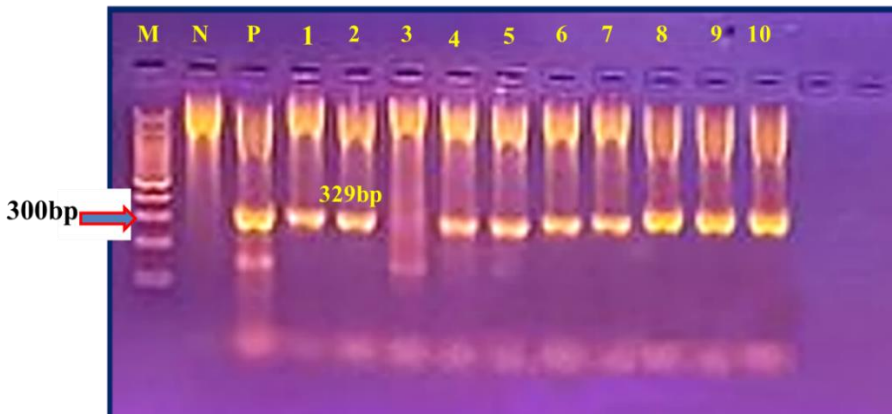


Figure 2. The P4b gene region was examined in tissue samples for the fowlpox virus using a conventional PCR method. Oligonucleotide primers were designed using NCBI to specifically amplify a 329 bp product size.

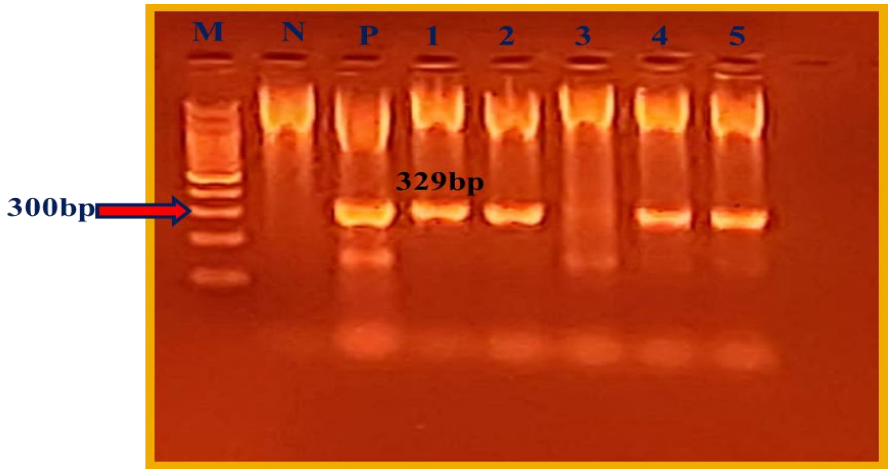


Figure 3. Tissue samples from pigeons and fowl suspected with avian pox virus were screened by PCR using designed oligonucleotide primers targeting the selected gene (p4b) to amplify a specific 329 bp band

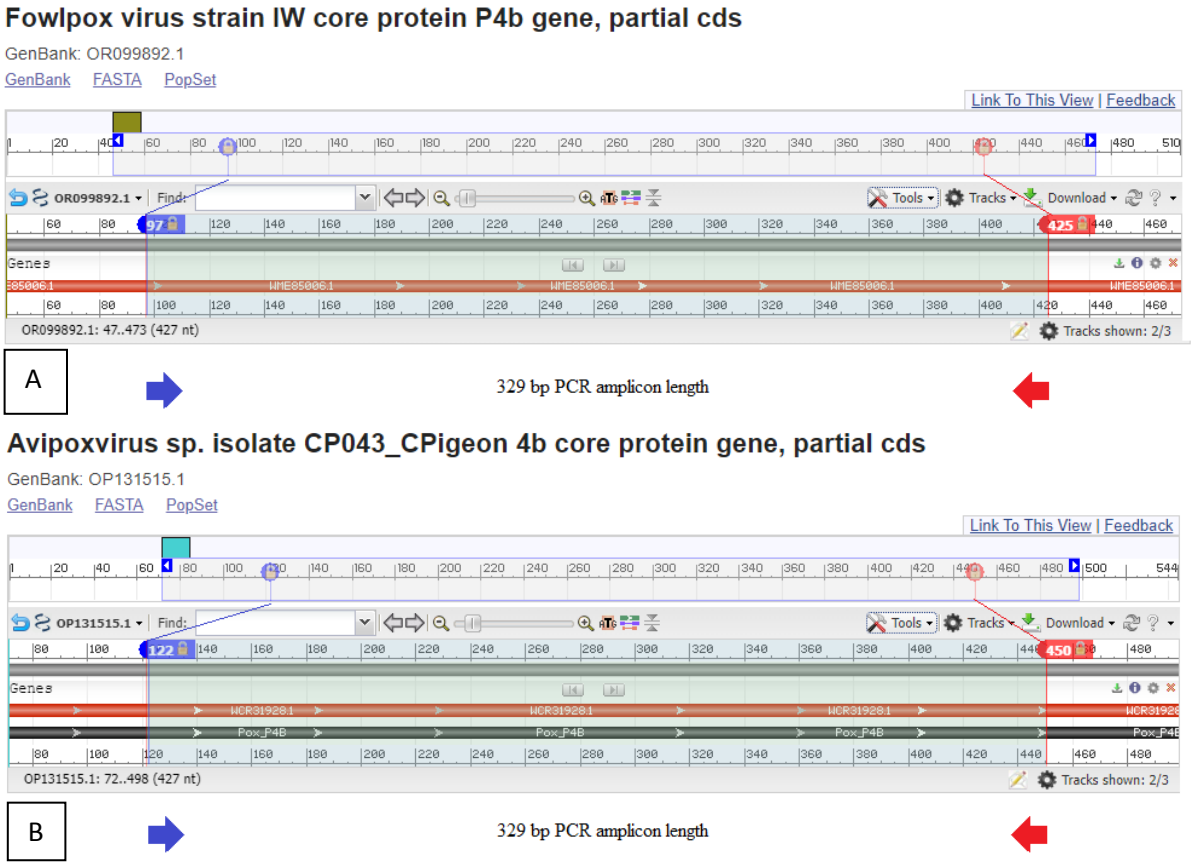


Figure 4. The retrieved 329 bp amplicon position partially encompassed a section of the P4b gene within fowlpox virus genomic sequences (GenBank acc. no. OR099892.1) and avipox virus genomic sequences (GenBank acc. no. OP131515.1) in branched A and B, respectively. The starting point of this amplicon is indicated by the blue arrow, while its endpoint is indicated by the red arrow

Table 2. The PCR amplicons, which measure 329 base pairs in length, were used to amplify a portion of the coding sequences of the P4b gene within the genomic sequences of fowlpox virus (GenBank acc. no. OR099892.1) and pigeon pox virus (GenBank acc. no. OP131515.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
A) Fowlpox P4b gene sequences	*AATCTTAGAAAAGACGCAGATGCTATAGTAAGATATCTCATGGATAGAAAATGTGA TATAAATAACTTTACGATACAAGACCTTATTCGCGTTATGAGGGAATTAAATATTAT TAGAAATGAAAGACAAGAGTTATTCGAGTTACTATCTCATGTCAAAGGATCACTTTC TAGTAATAGCGTTTCGGTAAAAACGAGTCATCCTCTAATGGTTATTTATTCGCACTC AGATAATAAGATAGGAGAACAGTTAAAACTACTAGAAAATACATATGATCCATCCAG ATATCAAGCGTTGATAGATACGACTAGGTTTCAATCAACAAACTT**	329 bp
B) Avipox P4b gene sequences	*AATCTCAGAAAAGATGCAGATGCTATAGTAAGATATCTTATGGATAGAAAATGTGA CATAAATAACTTTACGATACAGGATCTTATTCGAGTTATGAGAGAATTAAATATTAT TAGGAATGAAAGACAAGAGTTATTCGAGTTACTATCTCACGTCAAAGGATCTCTTTC TAGTAATAGTGTTTCGGTCAAAACTAGTCATCCTCTAATGGTTATTTATTCACATTC AGATAACAAGATAGGAGAACAGTTAAAACTACTAGAAAATACTTACGATCCATCTAG ATATCAGGCTCTAATAGATACTACGAGGTTTCAATCTACAAACTT**	329 bp

A)


10 20 30 40 50 60 70 80 90 100
ref.
AATCTTAGAAAAGACGCAGATGCTATAGTAAGATATCTCATGGATAGAAAATGTGATATAAATAACTTTAC
GATACAAGACCTTATTCGCGTTATGAGGG
S2 .....
S3 .....
110 120 130 140 150 160 170 180 190 200
ref.
AATTAAATATTATTAGAAATGAAAGACAAGAGTTATTCGAGTTACTATCTCATGTCAAAGGATCACTTTCT
AGTAATAGCGTTTCGGTAAAAACGAGTCA
S2 .....
S3 .....
210 220 230 240 250 260 270 280 290 300
ref.
TCCTCTAATGGTTATTTATTCGCACTCAGATAATAAGATAGGAGAACAGTTAAAACTACTAGAAAATACAT
ATGATCCATCCAGATATCAAGCGTTGATA
S2 ...G.....
S3 ...G.....
310 320
ref. GATACGACTAGGTTTCAATCAACAAACTT
S2 .....
S3 .....

B)

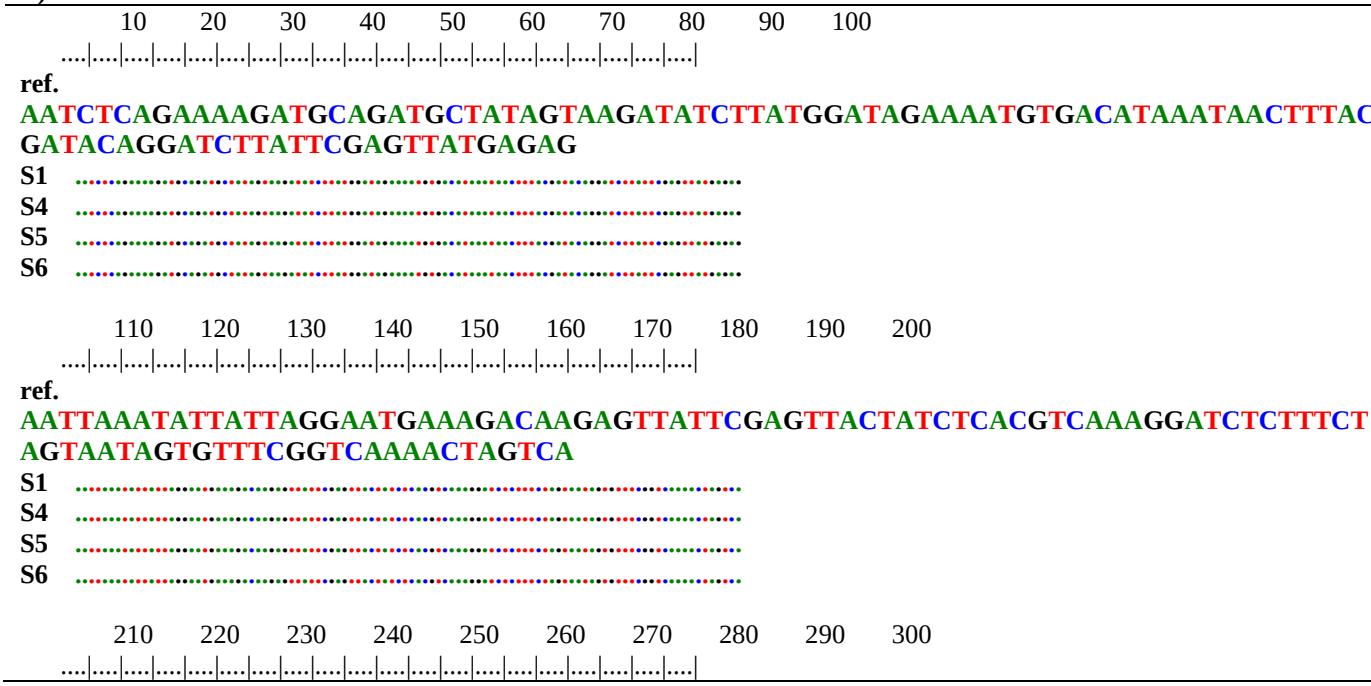
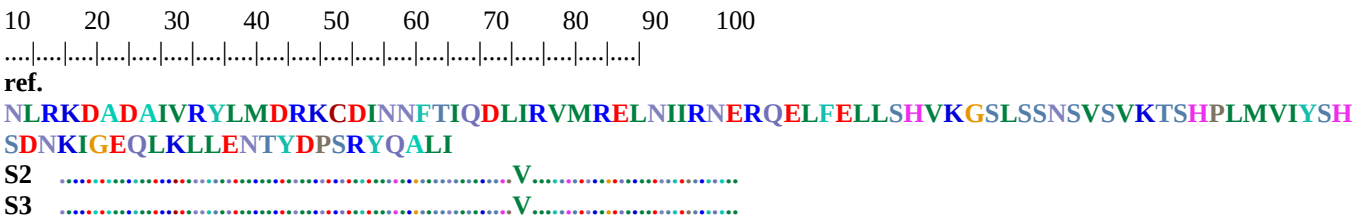

10 20 30 40 50 60 70 80 90 100
ref.
AATCTCAGAAAAGATGCAGATGCTATAGTAAGATATCTTATGGATAGAAAATGTGACATAAATAACTTTAC
GATACAGGATCTTATTCGAGTTATGAGAG
S1 .....
S4 .....
S5 .....
S6 .....
110 120 130 140 150 160 170 180 190 200
ref.
AATTAAATATTATTAGGAATGAAAGACAAGAGTTATTCGAGTTACTATCTCACGTCAAAGGATCTCTTTCT
AGTAATAGTGTTTCGGTCAAAACTAGTCA
S1 .....
S4 .....
S5 .....
S6 .....
210 220 230 240 250 260 270 280 290 300

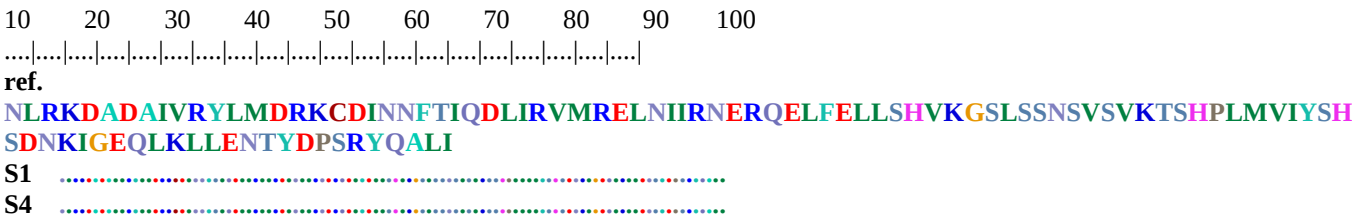
Figure 5. Nucleic acid sequences alignment of two samples with their corresponding reference sequences of P4b gene of fowlpox viruses in branch A, and avianpox viruses in branch B. The letter "S" followed by a number refers to the local sample number, whereas the sign "ref" refers to the NCBI referencing sequence.

A) sequences of amino acid residues of fowlpox virus P4b core protein



>WME85006.1 core protein P4b, partial [Fowlpox virus]
KPDICKGVSDSVKQKNTIINIDEITSTHDWQYNLRKDADAIVRYLMDRKCDINNFTIQDLIRVMRELNIIRNERQELFE
LLSHVKGSLSSNSVSVKTSHPLMVIYSHSDNKIGEQLKLLENTYDPSRYQALIDTTRFQSTNFVDMSTSSDMLFRFKD
QDSIGYVHPILVA

A) sequences of amino acid residues of avipox virus P4b core protein



ref. DTTRFQSTNF
S1 .....
S4 .....
S5 .....
S6 .....

>WCR31928.1 4b core protein, partial [Avipoxvirus sp.]
RLRSSSKPKPDICKGVSDSGKQKNTIINIDEITSTHDWQYNLRKDADAIVRYLMDRKCDINNFTIQDLIRVMRELNIIRN
ERQELFELLSHVKGSLSSNSVSVKTSHPLMVIYSHSDNKIGEQLKLLENTYDPSRYQALIDTTRFQSTNFVDMSTSSDM
LFRFKDQDSIGYVHPILVALFG

Figure 6. The amino acid residue alignment for variations in the P4b of fowlpox virus (branch A) and pigeonpox virus (branch B) samples. The positions of substitutions are highlighted within the 329 bp locus and the viral protein. Gray highlights indicate the amplified region. Missense and silent variants are highlighted in yellow and cyan, respectively, marking their specific positions on the amino acid residues in the chart.

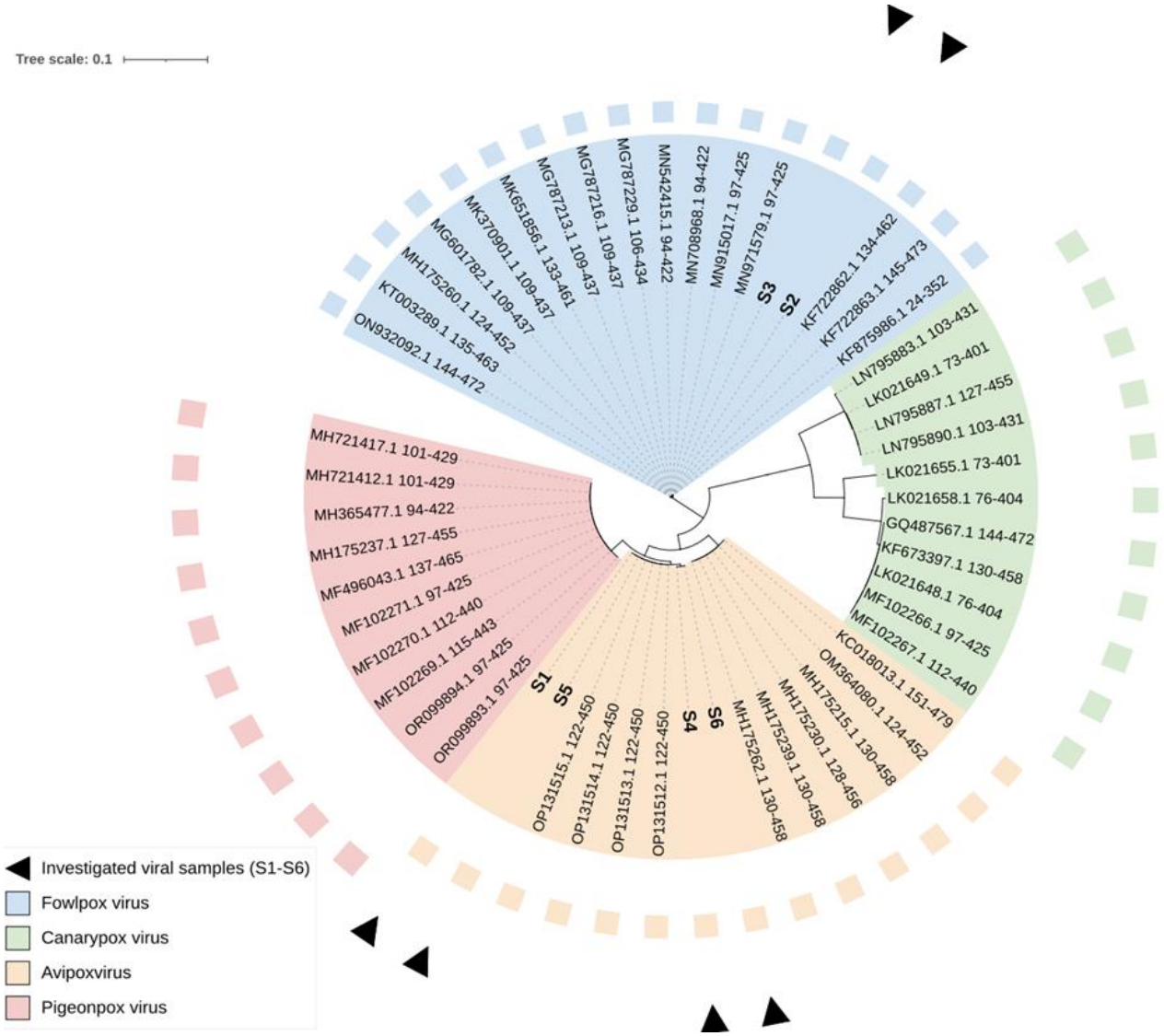


Figure 7. The circular cladogram phylogenetic tree illustrates the genetic variants of the P4b gene fragment in fowlpox and avipox viral samples. The analyzed viral variants are represented by black-colored triangles. The accompanying numbers correspond to the GenBank accession numbers of each respective species.

DISCUSSION

Avian pox is a highly contagious disease that affects domestic and wild birds worldwide. It is particularly prevalent in tropical and subtropical regions (Beukema et al., 2006).

The current study observed clinical signs in infected birds with the cutaneous form of the APV. These signs included an increase in small papules, pustules, or nodular lesions, which progressed to thick scabs resembling warts on featherless areas of the body. Initially, the lesions appeared as white or yellow patches and then converted into crusts that spread around the beak, legs, feet, combs, wattles, and other skin areas. Erosions, crusts, or nodules were also present in the vent area and on the skin of the head, specifically in the cere, beak, and eyelids of the bird (Figure 1). The present findings align with prior studies conducted by Mohan and Fernandez (2008) and Hibl et al. (2019), which highlight crusted and nodular lesions as characteristic features of the cutaneous form of fowl and pigeon pox virus. These manifestations were observed to be disseminated on the skin of the head and cranial cervical region. Notably, the majority of affected birds did not display additional clinical signs, and there was no notable increase in mortality within the examined flock. The primary focus of this study was on the core protein P4b shared by Fowlpox and avipox viruses. The researchers successfully amplified conserved regions within the gene responsible for encoding the P4b core proteins found in hen and pigeon virus strains. These regions are vital for the replication and structure of the viruses (Jarmin et al., 2006). The PCR assay, which was used in this investigation, proved to be a quick, incredibly sensitive, easy, and reliable diagnostic technique. The chosen oligonucleotide primers in the current study ensured a high sensitivity for APV particularly by targeting a highly conserved region of 4b. The P4b core protein gene of Apv was found to be present in all 40 samples taken from pigeons and hens in the current investigation using PCR analysis. The obtained result aligns with previous research by Fallavena et al. (2002) and Fagbohun, and Alaka (2021), demonstrating the practicality and effectiveness of PCR techniques in the identification of DNA viruses responsible for avipox and fowlpox.

In other circumstances, the intensity and diversity of the bird infection may be connected with the biological diversity of the P4b gene, which encodes the P4b core protein.

In this study, a particular PCR fragment was used to target the coding regions of the 4b gene in APV present in both pigeons and chickens. The amplified fragments were then subjected to direct sequencing to analyze the genetic variations in the local samples. All 40 samples obtained from pigeons and chickens tested positive for APV. Further examination was conducted on six selected samples, where two (S2 and S3) were associated with fowlpox virus, while the remaining four samples (S1, S4, S5, and S6) were linked to avipox virus specifically in pigeons. To amplify the P4b core protein gene sequences for the avipox and fowlpox viruses, respectively, these samples underwent a rigorous screening process. Since the P4b core protein gene may be able to adapt to varying genetic variety, as observed in various viral variations, it can therefore be utilized to explain the polymorphism of both viruses. Manarolla et al. (2010) study highlighted the avipoxvirus P4b core protein gene's molecular biological study. It was noted that this gene is frequently amplified for comparative genetic study and that it encodes a protein of 75.2 kDa. This study examined the 329 bp amplicons from the local viruses, and the findings revealed a striking degree of sequence similarity. The sequenced sample indicated over 99% sequence similarity with the corresponding nucleic acid sequences that were obtained from GenBank (OR099892.1 and OP131515.1), according to the NCBI BLASTn engine. Regarding the fowlpox and avipox viruses, the alignment results of 329bp revealed the presence of a single nucleic acid variation, represented by one nucleic acid substitution. This variation was observed in two fowl samples and two pigeon samples compared to reference sequences from NCBI. The present study identified a variant in fowlpox viruses (205 C>G) that caused a missense effect at position 101 (p.101Leu>Val) in the core protein sequences. However, a variant (204 T>C) in avipox viruses had a silent effect at position 108 (p.108Pro) in P4b. However, these alterations in the P4b may be a result of the invading organism adapting to drug treatments or implementing a preventative program using vaccinations for poultry and birds targeted against it (Topalis et al., 2016).

The construction of the phylogenetic tree relied on the nucleic acid variations identified in the amplified 329 bp region of the P4b gene amplicons from the investigated viral particles. Additionally, the study incorporated a phylogenetic analysis based on the nucleic acid variations in the P4b gene. The analyzed samples, along with other viral sequences, formed two distinct clades representing fowlpox viruses and pigeon pox viruses. The inclusion of out-group sequences expanded the scope of understanding regarding genetic diversity and evolutionary relationships

within the viral populations. These findings align with a prior local study conducted in Iraq by Hasan et al. (2021), which categorized f virus into two distinct clades. Clade A predominantly consisted of FPV strains from chickens (Gallus gallus), while the current study observed that pigeons were grouped within clade B. These results support previous research indicating the classification of fowlpox viruses into distinct clades, with chickens primarily belonging to clade A and pigeons grouped within clade B. In the main clade of the study, both the S1 and S2 samples showed a slight deviation compared to their neighboring positions. This deviation was attributed to a single genetic variation (205 C > G) present in both samples.

The positioning indicated that the 205 C > G variant had a minimal impact on causing a noticeable deviation from the original placement of other related viral samples within the main clade of the cladogram. The current study indicated that S2 and S3 were closely related to various strains of fowlpox virus isolated from different geographic locations worldwide. Many of these strains have been previously identified and deposited in GenBank from countries such as Iraq (Tikrit, Salah Al Deen), Egypt, Iran, Brazil, India, USA, Turkey, Tanzania, and China. The close positioning of the investigated S2 and S3 viral samples to strains from Asia, Africa, Europe, and the Americas suggests their international origins. This similarity could be attributed to factors, such as close borders and trade between neighboring countries, particularly Iran and Turkey. Moreover, the genetic similarity between local samples and globally distributed strains indicates the widespread presence of fowlpox virus variants in different regions, as noted in a study by Yeo et al. (2019). Furthermore, the observed similarity may arise from the importation of hens from infected countries to Iraq after completing their growth. Alternatively, it could indicate the presence of these isolates within Iraq due to their international distribution, as mentioned in the study by Jarmin et al. (2006).

In contrast, the analyzed viral isolates (S1, S4, S5, and S6) displayed a close relationship with adjacent sequences within the avipox virus clade, specifically those associated with pigeons. These neighboring sequences originated from various regions. The viral isolates occupied similar positions within this avipox virus clade, which included 14 sequences representing different strains. Among these sequences, the altered S4 and S6 local samples showed a slight deviation compared to the positions of their neighboring sequences. This deviation can be attributed to a single genetic variation (204 T > C) present in both of these local samples. The presence of this variation suggests that it has a limited impact on deviating from the original positions of other related viral samples within the main clade of the cladogram. The clustering of the majority of incorporated viral sequences further indicates a close phylogenetic distribution pattern among them. However, it is important to note that the examined S1, S4, S5, and S6 samples are closely associated with variable strains isolated from Australia (GenBank OP131512.1, OP131513.1).

Hence, the single nucleic acid variation identified in the local sample study indicates a minor deviation within the same viral clade, without imparting any notable evolutionary effects on the current viral positioning. The local samples S2 and S3, which are associated with the fowlpox virus, belong to the first clade, while samples S1, S4, S5, and S6, which are specific to avipox viruses that infect pigeons, form the second clade.

Although insignificant differences were observed in the current study, two outgroup clades were incorporated. These outgroup clades, namely S1 and S5, were attributed to the canarypox virus and the mixture of pigeon pox and mallard duck virus, respectively. Both outgroups were positioned in distinct phylogenetic positions, separate from the major clades of fowlpox and pigeons' viral sequences. The closest outgroup clade to the fowlpox virus was identified as the canarypox virus clade, while the closest outgroup clade to the avipox virus was the pigeonpox virus clade**.** Interestingly, the fowlpox viral sequences were located in close proximity to the roots of the phylogenetic tree, indicating their ancestral positioning compared to the canarypox virus, avipoxvirus, and pigeonpox viral sequences, respectively. Furthermore, the positioning of the three incorporated outgroups away from the root indicated that they are descendants of the ancestral fowlpox viral sequences. The results of the current study align with previous research conducted by Lüschow et al. (2004) and Jarmin et al. (2006). These studies reported that upon sequencing the amplicons, the majority of the isolates, excluding the QP-241 poxvirus from the Japanese quail, formed distinct clusters within two main Clades of avian poxviruses (APVs) including Clade A (Fowlpox virus) and Clade B (Pigeonpox virus), based on their genetic similarities. It is worth noting that the Japanese quail (QP-241) exhibited unique characteristics that distinguished it from other avian poxviruses.

CONCLUSION

In conclusion, the data presented in this study indicated that the analyzed P4b gene sequences offer a precise

description of the targeted fowl and pigeon poxviruses, without any confusion from closely related viral outgroup sequences. The use of P4b gene sequences in this study provided additional evidence for the accurate identification of viral serotypes. These findings support the observed divergence among pathogenic viral sequences infecting birds from various sources worldwide. The comprehensive phylogenetic tree constructed based on the P4b gene serves as a valuable tool for effectively identifying viral serotypes. This highlights the capability of the currently employed P4b gene-specific primers to accurately depict the studied fowl and pigeon poxviruses and their phylogenetic positions. Overall, the effectiveness of these P4b gene-based fragments in identifying viral sequences has been demonstrated, underscoring the precision of the currently utilized P4b gene-specific primers in characterizing the investigated fowl and pigeon poxviruses and determining their phylogenetic relationships.

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Availability of data and materials

The data and materials utilized in this study can be obtained by contacting the corresponding author directly or accessing them through a designated data repository.

Ethical considerations

Ethical issues (plagiarism, consent to publication, misconduct, data fabrication and/ or forgery, double publication and/ or submission and replication) were checked by the authors.

Authors' contributions

Aisha Faisal and Amer Al-Azzawi jointly proposed the hypothesis, designed the study, collected samples from various poultry farms, conducted the molecular work Both authors contributed to the preparation of the manuscript, and approved the final edition of the article.

Competing interests

The Authors declare that they have no competing interests.

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