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Molecular Detection of Pox virus in Diyala Province from Pigeons

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Abstract

An infectious viral illness called pigeon chicken pox develops lesions that are fibro-necrotic, proliferating, or nodular in the feather-free parts of the skin or mucous membranes in the mouth, esophagus, and upper respiratory tract. The goal of this work was to search the Diyala pigeon for the pigeon pox virus (PPV) and its sequences molecularly. In this investigation, nodular lesions from Six pigeons from various Diyala locations that had the pox were gathered. For the purpose of PCR-based P4b gene identification, DNA materials were extracted. PCR was used to validate the virus detection, sequenced and BLST search was carried out and pairwise distance was inferred. And all of the field samples tested positive for PPV. Pigeon PPV was verified by PCR. Diyala birds have pigeon pox, and PCR should be used to diagnose the condition.

Key Words : Molecular, Detection, Pox virus, Pigeons

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Introduction

The pox virus, which is a member of the subfamily Chordopoxvirinae and family Poxviridae, affects both domestic and wild bird species of different breeds, ages, and sexes(1). There are 25 different species in

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the genus Avipoxvirus (2). It is a common viral illness that affects commercial, wild, and domestic chickens. There are three main strains of the virus: pigeon, chicken, and canary (3). Lesions that are proliferative and nodular in featherless regions of the skin or fibro-necrotic and proliferating in the mucous membrane of the upper respiratory tract, mouth, and esophagus are associated with the disorder (2). The disease is divided into two types: wet or dipheria pox and dry or cutaneous pox. There is a higher mortality rate within infected birds with the more severe version of the sickness, called wet pox. Wet pox simply can cause substantial death rates in exposed birds, as high as 50–60%. The sickness inhibits the expansion and growth of chicks also pullets and reduces egg production in layers (4). The virus takes between 4 and 10 days in hens to fully incubate. Depending on the host's susceptibility, the virus strain's virulence, and the spread of the lesion, the disease's clinical symptoms may differ (5). The virus can spread by sharing food and water, as well as through microscopic tears in the skin or mouth. It can also be spread by mosquito bites. Infections are spread via mosquitoes, carrier birds, and polluted environments. The virus can persist in the environment for a very long time. Numerous factors have a significant impact on the rise in the disease's occurrence. These include breed variations, management techniques, and environmental circumstances (6). Economic losses caused by fowl pox include reduced growth, decreased egg production, and increase mortality (6, 7). Vaccination with live fowl pox virus (FPV) or pigeon pox virus has been used in Iraq, for control of the disease in the poultry industry, and economic losses have been minimized (8). Fast and specific confirmation of an infection is necessary, PCR was used to diagnose fallowed by sequencing in this study in Diyala province from pigeon samples for confirmation PPV.

Materials and methods

Collection of the samples

Six samples (of the pigeon pox) were collected

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from Diyala. Sick, infected birds' nodular lesions (Figure 1) were checked with a sterile blade before being placed in an Eppendorf tube containing 0.5 ml of sterile viral transfer media (VTM) (9). Samples were sent immediately to the laboratory of the Alqma in Baghdad.

Figure 1.Nodular lesion of PPV

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Molecular detection

In compliance with the manufacturer's procedure (QIAGEN, Germany), 200 µl samples were used to extract the viral DNA using the QIAamp. Through the use of 2% NAAgarose gel electrophoresis, each PCR product was found. In a thermal cycler (Mastercycler, Eppendorf, Germany) utilizing the (10) condition with slide modification, P4b gene specific PCR was carried out. the above-mentioned P4b gene primer sequence The green PCR master mix (Promega®, USA), forward and reverse primers, nuclease-free water, and template DNA were all combined to make a 25 l PCR mixture. The P4b gene was amplified using a thermal profile that included initial denaturation at 94°C for 5 minutes, followed by 35 cycles of reaction that each lasted 94°C for 45 seconds and 48°C for 1.5 minutes. A 35-cycle reaction consisting of 94°C for 45 seconds, 48°C for 1.5 minutes, 60°C for 2 minutes, and final extension at 60°C for 10 minutes comprised the thermal profile used for the amplification of the P4b gene. 1.5% agarose gel was used to evaluate the PCR results. (Sigma-Aldrich, USA). Lee & Lee (1997) employed two sets of primers as fallow: 2fPF CAGCAGGTGCTAAACAACAA and p2fPR CGGTAGCTTAACGCC GAATA. The PPV amplicon size was 578 bp (11, 12). The purpose of this work was to employ molecular methods to distinguish Pigeon pox virus.

Partial sequencing and phylogenetic analysis:

To guarantee good read data, ChromasPRO (Technelysium Pty Ltd, Helens vale, Australia) was used to initially assess the incomplete p4b sequences. Next, a BLAST search against the NCBI database (GenBank) was used to establish the identities of the sequences and homology(13).

Every pox positive sample was used its alignments to reference genomes performed in MEGA6 (14), and phylogenetic analysis was inferred using the neighbor-joining approach with 1000 bootstrap repetitions.

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Reference strains that were employed in the investigation with default parameters and 1000 bootstrap resampling, the highest-likelihood analysis was used to estimate the tree structure of both isolate & reference sequences. Identify which samples proved positive by running a PCR using the P4b gene as the target(15).

Results

Molecular detection of the viruses from field samples

In this investigation, six suspected field samples of fowl pox (nodular lesions) from infected pigeons were collected and used for molecular identification. DNA was isolated from the suspected field samples of pigeon pox, and PCR was then carried out to detect a unique P4b gene specific primer. 100% of the four samples examined were PCR positive (Figure 2).

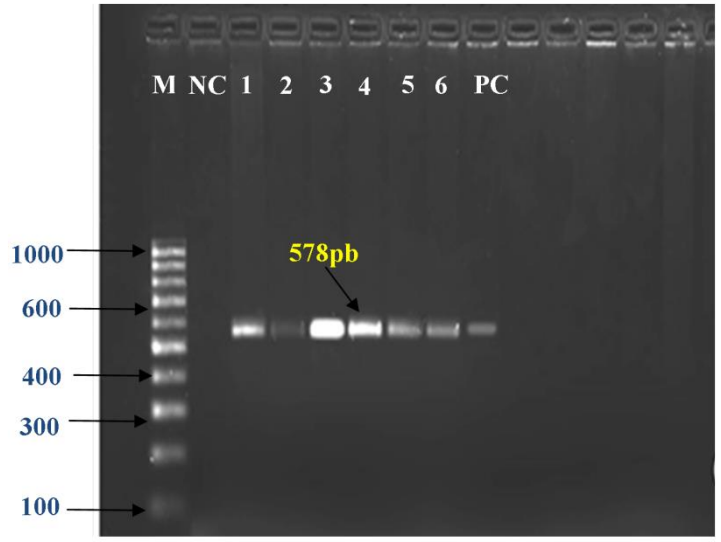

Figure. 2. Electrophoresis results of PCR products of PPVshowing specific bands for *P4b* gene on 1.5% agarose gel. M= 1kb DNA marker; L1 to L6 = PPVm=, L7, NC+ negative control, PC=positive control.

Phylogenetic Analysis

Based on the sequences (all six sample gave the same sequences), representative individuals from related viral sequences, and the closest virus relatives determined by the best BLAST, phylogenetic analysis was carried out. Using MEGA 6, sequence

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alignment was carried out using Clustal W at its default settings [11]. In order to match the incomplete (P4b) genomic sections of the viruses acquired for the study, aligned sequences were cut. The neighbor-joining approach, which depends on the p-distances model developed by MEGA, was used to create a tree of phylogeny with 1000 bootstrapping resamples from the alignment data sets [12]. Every node's bootstrap values are provided. Names matched those of NCBI strains and shared 99% of their similarities (Table 1).

Nucleotide Sequence

The sequences of the viral genome were uploaded to GenBank and are awaiting an entry number (Diyala Akram P1). Table 2 lists the additional reference genes that were employed in this investigation: MT499376.1, MT499377.1, OR099895.1, OR0270230.1, and OR0270235.1. (Table 2 Figure 3).

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Table 1: Matching identities of the PPV strain in this study (subjected) against the query in the NCBI.

Identities:456/458(**99%**), Gaps:0/458(0%), Strand: Plus/Plus

```
Query  1    GATATATGTAAAGGAGCTTTAGATTCTGGAAAACAAAAAAATACAATTATCAATATAGAT  60
            |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  49   GATATATGTAAAGGAGTTTTAGATTCTGGAAAACAAAAAAATACAATTATCAATATAGAT  108

Query  61   GAAATTACATCAACTCATGACTGGCAATATAATCTCAGAAAAGATGCAGATGCTATAGTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  109  GAAATTACATCAACTCATGACTGGCAATATAATCTCAGAAAAGATGCAGATGCTATAGTA  168

Query  121  AGATATCTTATGGATAGAAAATGTGACATAAATAACTTTACGATACAGGATCTTATTAGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  169  AGATATCTTATGGATAGAAAATGTGACATAAATAACTTTACGATACAGGATCTTATTAGA  228

Query  181  GTTATGAGAGAATTAAATATTATTAGGAACGAAAGACAAGAGTTATTCGAGTTACTATCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  229  GTTATGAGAGAATTAAATATTATTAGGAACGAAAGACAAGAGTTATTCGAGTTACTATCT  288

Query  241  CACGTTAAAGGATCGCTTTCTAGTAATAGTGTTTCTGTCAAAACTAGTCATCCACTAATG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  289  CACGTTAAAGGATCGCTTTCTAGTAATAGTGTTTCTGTCAAAACTAGTCATCCACTAATG  348

Query  301  GTTATTTATTCACATTCAGATAACAAGATAGGGGAACAGTTAAAACTACTAGAAAATACT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  349  GTTATTTATTCACATTCAGATAACAAGATAGGGGAACAGTTAAAACTACTAGAAAATACT  408

Query  361  TACGATCCATCTAGATATCAGGCTCTGATAGATACGACGAGGTTTCAATCTACAAACTTT  420
            |||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  409  TACGATCCATCTAGATATCAGGCTCTGATAGATACTACGAGGTTTCAATCTACAAACTTT  468

Query  421  GTGGATATGTCAACGTCTAGTGATATGTTGTTTAGATT  458
            |||||||||||||||||||||||||||||||||||||
Sbjct  469  GTGGATATGTCAACGTCTAGTGATATGTTGTTTAGATT  506
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Table 2: References strains that used in this study and their isolated countries of Pigeon pox virus.

Strains	Country
MT499376.1	Iraq
MT499377.1	Iraq
OR099895.1	Libya
OR0270230.1	Egypt
OR0270235.1	Egypt

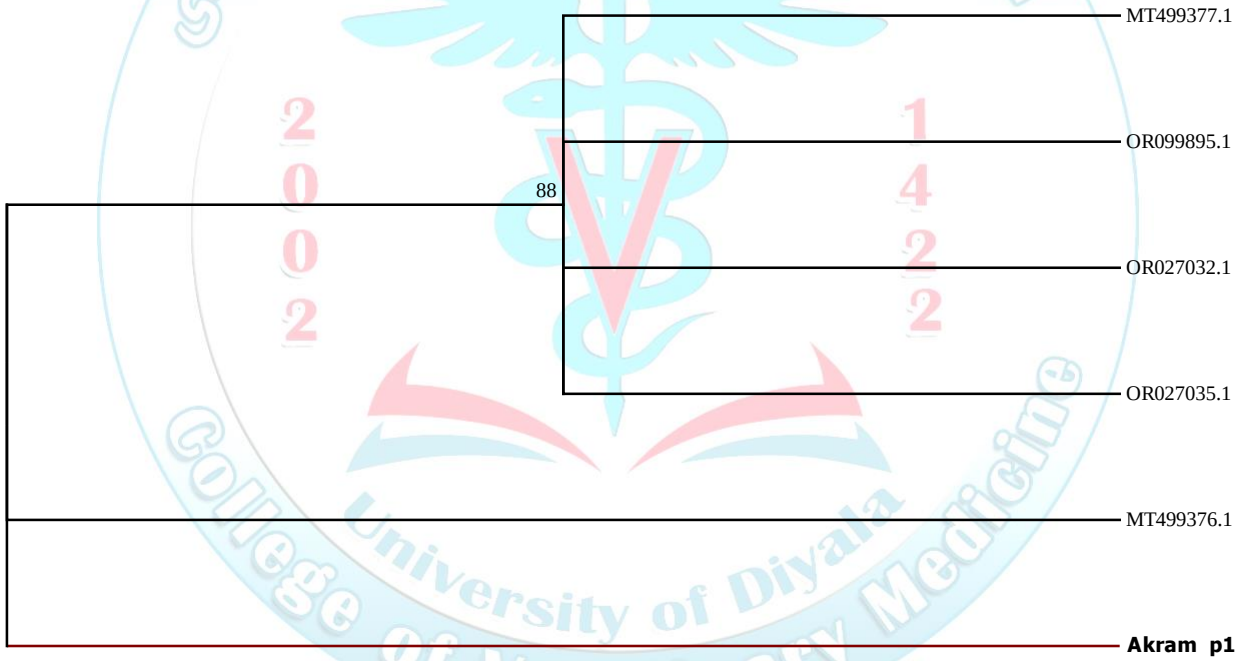

Figure 3: Using MEGA 6.0 and the Clustal W technique, the partial P4b gene nucleic acid sequences of the collected Pox virus strains and a selection of reference strains were used to identify their evolutionary connections.

Discussion

Pigeons from the Diyala districts were gathered, and their nodular lesions were treated to PCR in order to confirm the illnesses. Every instance happened in Diyala's eastern and central regions, and the majority of diagnoses came from standard clinical examinations. PCR results for all samples subjected to testing were positive.

PCR has been shown in several studies to be a useful method for identifying strains of the avian

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pox virus (10, 16, 17). Following infection confirmation, the P4b gene was amplified, resulting in the study's predicted sizes. Thus PCR is an extremely valuable method for the confirmation of PPV infections(18). Histopathological analysis, microscopy with electrons, the isolation of the virus on chorioallantoic membrane (CAM) of formed chicken eggs or cell cultivated, and serological tests were the traditional laboratory techniques used to diagnose the Pox virus (11, 19). These methods are labor-intensive and require specialized laboratory supplies (20, 21). Molecular biology approaches have shown to be among the most accurate ways for routine diagnosis in recent times. In particular, during the past several years, the use of PCR, which depends on the amplified of a 578 bp area of the extremely stable P4b gene of APVs, for diagnosis has increased (22).The current study's findings diverge from those reported by Masola et al. (2014) since in their research, 66 (42.86%) of the 154 samples that were examined were found to be positive for PPV by PCR. This variation might have its origin in the significant differences in sample sizes across these studies. Compared to the 154 samples analysed by Masola et al. (2014), we only looked at six samples in our inquiry. The detection of molecules rate of PV in the line of this investigation was reported by Roy et al. (2013) to be almost 100% by PCR, which is and the molecular findings of the current study are almost exactly the same as those published by Roy et al. The present investigation's findings are somewhat different from the results from Kabir et al.'s (2015) investigation, which found that 32 (80%) among the 40 samples tested tested positive for the pigeons pox virus. According to the evolutionary trees for the P4b encoding sequences, Akram p1 are divided into two different sub-genotypes. A distinct clade was generated by the five strains (MT499376.1, MT499377.1, OR099895.1, OR0270230.1, and OR0270235.1). Akram P1 was phylogenetically more connected to the isolated strain MT499376.1, and those 5 viruses were more similar to the strain MT499376.1 in terms of their genetic distance. It was discovered using PCR that PPV was present in all four field specimens collected for pigeon pox. The pigeon pox virus is rife in the birds of Diyala province.

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