KJVR
Korean Journal of Veterinary Research

Original Article

Virology

pISSN 2466-1384 · eISSN 2466-1392
Korean J Vet Res 2024;64(1):e7
https://doi.org/10.14405/kjvr.20230048

*Corresponding author:
Harith Abdulla Najem
Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Basrah, Karmat Ali 61001, Iraq
Tel: +964-40-412714
E-mail: harith.najem@uobasrah.edu.iq
https://orcid.org/0000-0002-4203-5282

Conflict of interest:
The authors declare no conflict of interest.

Received: Dec 19, 2023
Revised: Mar 3, 2024
Accepted: Mar 6, 2024

Clinical and molecular detection of fowl pox in domestic pigeons in Basrah Southern of Iraq

Isam Azeez Khaleefah[1], Hassan M. Al-Tameemi[2], Qayssar Ali Kraidi[3], Harith Abdulla Najem[1], Jihad Abdulameer Ahmed[1], Haider Rasheed Alrafas[2]

[1]Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Basrah, Basrah 61001, Iraq
[2]Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah 61001, Iraq
[3]Department of Basic Sciences, College of Nursing, University of Misan, Maysan 62001, Iraq

Abstract

Bird species, particularly poultry and other bird types, including domestic pigeons, are susceptible to fowl pox, a contagious viral disease. The main goal of this study was to validate clinical avipoxvirus diagnoses using molecular analytical methods. The essential components of the investigation were the clinical signs, visible abnormalities, histological changes, and polymerase chain reaction analysis. Twenty out of 120 pigeons had clinical symptoms, which included yellowish crust or nodules near the feet, eyes, and beak. An erosive epidermal lesion and an epidermal acanthotic papular lesion with basal vacuolation were maculopapular evidence associated with significant epidermal hyperkeratosis, as confirmed by histological analysis. In addition, the results showed keratinocyte necrosis beneath the hyperkeratotic epidermal layer, together with superficial and deep dermal perivascular lymphocytic infiltration. In addition, the P4b core protein gene underwent phylogenetic analysis. The sequence analysis results indicated a high degree of similarity across the local strains, with just minor variations observed. Five sample sequences were selected and submitted to the NCBI database. These sequences were identified as OR187728, OR187729, OR187730, OR187731, and OR187732. All the various strains in this research may be classified under clade A of the chicken pox virus phylogenetic classification. This study presents the first description and characterization of pox virus infections in domestic pigeons inside the Basrah governorate.

Keywords: bird pox virus; pigeon pox; polymerase chain reaction; phylogeny

Introduction

Avian pox (AP) is a viral disease that affects birds. The genus *Avipoxvirus* belongs to the subgroup *Chordopoxvirinae* of the *Poxviridae* family of viruses [1]. The genus *Avipoxvirus* has enveloped viruses. The genomes are linear and 300 kb in length. The 12 different species that comprise this family are the canarypox, flamingopox, fowlpox, juncopox, Mynahpox, penguinpox, pigeonpox, psittacinepox, quailpox, sparrowpox, starlingpox, and turkeypox viruses. [2].

More than 230 species of domestic and wild birds are global victims of avipoxviruses. It affects many birds but rarely occurs in waterfowl or shorebirds [3]. These include pets, seabirds, songbirds, upland game birds, chickens, turkeys, and occasionally raptors. In addition, the disease is more prevalent in warm and humid re-

gions. This suggests that AP is a recurrent viral disease [4]. The disease characteristics include unique, proliferative lesions on the epidermis of the head, legs, or toes and on the mucous membranes of the mouth and upper respiratory tract. The progression of the disease in birds is typically slow, and there is the possibility of systemic disease. The infection is spread when a mosquito bites a bird with the virus in its circulation or bites close to lesion secretions. The virus is transferred to a new host when the mosquito bites and feeds on a vulnerable host. Airborne particles or contaminated surfaces can also spread the virus indirectly. Infections can result when contaminated particles come into contact with the mucous membranes or skin abrasions [5]. Although the virus has been effectively disseminated within a limited area via mosquito transmission, long-distance transmission between sick birds has allowed the virus to proliferate [6].

The clinical symptoms can be divided into cutaneous and systemic. The cutaneous forms can occasionally be seen in large clusters and manifest as wart-like growths around the eyes and other (feather-free) places [7]. Lesions from a poc might be dispersed or localized. The degree of infection determines the size and quantity of these growths [8]. Yellowish, elevated lesions on the mucous membranes of the mouth, esophagus, trachea, and lungs are the hallmarks of diphtheritic, or wet, pox. Breathing or swallowing difficulties may result from them. Birds may appear frail and malnourished in both situations [9]. The circulating strains remain unclear because of a lack of research on the prevalence of pigeon pox among wild birds and domestic populations. The present study monitored domestic pigeons in Basrah, Iraq, to determine infected birds with pox. Molecular analysis was then performed to confirm the spread of avipoxvirus in Basrah, Iraq.

Materials and Methods

Clinical monitoring and sample collection

Samples were collected from suspected cases observed in Veterinary Clinics and reported by pigeon owners in Basrah city center. The owner's name, bird's tag, and color were recorded. The study duration was from May to June 2022. A comprehensive examination of 120 domestic pigeons revealed 20 that displayed the clinical symptoms of fowl pox. The focus on pigeons with skin lesions, a prevalent and diagnostically valuable indication of the disease, underscored the importance of accurate sample collection and analysis. This endeavor was crucial for the well-being of the pigeon population and the overall management of potential disease outbreaks within the district.

The suspected cases were sent to the Department of Veterinary Pathology and Poultry Diseases at the University of Basrah for examination. Tissue samples from the skin around the eye and peck were pinched and pulled out using a scalpel, and each piece was divided into portions. The first portion was pooled in a sterile container with 5 ml of a 50% glycerol phosphate buffer solution and stored at -20°C for the polymerase chain reaction (PCR). The second portion was fixed in a 10% formalin solution for the histopathological examination, according to Luna [10].

DNA extraction and PCR amplification

Virus DNA was extracted from dermal lesions and a vaccine for fowl pox using a commercial kit (Wizard Genomic DNA Purification Kit; Promega, USA) according to the manufacturer's instructions. DNA was eluted and stored at -20°C until used. For all PCR reactions, the mixture contained 25 μL of GoTaq G2 Colorless PCR Master Mix (Promega), 5 μL of the extracted DNA, 1.5 μL of each primer, and nuclease-free water that was added to a final volume of 50 μL. A control positive tube (DNA extracted from a standard Fowl Pox vaccine) and a negative control tube (DNA extracted from a control negative sample) were included. The presence of pox virus in the DNA extracted from clinical samples was confirmed by performing poxvirus-specific PCR with the primers designed for amplifying the 578 bp pox virus 4b gene (virus core protein) (4b forward, 5'-CAGCAGGTGCTAAACAACAA-3', and 4b reverse, 5'-CGGTAGCTTAACGCCGAATA-3') [11,12]. Macrogen (Korea) was then used to purify and sequence the PCR products of the pox virus 4b gene. The PCR cycles for the *Avipoxvirus* 4b gene consisted of one cycle at 94°C for 5 minutes, 35 cycles at 94°C for one minute, 61°C for one minute, and 72°C for one minute. One cycle was performed at 72°C for 10 minutes after these cycles. The clade of the poxvirus-positive sample was determined by amplifying the locus fpv140 by PCR using the forward primer (5'-GAAGTAGAGTTATCGGTTC-3') and the reverse primer M2912 (5'-GGTGATCCATTTCCATTTC-3'), as described elsewhere [13,14]. The fpv140 PCR distinguishes between clades A and B, which are 1,800 and 2,400 base pairs long, respectively. Amplification was performed after initial denaturation for 5 minutes at 94°C for 35 cycles and consisted of one minute of denaturation at 94°C, one minute of annealing at 55°C, and 2 minutes of extension at 72°C. A final extension step was performed for 15 minutes at 72°C. Subsequently, 2% (for P4b) and 1.2% (for fpv140) agarose gel electrophoresis was used to separate 5 microliters of the amplified PCR products. Ethidium bromide was then used to stain the samples, and an ultraviolet transilluminator was used to observe them next to a 1 K

https://doi.org/10.14405/kjvr.20230048

DNA ladder (Promega). Evolutionary analyses were conducted using MEGA11, and the evolutionary history was inferred using the neighbor-joining method.

Results

Clinical signs

In this study, 120 pigeons were examined. Twenty suspected cases of pigeon pox showed the cutaneous forms of pox infection, characterized by the development of wart-like growths on the skin and mucous membranes of the bird. These growths were observed on the head, face, beak, eyes, legs, feet, and wings. The growths were grayish-white in color and ranged in size from small papules to large nodules. These lesions were associated with swelling, redness, and ocular discharge, which led to impaired vision and difficulty in feeding in some cases (Fig. 1). These signs were more common in young pigeons. The suspected cases were lethargic and less active, tended to spend more time resting or sleeping, and showed less interest in their surroundings.

Histopathological study

Histopathology revealed severe epidermal hyperkeratosis, and there was epidermal maculopapular evidence of an erosive epidermal lesion (Fig. 2). In addition, epidermal acanthotic papular lesions with basal vacuolation and superficial and deep dermal perivascular lymphocytic infiltration were observed (Fig. 3). An epidermal acanthotic papular lesion with basal vacuolation and superficial and deep dermal perivascular lymphocytic infiltration were noted (Fig. 4). An area of keratinocyte necrosis was observed beneath the hyperkeratotic epidermal layer, as shown in Fig. 5.

DNA extraction and PCR amplification

Pox virus-specific DNA (amplification of the 578 bp region of

Fig. 1. Pigeon pox. (A) Cutaneous forms in young. (B) Cutaneous forms in adults.

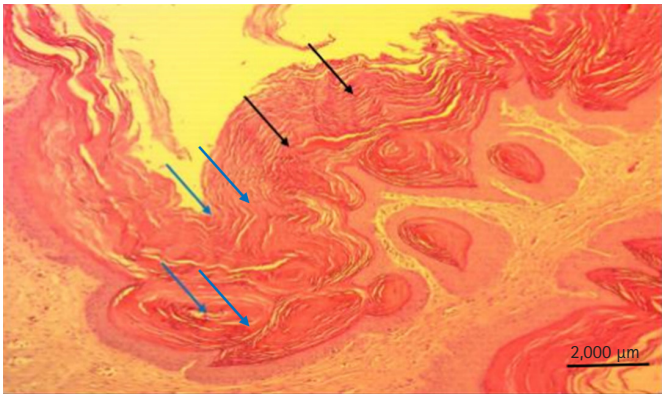

Fig. 2. Histopathological micrograph of the skin of a poxvirus-diseased bird shows severe epidermal hyperkeratosis (black arrows) and epidermal maculopapular evidence of an erosive epidermal lesion (blue arrows). Hematoxylin and eosin stain, scale bar = 2,000 μm.

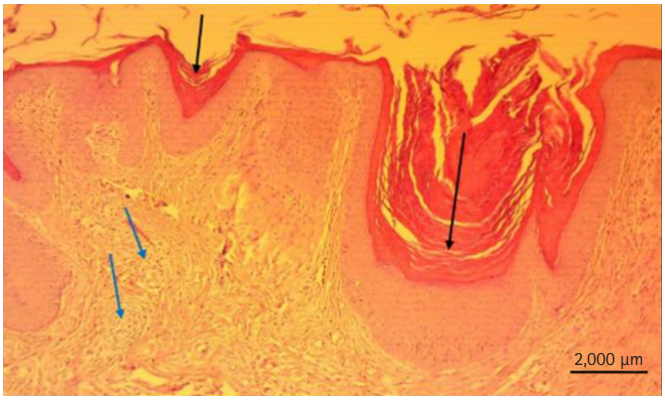

Fig. 3. Histopathological micrograph of an infected bird's skin caused by the poxvirus shows an epidermal acanthotic papular lesion with basal vacuolation (black arrows). There is superficial and deep dermal perivascular lymphocytic infiltration (blue arrows). Hematoxylin and eosin stain, scale bar = 2,000 μm.

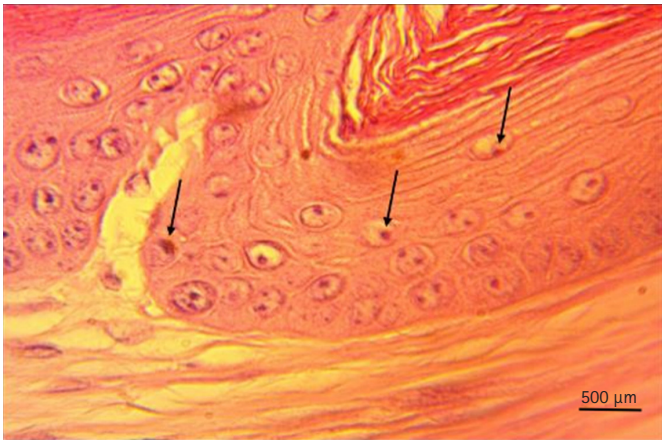

Fig. 4. Histopathological micrograph of the skin of a poxvirus-diseased bird shows intracellular eosinophilic cytoplasmic inclusion bodies in the epidermal keratinocytes (arrows). Hematoxylin and eosin stain, scale bar = 500 μm.

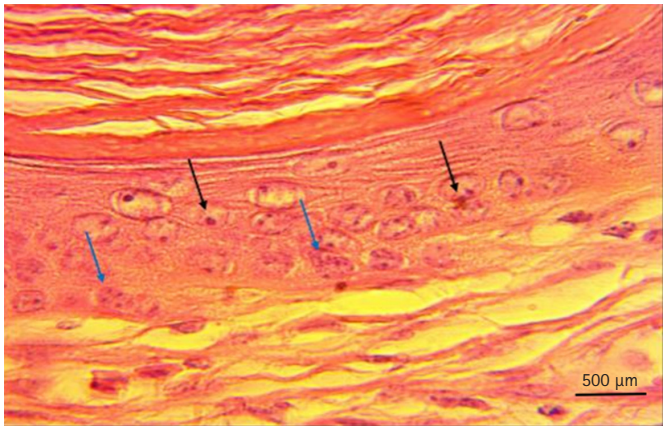

Fig. 5. Histopathological micrograph of the skin of a poxvirus-diseased bird shows intracellular eosinophilic cytoplasmic inclusion bodies in the epidermal keratinocytes (black arrows). An area of necrosis of keratinocytes can be observed beneath the hyperkeratotic epidermal layer (blue arrows). Hematoxylin and eosin stain, scale bar = 500 μm.

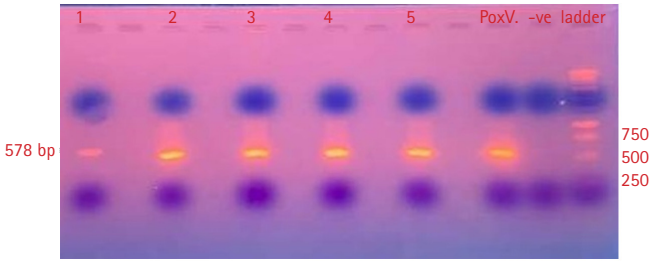

Fig. 6. Polymerase chain reaction amplification of the pox virus 4b gene-virus core protein gene from the isolates of this study, which shows 578 bp DNA bands. Lanes 1-5 represent samples, and Lane 6 (PoxV) represents the Pox vaccine, followed by the negative control. The last lane is the DNA size marker (1 kbp DNA ladder).

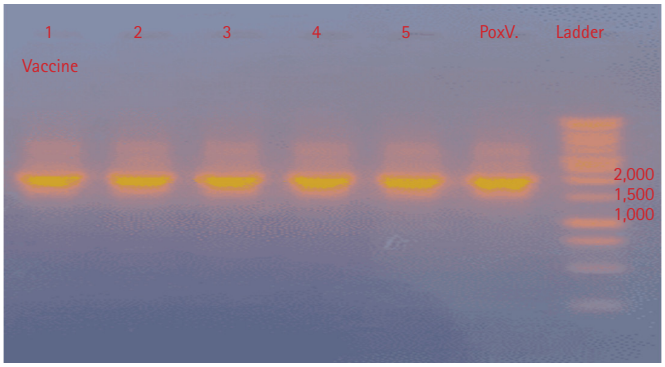

Fig. 7. Fpv140 gene locus amplified by polymerase chain reaction from the isolates, which show 1,800 bp DNA bands. Lanes 1-5 represent the samples, Lane 6 (PoxV) represents the Pox vaccine, and the last lane is the DNA size marker.

the highly conserved P4b gene of the pox virus core protein) was detected in the vaccine and the 20 suspected samples. Five random positive PCR samples were extracted and sent for sequencing to acquire a cross-sectional representation of the observed cutaneous symptoms and enable a more precise and nuanced analysis of the underlying disease.

Sequence analysis showed that all 5 strains were closely related, with relatively minor differences. Five representative sequences were selected and deposited in the NCBI database (OR187728, OR187729, OR187730, OR187731, and OR187732). Fig. 6 shows the derived phylogenetic trees. All the strains of this study were included in clade A (fowl pox virus) according to the phylogenetic classification by Jarmin et al. [15] (Fig. 7). Consequently, amplification of the gene fpv140 gave 1800 bp PCR-positive results, including the vaccine-positive control (Fig. 8).

Discussion

The virus known as fowl pox produces proliferative and nodular lesions on the featherless areas of the skin or fibrino-necrotic and proliferative lesions in the esophagus, mouth, and upper respiratory tract mucous membranes [15]. The frequency of pigeon pox in Iraq has not been well documented. Research on pigeon pox in Basrah is needed to describe the most current strain in circulation, monitor their evolution, and ascertain their degree of similarity to vaccine strains. This study assessed a group of 120 pigeons from different flocks. Among the pigeons screened, 20 showed clear clinical indications of the cutaneous form of pigeon pox, as evidenced by the lesions on their skin in feathers-free locations. Some of these birds even partially or completely lost one or both eyes. The diagnostic signs revealed a characteristic pattern typical of pigeon pox infection, the most common symptom being the appearance of wart-like growths on the skin and mucous membranes of the pigeons. These growths, ranging in size from small papules to large nodules, were spread uniformly across different anatomical sites such as the head, face, beak, eyes, legs, feet, and wings. In terms of color, these growths are generally gray and white and include a comprehensive range of sizes and locations. These outcomes were comparable to a previous study [16] that also noted lesions in the skin, mouth, and upper respiratory tract membranes.

The histopathological examination of affected tissues provides valuable insights into the characteristic lesions and changes associated with the disease. In this study, a microscopic examination of the pox-infected skin of a pigeon showed that it had acanthosis, which is a thickening of the epidermis caused by the growth of keratinocytes. There was also epidermal maculopapular evidence of an erosive epidermal lesion. These re-

https://doi.org/10.14405/kjvr.20230048

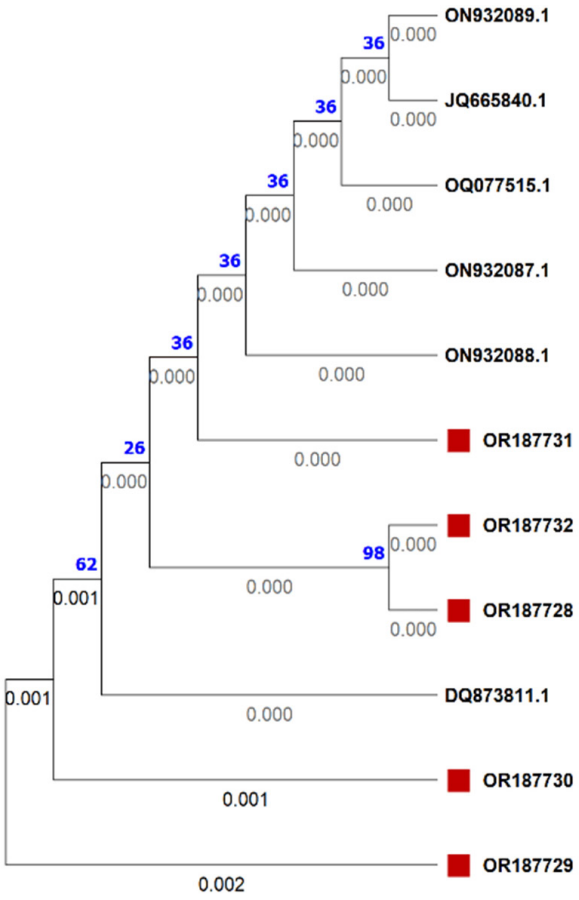

Fig. 8. Phylogenetic tree analysis of 5 pigeon pox viruses (this study) constructed based on *Avipoxvirus* 4b gene sequence analysis, showing the phylogenetic relation of strains isolated from pigeons (red square) and closely related pigeon pox strains from NCBI database. Evolutionary analyses and the tree was constructed using the neighbor-joining method with MEGAX11 software.

sults concurred with those reported elsewhere [17]. The affected keratinocytes had larger nuclei, eosinophilic intracytoplasmic inclusion bodies, and vacuolation. There was also an area of necrosis of keratinocytes [18]. The affected skin had keratinization, hyperplasia, and several phases of intracytoplasmic inclusion bodies.

In addition to the changes in the epidermis, a pigeon pox infection often induces an inflammatory response in the underlying dermis. The infiltration of mononuclear cells—mainly lymphocytes, macrophages, and plasma cells—defines the inflammation. The presence of these inflammatory cells is indicative of the host's immune response to the viral infection. Necrosis and a complicated buildup of inflammatory cells (macrophages and heterophils) were observed in the hyperplastic epidermis [19].

A specific pan-gene marker was the highly conserved P4b core protein gene in diagnostics and phylogenetic analysis of the poxvirus. Regarding molecular biological analysis, all isolates in this investigation generated gene P4b amplification products of the anticipated size. Hence, PCR is an excellent method for diagnosing pox virus infections. According to previous studies [14,20], 3 main clades of AP viruses have been identified using phylogenetic analysis using the P4b gene (fpv167) sequence: clade A (psittacine pox-like viruses), clade B (canarypox-like viruses), and clade C (fowlpox-like viruses). Clade A can be split into subclades A1 (fowlpox virus), A2 (turkey pox virus), A3, and A4, while clade B remains intact (starling pox virus) and can be further divided by subclades B1 (canarypox virus) and B2. The size of the primary product of the fpv140 locus PCR, which is 1,800 bp for clade A and 2,400 bp for clade B, allows for simple differentiation. After these amplicons were sequenced, all isolates clustered within the A (fowlpox virus) clade.

In conclusion, the pigeon pox pathogen poses a hazard to the poultry industry in Iraq. On a phylogenetic level, all pigeon pox genotypes analyzed were related to fowlpox-like viruses (subclade A). No genotypes belonged to clades B or C of the canarypox-like or psittacine pox viruses. Nevertheless, more research will be needed to complete whole genome sequencing and assess the effectiveness of the present vaccination.

Acknowledgments

The staff of the University of Basra's College of Veterinary Medicine is thanked by the authors for providing the necessary assistance and resources for the study project.

Funding

All expenses were covered by the authors. The initial work and genomic isolation was conducted at University of Basrah laboratories.

Data Availability Statement

Contact the corresponding author for data availability.

Author's Contributions

Data curation: Khaleefah IA; Formal analysis: Khaleefah IA; Investigation: Khaleefah IA, Ahmed JA; Methodology: Khaleefah IA; Project administration: Najem HA; Software: Al-Tameemi HM; Validation: Kraidi QA; Visualization: Alrafas HR, Kraidi QA; Writing–original draft: Al-Tameemi HM, Khaleefah

IA, Kraidi QA, Najem HA, Ahmed JA; Writing–review & editing; Al-Tameemi HM, Alrafas HR.

ORCID

Isam Azeez Khaleefah, https://orcid.org/0000-0001-8050-7896
Hassan M. Al-Tameemi, https://orcid.org/0000-0002-1947-798X
Qayssar Ali Kraidi, https://orcid.org/0000-0003-4053-1386
Harith Abdulla Najem, https://orcid.org/0000-0002-4203-5282
Jihad Abdulameer Ahmed, https://orcid.org/0000-0002-8826-6289
Haider Rasheed Alrafas, https://orcid.org/0000-0002-5564-8969

References

1. Kim TJ, Schnitzlein WM, McAloose D, Pessier AP, Tripathy DN. Characterization of an avianpox virus isolated from an Andean condor (Vultur gryphus). Vet Microbiol 2003;96:237–246.
2. Bolte AL, Meurer J, Kaleta EF. Avian host spectrum of avipoxviruses. Avian Pathol 1999;28:415–432.
3. Weli SC, Tryland M. Avipoxviruses: infection biology and their use as vaccine vectors. Virol J 2011;8:49.
4. Alehegn E, Chanie M, Mengesha D. A systematic review of serological and clinicopathological features and associated risk factors of avian pox. Br J Poult Sci 2014;3:78–87.
5. Vanderplasschen A, Pastoret PP. The uses of poxviruses as vectors. Curr Gene Ther 2003;3:583–595.
6. Hernandez M, Sanchez C, Galka ME, Dominguez L, Goyache J, Oria J, Pizarro M. Avian pox infection in Spanish Imperial eagles (Aquila adalberti). Avian Pathol 2001;30:91–97.
7. Buenestado F, Gortázar C, Millán J, Höfle U, Villafuerte R. Descriptive study of an avian pox outbreak in wild red-legged partridges (Alectoris rufa) in Spain. Epidemiol Infect 2004;132:369–374.
8. Mosad SM, El-Tholoth M, El-Kenawy AA. Conventional and molecular detection of avipoxviruses from chickens, pigeons and turkeys. Mansoura Vet Med J 2019;20:85–91.
9. Kabir ML, Haque ME, Borty SC, Mustafa K, Kamal MM, Khasruzzaman AK, Khan MS, Islam MA. Isolation and molecular detection of fowl pox and pigeon pox viruses from recent outbreak in Bangladesh. Indian J Life Sci 2015;5:1–7.
10. Humason GL. Animal Tissue Techniques. 2nd ed. W. H. Freeman Co., San Francisco, 1967.
11. Huw Lee L, Hwa Lee K. Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. J Virol Methods 1997;63:113–119.
12. Lüschow D, Hoffmann T, Hafez HM. Differentiation of avian poxvirus strains on the basis of nucleotide sequences of 4b gene fragment. Avian Dis 2004;48:453–462.
13. Bertelloni F, Ceccherelli R, Marzoni M, Poli A, Ebani VV. Molecular detection of Avipoxvirus in wild birds in Central Italy. Animals (Basel) 2022;12:338.
14. Manarolla G, Pisoni G, Sironi G, Rampin T. Molecular biological characterization of avian poxvirus strains isolated from different avian species. Vet Microbiol 2010;140:1–8.
15. Jarmin S, Manvell R, Gough RE, Laidlaw SM, Skinner MA. Avipoxvirus phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades. J Gen Virol 2006;87(Pt 8):2191–2201.
16. Hsieh YC, Chen SH, Wang CW, Lee YF, Chung WC, Tsai MC, Chang TC, Lien YY, Tsai SS. Unusual pox lesions found in Chinese jungle mynahs (Acridotheres cristatellus). Avian Pathol 2005;34:415–417.
17. Beytut E, Haligur M. Pathological, immunohistochemical, and electron microscopic findings in the respiratory tract and skin of chickens naturally infected with avipoxvirus. Turk J Vet Anim Sci 2007;31:311–317.
18. Hasan II, Rasheed ST, Shakor MK. Pathological, molecular and phylogenic study of fowlpox virus in domesticated chickens of Tikrit City, Iraq. Braz J Vet Res Anim Sci 2021;58:e176255.
19. Dharanesha NK, Baghel KR, Pathak M, Saminathan M. Gross and histopathology of avian pox in a feral pigeon (Columba spp): a case report. Indian J Vet Pathol 2020;44:268–270.
20. Prukner-Radovcić E, Lüschow D, Ciglar Grozdanić I, Tisljar M, Mazija H, Vranesić D, Hafez HM. Isolation and molecular biological investigations of avian poxviruses from chickens, a turkey, and a pigeon in Croatia. Avian Dis 2006;50:440–444.

https://doi.org/10.14405/kjvr.20230048