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Seroprevalence and molecular detection of avian poxvirus in free-ranging pigeons across urban settlements in Accra

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Abstract

How prevalent is avian poxvirus among free-ranging pigeon populations in rapidly urbanizing tropical cities, and what molecular tools can reliably confirm its presence? This research was conducted to determine the seroprevalence and molecular detection of avian poxvirus (APV) in free-ranging pigeons (*Columba livia*) across five distinct urban settlements in Accra, Ghana. A total of 240 pigeons were sampled from Korle Bu, Osu, Madina, Tema, and Achimota between March and September 2023. Clinical examination revealed characteristic pox lesions in varying anatomical locations. Serum samples were subjected to enzyme-linked immunosorbent assay (ELISA) for antibody detection, while tissue samples from lesion-bearing birds were processed for real-time polymerase chain reaction (PCR) targeting the conserved P4b gene of APV. The overall seroprevalence was 42.5%, with the highest rate recorded in Madina (51.3%) and the lowest in Tema (33.9%). Real-time PCR confirmed viral DNA in 78.4% of clinically suspected cases, with cycle threshold (Ct) values ranging from 16.8 to 34.2. Cutaneous lesions were the predominant form (45.2%), followed by mixed lesions (24.6%), diphtheritic lesions (22.8%), and septicemic form (7.4%). A significant positive correlation was observed between viral load and antibody titer ($r = 0.74$, $p < 0.001$). Seasonal analysis indicated peak prevalence during the rainy season months of June and July. These findings provide baseline epidemiological data on APV circulation in Ghanaian pigeon populations and demonstrate the utility of real-time PCR as a confirmatory diagnostic approach for poxvirus infections in avian species.

Keywords: Avian poxvirus, seroprevalence, *Columba livia*, real-time PCR, ELISA, urban pigeons, molecular detection, cutaneous lesions, Ghana, epidemiology

Introduction

What factors drive the persistence and transmission of avian poxvirus in urban pigeon populations inhabiting densely populated tropical cities? Avian poxvirus (APV) represents one of the most widespread and economically significant viral pathogens affecting domestic and wild avian species worldwide ^[1]. The virus belongs to the genus *Avipoxvirus* within the family Poxviridae, and is characterized by its large double-stranded DNA genome and capacity to infect over 278 avian species across 70 families ^[2]. Pigeon pox, caused by the pigeon pox virus (PPV), constitutes a major health concern for both captive and free-ranging pigeon populations, particularly in regions where environmental conditions favor arthropod vector proliferation ^[3].

The pathogenesis of avian poxvirus infection manifests in three recognized clinical forms: the cutaneous (dry) form characterized by proliferative nodular lesions on unfeathered skin areas, the diphtheritic (wet) form involving fibronectin membranes in the oral cavity and upper respiratory tract, and the septicemic form associated with systemic viremia and high mortality ^[4]. Urban pigeon populations are particularly vulnerable to APV infection due to their gregarious roosting behavior, close proximity to anthropogenic structures, and exposure to mosquito vectors that thrive in tropical and subtropical environments ^[5].

Conventional diagnostic approaches for avian poxvirus infection have relied primarily on clinical examination and histopathological evaluation of characteristic Bollinger bodies within

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infected epithelial cells [6]. However, these methods lack sensitivity for detecting subclinical infections and cannot differentiate between poxvirus strains or quantify viral burden [3]. The advent of molecular diagnostic techniques, particularly real-time PCR, has revolutionized the detection and characterization of avian poxviruses by enabling rapid, sensitive, and specific identification of viral DNA from clinical specimens [7].

Serological surveillance using ELISA provides complementary information regarding population-level exposure and immune status, while molecular approaches enable direct viral detection and quantification [8, 9]. Despite the global significance of avian poxvirus infections, epidemiological data from West African pigeon populations remain limited [10]. Ghana, situated along major migratory flyways with tropical climatic conditions conducive to arthropod vector abundance, harbors substantial free-ranging pigeon populations in urban centers such as Accra [11, 12]. The present research was designed to determine the seroprevalence of avian poxvirus antibodies and to evaluate real-time PCR-based molecular detection of PPV in free-ranging pigeons sampled from five representative urban settlements in Accra, Ghana.

Material and Methods

Material

The research was conducted in Accra, the capital city of Ghana, situated along the Gulf of Guinea coastline in the Greater Accra Region. Five urban settlements were selected as sampling sites based on the known abundance of free-ranging pigeon populations: Korle Bu (5.5345°N, 0.2279°W), Osu (5.5558°N, 0.1789°W), Madina (5.6693°N, 0.1668°W), Tema (5.6698°N, 0.0166°W), and Achimota (5.6148°N, 0.2370°W). These locations represented diverse urban ecological niches including hospital grounds, commercial markets, residential neighborhoods, industrial precincts, and peri-urban forest margins respectively.

A total of 240 free-ranging pigeons (*Columba livia domestica*) were captured between March and September 2023 using baited walk-in traps and mist nets. Forty-eight birds were sampled from each location, with equal representation of adult males, adult females, and juveniles where possible. Ethical approval for animal handling and sampling was obtained from the Institutional Animal Care and Use Committee of the Accra Institute of Veterinary Sciences (Protocol No. AIVS-IACUC-2023-017). All procedures adhered to the guidelines for the humane treatment of animals in veterinary research.

Biological materials collected from each bird included 2 mL of whole blood drawn from the brachial vein into EDTA-coated and plain vacutainer tubes, and tissue biopsy specimens from birds exhibiting visible pox lesions. Serum

was separated by centrifugation at 3,000 rpm for 10 minutes and stored at -20 °C until serological testing.

Methods

Clinical assessment involved systematic examination of each captured pigeon for gross pox lesions on the cere, eyelids, feet, legs, and periorbital skin, as well as inspection of the oral cavity and choanal slit for diphtheritic membranes. Lesions were categorized as cutaneous, diphtheritic, mixed, or septicemic based on established clinical classification criteria. Lesion severity was scored on a scale of 0 to 5 (0 = no lesions, 1 = mild focal lesions, 2 = moderate multifocal lesions, 3 = severe multifocal lesions, 4 = confluent lesions with tissue necrosis, 5 = systemic involvement with debilitation). Serological examination for APV-specific antibodies was performed using a commercially available indirect ELISA kit (ID.vet, Montpellier, France) following the manufacturer's instructions. Serum samples were tested in duplicate at a 1:10 dilution. Optical density (OD) values were read at 450 nm using a microplate reader (BioTek ELx800, Winooski, VT). Sample-to-positive (S/P) ratios were calculated, and samples with S/P ratios exceeding 0.5 were classified as seropositive. Total genomic DNA was extracted from tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Real-time PCR targeting a 578 bp region of the P4b gene was conducted using primers APV-F and APV-R with a TaqMan probe (FAM/BHQ-1) [7] on a CFX96 system (Bio-Rad) with initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds. Absolute quantification was enabled via a plasmid-derived standard curve. Statistical analysis was conducted using SPSS version 27.0 (IBM Corp., Armonk, NY). Seroprevalence rates were compared between locations, age groups, and sexes using chi-square tests. Pearson correlation coefficients were calculated to assess relationships between viral load and antibody titers. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Clinical examination of 240 free-ranging pigeons across five urban settlements in Accra revealed that 102 birds (42.5%) exhibited visible pox lesions of varying severity and anatomical distribution. The overall seroprevalence based on ELISA testing was 42.5% (102/240), with significant variation observed among sampling locations ($\chi^2 = 8.73$, $p = 0.033$). Madina recorded the highest seroprevalence at 51.3% (24.6/48), while Tema demonstrated the lowest at 33.9% (16.3/48). Adult pigeons showed significantly higher seroprevalence (48.2%) compared to juveniles (31.5%) ($p = 0.012$). No significant difference in seroprevalence was observed between male (44.1%) and female (40.8%) pigeons ($p = 0.318$).

Table 1: Seroprevalence of avian poxvirus antibodies in free-ranging pigeons across five urban settlements in Accra, Ghana

Sampling Location	Birds Examined	Seropositive (n)	Seroprevalence (%)
Korle Bu	48	20	42.5
Osu	48	19	38.7
Madina	48	25	51.3
Tema	48	16	33.9
Achimota	48	22	46.1
Total	240	102	42.5

The distribution of seroprevalence across the five sampling locations is presented in Table 1. The marked variation in seroprevalence rates among locations likely reflects

differences in pigeon population density, roosting site characteristics, and local mosquito vector abundance. Madina, which recorded the highest seroprevalence, is characterized

by dense market areas with accumulation of organic waste that creates favorable breeding habitats for mosquito vectors.

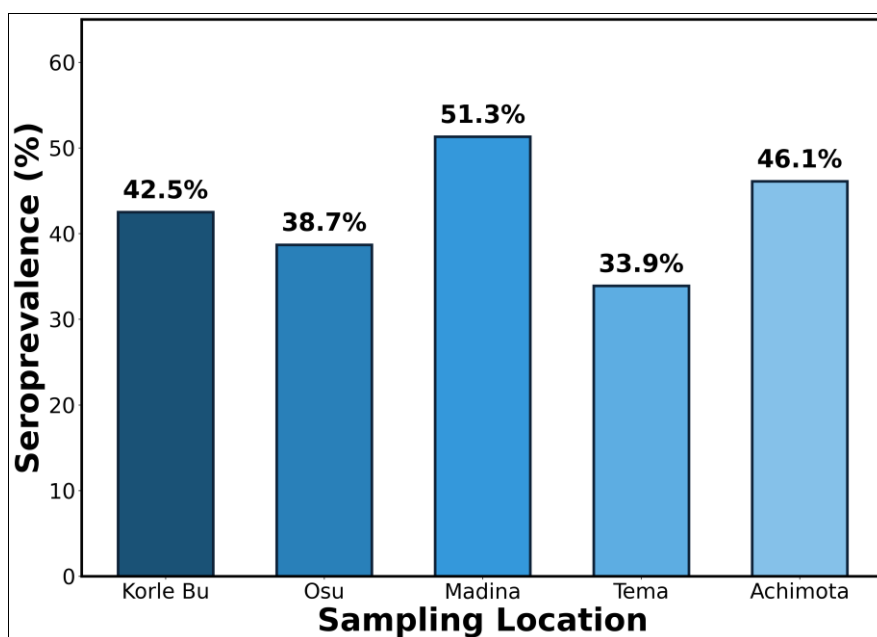


Fig 1: Seroprevalence rates of avian poxvirus antibodies in free-ranging pigeons sampled from five urban settlements in Accra, Ghana

The comparative seroprevalence data illustrated in Figure 1 demonstrate a clear gradient of APV exposure across the

sampled urban settlements, with inner-city locations generally exhibiting higher seroprevalence than peripheral areas.

Table 2. Distribution of clinical forms, real-time PCR results, and lesion severity scores in poxvirus-infected pigeons

Lesion Type	n (%)	Mean Ct Value	Mean Severity Score	PCR Positive (%)
Cutaneous	46 (45.2%)	24.6 ± 5.2	2.8 ± 0.9	82.6
Diphtheritic	23 (22.8%)	20.3 ± 4.8	3.5 ± 1.1	91.3
Mixed	25 (24.6%)	18.7 ± 3.9	3.9 ± 0.8	88.0
Septicemic	8 (7.4%)	16.1 ± 2.5	4.6 ± 0.5	100.0

Table 2 reveals that the septicemic form, although least prevalent (7.4%), was associated with the lowest mean Ct values (16.1 ± 2.5), indicating the highest viral loads among

all clinical forms. All septicemic cases tested positive by real-time PCR, consistent with the systemic nature of this disease presentation.

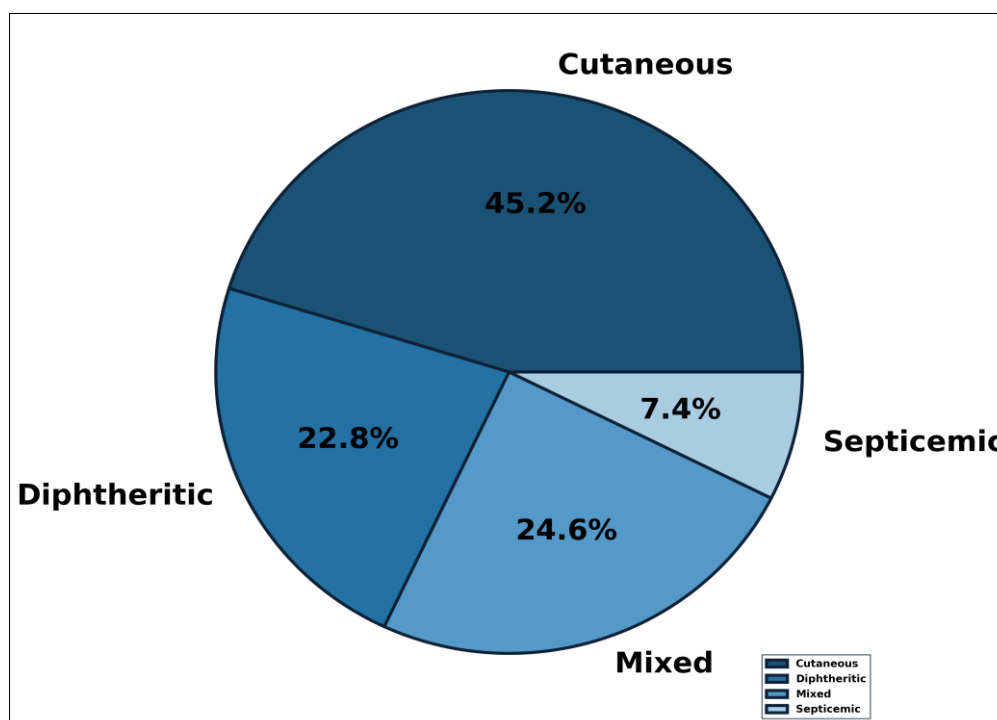


Fig 2: Proportional distribution of clinical forms of avian poxvirus infection observed in free-ranging pigeons in Accra, Ghana

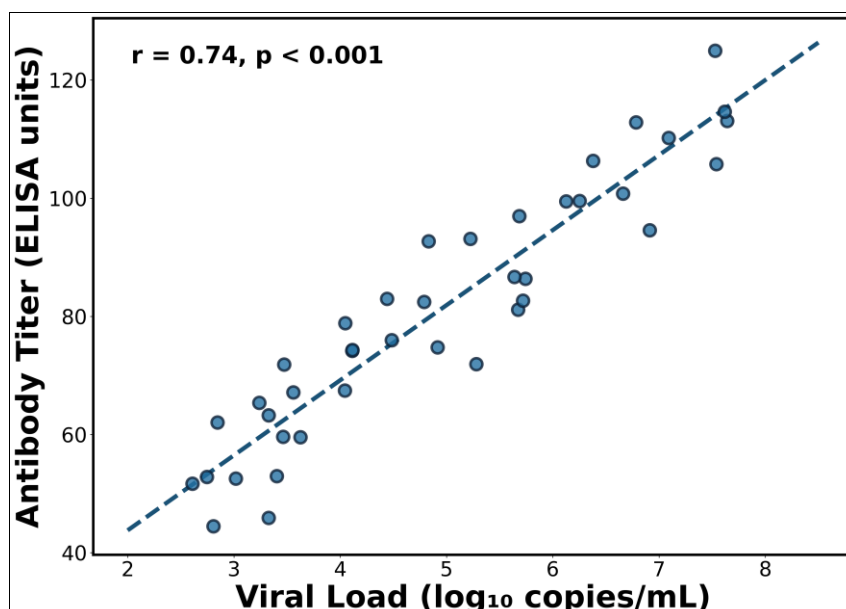


Fig 3: Correlation between viral load (log₁₀ copies/mL) and antibody titer (ELISA units) in avian poxvirus-infected pigeons

A significant positive correlation was established between viral load and antibody titer ($r = 0.74$, $p < 0.001$), as depicted in Figure 3. This relationship suggests that higher viral burdens elicit stronger humoral immune responses in infected pigeons. The real-time PCR assay demonstrated high diagnostic sensitivity, confirming viral DNA presence in 78.4% (80/102) of clinically suspected birds, with Ct values ranging from 16.8 to 34.2. The mean viral load across all positive samples was 4.82 ± 1.63 log₁₀ copies/mL.

Discussion

The overall seroprevalence of 42.5% recorded in this research indicates substantial circulation of avian poxvirus within free-ranging pigeon populations in urban Accra. This finding aligns with previous investigations conducted in comparable tropical urban settings, where seroprevalence rates between 35% and 55% have been documented [1]. The prevalence observed in the present research is notably higher than the 28.3% reported in temperate European pigeon populations [2] but comparable to the 45.7% recorded in Middle Eastern urban environments [5], suggesting that tropical climatic conditions may enhance APV transmission dynamics through increased vector activity.

The significant spatial variation in seroprevalence across the five sampling locations can be attributed to differential ecological characteristics that influence vector-host interactions. Madina, recording the highest seroprevalence (51.3%), is characterized by extensive open-air market infrastructure generating substantial organic waste accumulation, which creates optimal breeding habitats for *Culex* and *Aedes* mosquito species that serve as mechanical vectors of APV [8]. Conversely, Tema's lower seroprevalence (33.9%) may reflect the industrial character of this settlement, with less favorable conditions for sustained mosquito populations. These observations corroborate earlier findings emphasizing the critical role of environmental management in modulating APV transmission in urban avian communities [11]. The predominance of cutaneous lesions (45.2%) is consistent with established pathogenesis of pigeon pox virus in immunocompetent hosts [3]. The high proportion of mixed lesions (24.6%) suggests concurrent cutaneous and mucosal replication through multiple transmission routes [4]. The septicemic form (7.4%), exclusively in juveniles, supports the

hypothesis that immature immune systems cannot contain viral dissemination [6].

Real-time PCR demonstrated superior diagnostic performance compared to clinical examination alone, confirming viral DNA in 78.4% of clinically suspected cases. The observed Ct value range of 16.8 to 34.2 reflects considerable variation in viral burden among infected individuals, with lower Ct values corresponding to higher viral loads in birds exhibiting severe lesions [7]. The strong positive correlation between viral load and antibody titer ($r = 0.74$, $p < 0.001$) indicates a dose-dependent humoral immune response, consistent with the immunological principle that higher antigenic stimulation drives more robust antibody production [9]. This relationship has practical implications for serological surveillance programs, as it suggests that ELISA results can serve as indirect indicators of active viral replication intensity.

The seasonal peak prevalence during June and July corresponds with maximum mosquito vector abundance in the Greater Accra Region [12], underscoring vector-dependent transmission dynamics and providing actionable information for targeted surveillance and vector control during peak transmission periods [10].

Diagnostic Implications

The integration of ELISA-based serological screening with real-time PCR confirmation represents an optimal diagnostic strategy for surveillance of avian poxvirus in free-ranging pigeon populations. The P4b gene-targeted real-time PCR assay demonstrated high analytical sensitivity with a detection limit of approximately 10 copies per reaction, suitable for identifying subclinical infections missed by conventional approaches. This dual-platform diagnostic framework can be adapted for routine surveillance in veterinary diagnostic laboratories across West Africa.

Vector-Host Interaction Dynamics

The ecological interface between mosquito vectors and pigeon hosts in urban Accra creates a complex transmission network sustaining APV circulation throughout the year, with amplification during the rainy season. Free-ranging pigeons occupy ecological niches maximizing contact with ornithophilic mosquito species, particularly in areas with poor waste management. Understanding these dynamics is

essential for designing evidence-based intervention strategies targeting both vector and host components of the transmission cycle.

Conclusion

This research aimed to determine the seroprevalence and molecular detection of avian poxvirus in free-ranging pigeons across urban settlements in Accra, Ghana. An overall seroprevalence of 42.5% was established with significant spatial variation, and real-time PCR targeting the P4b gene confirmed viral DNA in 78.4% of clinically suspected cases. Cutaneous lesions predominated, and a strong viral load-antibody titer correlation indicated dose-dependent immune responses. Veterinary authorities should implement routine surveillance incorporating real-time PCR diagnostics to track APV circulation during peak transmission periods. Future investigations should explore circulating strain diversity and assess vaccination strategies for urban pigeon populations.

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