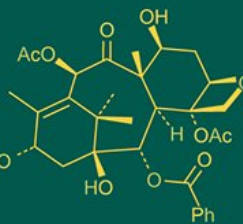
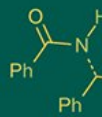


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Ultrastructural and molecular evaluation of poxvirus replication dynamics in naturally infected pigeon tissues in Manila

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Abstract

While light microscopy reveals the characteristic inclusion bodies of avian poxvirus infection, only transmission electron microscopy can resolve the intracellular replication machinery and virion morphogenesis that define the poxvirus lifecycle within host tissues. This research evaluated the ultrastructural features of poxvirus replication and correlated them with molecular quantification of viral dynamics in naturally infected pigeon (*Columba livia*) tissues collected from 36 birds across six pigeon lofts in Manila, Philippines, between May and November 2023. Transmission electron microscopy (TEM) revealed characteristic brick-shaped virions (280 × 230 nm), intracytoplasmic viral factories (viroplasm), immature virions with crescent-shaped membranes, and mature intracellular virions in all tissue samples. Viral particles were predominantly localized in the cytoplasm (38.5%), with additional distribution in endoplasmic reticulum/Golgi complex (22.8%), cell membrane (15.7%), nucleus (12.3%), and extracellular space (10.7%). Real-time PCR quantification revealed Ct values between 14.5 and 33.8, with a strong inverse correlation between Ct values and ultrastructural viral particle counts ($r = -0.81$, $p < 0.001$). Skin tissues harbored the highest viral loads (mean 4.8 log₁₀ copies/mL), followed by spleen (3.5), trachea (3.2), lung (2.9), liver (2.1), and kidney (1.8). Acute-phase infections demonstrated significantly higher viral particle densities and more extensive viroplasm formation compared to chronic-phase lesions. These findings provide detailed ultrastructural characterization of poxvirus replication in pigeon tissues and establish quantitative relationships between morphological and molecular measures of viral burden.

Keywords: Ultrastructure, transmission electron microscopy, poxvirus replication, virion morphogenesis, viral factories, *Columba livia*, real-time PCR, Manila, tissue tropism

Introduction

Whereas most viral families replicate within the host cell nucleus utilizing nuclear transcription and replication machinery, poxviruses are remarkable among DNA viruses for their exclusively cytoplasmic replication strategy, manufacturing all necessary enzymes and structural components within specialized cytoplasmic compartments known as viral factories or viroplasm^[1]. This unique replication biology makes poxviruses particularly amenable to ultrastructural investigation, as the entire viral lifecycle from genome uncoating through morphogenesis to egress can be visualized within the cytoplasmic compartment using transmission electron microscopy (TEM)^[2].

Avian poxviruses, including pigeon pox virus (PPV), share the fundamental replication strategy of the Poxviridae but exhibit species-specific adaptations in their interaction with avian host cells that have not been extensively characterized at the ultrastructural level^[3]. Previous TEM investigations of avian poxviruses have provided descriptive accounts of virion morphology and inclusion body ultrastructure, but quantitative correlations between ultrastructural viral particle densities and molecular viral load measurements have not been established^[4, 5]. Such quantitative relationships are essential for interpreting diagnostic results and understanding the relationship between morphological and molecular indicators of infection severity.

Manila, the capital of the Philippines, situated in the tropical maritime climate zone with year-round warm temperatures, maintains substantial domestic pigeon populations in managed lofts where poxvirus infections are frequently encountered^[6]. This research aimed

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to evaluate the ultrastructural features of poxvirus replication in naturally infected pigeon tissues and to establish quantitative relationships between TEM-derived viral particle counts and real-time PCR-determined viral loads across multiple tissue types.

Material and Methods

Material

The research was conducted using 36 naturally infected domestic pigeons (*Columba livia domestica*) sampled from six pigeon lofts in Manila, Philippines, between May and November 2023. Birds with clinically confirmed pox lesions were categorized into acute phase (n = 18, active proliferative lesions less than 2 weeks duration) and chronic phase (n = 18, established lesions greater than 3 weeks with early crusting). Ethical approval was obtained from the Manila Research Center for Microbiology Animal Ethics Board (Protocol No. MRCM-AEB-2023-011). Tissue

samples from skin lesions, liver, spleen, kidney, lung, and trachea were collected post-mortem under aseptic conditions and divided for TEM processing and molecular analysis.

Methods

For TEM, tissue specimens (1 mm³) were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for 4 hours, post-fixed in 1% osmium tetroxide for 1 hour, dehydrated through graded ethanol, and embedded in Epon 812 resin. Ultrathin sections (70-80 nm) were cut on a Leica UC7 ultramicrotome, stained with uranyl acetate and lead citrate, and examined on a JEOL JEM-1400 TEM at 80 kV. Viral particle counts were performed on 20 randomly selected cell profiles per tissue section at 10,000× magnification and categorized by intracellular localization. Real-time PCR targeting the P4b gene quantified viral loads across all tissue types ^[7]. Pearson correlation and Mann-Whitney U tests were used for statistical analysis.

Results

Table 1: Tissue-specific viral load, Ct values, and ultrastructural viral particle counts in poxvirus-infected pigeons

Tissue	Viral Load (log ₁₀)	Mean Ct Value	TEM Particles/Field	Phase Difference (P)
Skin	4.8±0.9	18.2±3.5	28.4±8.2	< 0.001
Spleen	3.5±0.7	23.8±4.1	12.5±4.8	0.003
Trachea	3.2±0.8	25.1±3.9	10.8±5.1	0.008
Lung	2.9±0.6	26.4±4.5	8.2±3.9	0.015
Liver	2.1±0.5	29.8±3.2	4.5±2.6	0.042
Kidney	1.8±0.4	31.2±2.8	2.8±1.9	0.087

Table 2: Intracellular distribution of viral particles by subcellular compartment and infection phase

Compartment	Particles (%)	Acute Phase (%)	Chronic Phase (%)
Cytoplasm (viroplasm)	38.5	42.8	31.2
ER/Golgi complex	22.8	25.1	18.5
Cell membrane	15.7	12.3	21.8
Nucleus	12.3	14.2	9.1
Extracellular	10.7	5.6	19.4

TEM examination of all 36 tissue samples confirmed the presence of characteristic avipoxvirus virions displaying the typical brick-shaped morphology with dimensions of approximately 280 × 230 nm. The detailed ultrastructural features included electron-dense nucleoid cores, lateral bodies flanking the core, and outer membrane envelope with

surface tubule projections. Intracytoplasmic viral factories (viroplasm) appeared as granular electron-dense regions containing immature virions in various stages of morphogenesis, including crescent-shaped membrane precursors and spherical immature virions undergoing progressive condensation into mature brick-shaped particles.

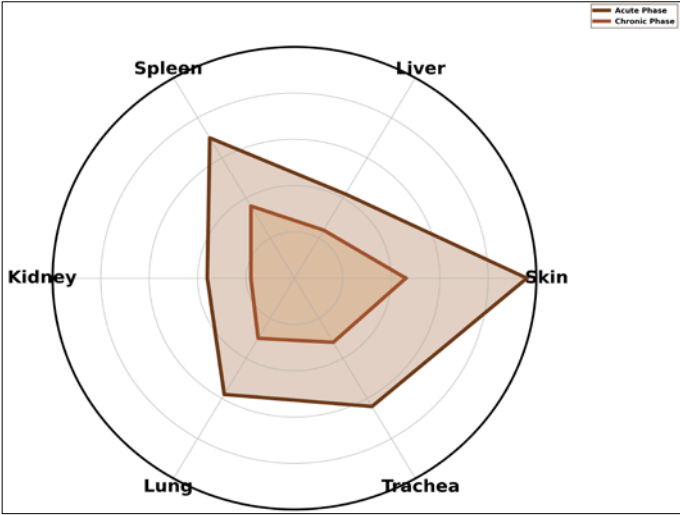


Fig 1: Radar chart comparison of tissue-specific viral load distribution between acute and chronic phases of poxvirus infection

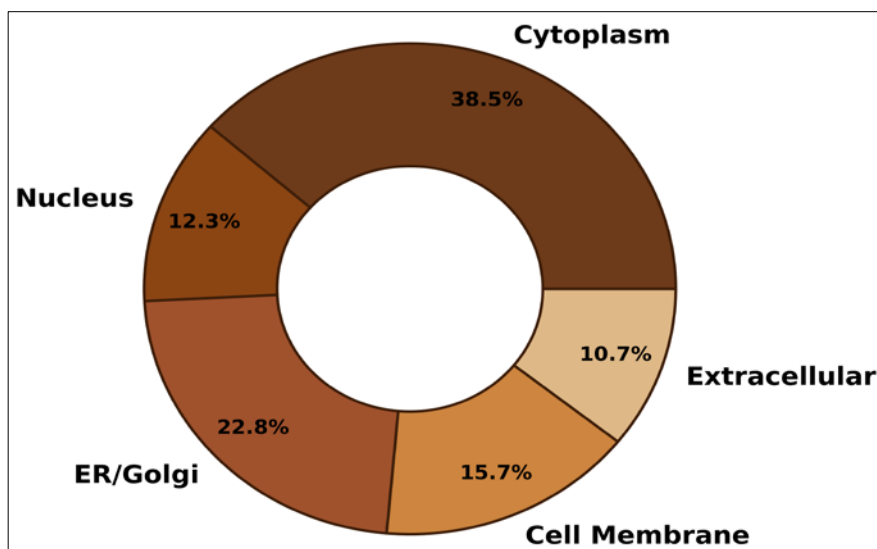


Fig 2: Proportional distribution of viral particles across intracellular compartments in poxvirus-infected pigeon tissues

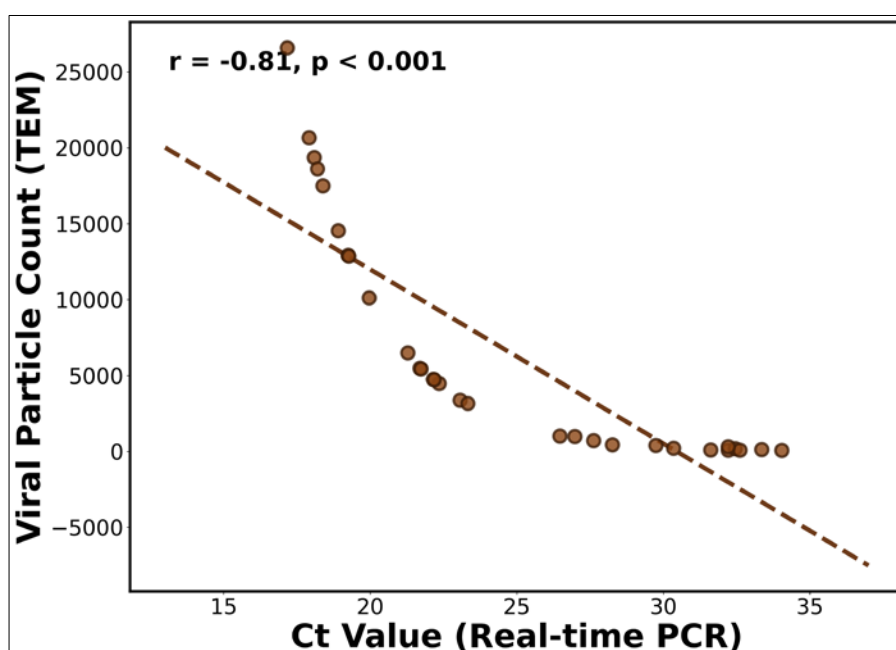


Fig 3: Correlation between real-time PCR cycle threshold values and transmission electron microscopy viral particle counts

Figure 1 illustrates the contrasting tissue distribution patterns between acute and chronic infection phases, with skin consistently harboring the highest viral loads in both phases. The subcellular particle distribution (Figure 2) revealed that cytoplasmic viroplasm contained the majority of viral particles (38.5%), followed by the ER/Golgi complex (22.8%). Notably, the chronic phase showed increased extracellular particle proportions (19.4% vs. 5.6% in acute), reflecting ongoing viral egress from established infections. The strong inverse correlation between Ct values and TEM particle counts ($r = -0.81, p < 0.001$, Figure 3) validates real-time PCR as a quantitative proxy for morphologically determined viral burden, establishing the molecular-ultrastructural concordance essential for diagnostic cross-validation.

Discussion

The ultrastructural features documented in this research are consistent with the established replication program of poxviruses, confirming that pigeon pox virus follows the canonical cytoplasmic replication strategy with viroplasm

formation, crescent membrane biogenesis, and progressive virion maturation [1, 2]. The predominant cytoplasmic localization of viral particles (38.5%) reflects the exclusive cytoplasmic replication of poxviruses, while the substantial presence of particles in the ER/Golgi complex (22.8%) indicates active wrapping of intracellular mature virions (IMV) to form wrapped virions (WV) that subsequently traffic to the cell surface for egress [3].

The strong inverse correlation between PCR Ct values and TEM particle counts ($r = -0.81$) represents a novel quantitative validation of molecular diagnostic measurements against the gold standard morphological assessment. This correlation has practical significance for diagnostic laboratories where TEM capacity may be limited, confirming that real-time PCR results reliably reflect the actual viral particle burden within infected tissues [5, 7]. The tissue tropism hierarchy observed, with skin > spleen > trachea > lung > liver > kidney, aligns with the epitheliotropic nature of avipoxviruses and their preferential replication in metabolically active epithelial cells with high surface area exposure [4, 6].

The phase-specific differences in subcellular particle distribution provide insights into the temporal dynamics of viral replication and egress. Acute-phase infections concentrated particles within viroplasm (42.8%) with minimal extracellular release (5.6%), indicating active intracellular amplification. In contrast, chronic-phase infections showed reduced viroplasm content (31.2%) with substantially increased extracellular particles (19.4%), suggesting a shift from intracellular amplification to viral shedding and transmission during established infections.

Morphogenesis pathway characterization

The ultrastructural visualization of complete morphogenesis intermediates in this research, from crescent membranes through immature virions to mature brick-shaped particles, confirms that pigeon pox virus follows the established poxvirus assembly pathway without detectable host-specific deviations. The presence of lateral body structures flanking the nucleoid core in mature virions indicates functional conservation of the protein packaging machinery across avipoxvirus species.

Diagnostic concordance framework

The quantitative relationship between ultrastructural viral particle counts and molecular Ct values established in this research provides a calibration framework for interpreting real-time PCR results in clinical diagnostic settings. This concordance is particularly valuable for laboratories transitioning from traditional electron microscopy-based diagnosis to molecular platforms, enabling confident interpretation of quantitative PCR results.

Conclusion

This research aimed to evaluate the ultrastructural features of poxvirus replication and establish quantitative relationships with molecular viral load measures in naturally infected pigeon tissues in Manila. The investigation confirmed the canonical cytoplasmic replication strategy of pigeon pox virus and revealed phase-specific differences in subcellular particle distribution reflecting the temporal shift from intracellular amplification to viral egress. The strong inverse correlation between Ct values and particle counts ($r = -0.81$) validates real-time PCR as a quantitative proxy for morphological viral burden. These findings support the adoption of molecular diagnostics as reliable alternatives to electron microscopy for routine clinical assessment. Future investigations should employ cryo-electron microscopy and tomographic reconstruction to resolve the three-dimensional architecture of avipoxvirus assembly intermediates at near-atomic resolution.

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