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CHALLENGES IN FIELD DIAGNOSIS OF AVIAN POXVIRUS IN WILD BIRDS

DESAFIOS NO DIAGNÓSTICO DE CAMPO DO POXVÍRUS EM AVES SILVESTRES

DESAFÍOS EN EL DIAGNÓSTICO DE CAMPO DEL POXVIRUS AVIAR EN AVES SILVESTRES

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ABSTRACT

Avian poxvirus, belonging to the family Poxviridae and the genus *Avipoxvirus* (APV), represents an important infectious disease affecting both wild and domestic birds and is characterized by proliferative cutaneous lesions and diphtheritic manifestations in mucosal tissues. The infection shows tropism for keratinocytes and epithelial cells, with viral replication occurring entirely within the cellular cytoplasm, resulting in epithelial hyperplasia and the formation of nodules or diphtheritic plaques. In wild birds, field diagnosis presents significant challenges, as the clinical manifestations of the disease may resemble other dermatological and respiratory conditions, potentially compromising early identification and the implementation of control measures. This study aimed to synthesize and analyze recent scientific literature regarding limitations and advances in the diagnosis of avian poxvirus in wild birds, characterizing the work as a narrative review conducted in the PubMed database, including publications from the last five years in Portuguese and English. The results indicate that although macroscopic evaluation is useful as an initial assessment, diagnostic confirmation depends on laboratory methods such as histopathology and molecular techniques, particularly Polymerase Chain Reaction (PCR), which enables differentiation of circulating viral clades and phylogenetic monitoring. Due to its high genetic diversity and environmental persistence in contaminated scabs, the epidemiological complexity of the virus is considerable, reinforcing the need for continuous molecular surveillance.

Keywords: Avian Poxvirus. *Avipoxvirus*. Wild Birds. Diagnosis. PCR. Histopathology.

RESUMO

O poxvírus aviário, pertencente à família Poxviridae e ao gênero *Avipoxvirus* (APV), representa uma importante enfermidade infecciosa em aves silvestres e domésticas, e

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caracteriza-se por lesões cutâneas proliferativas e manifestações diftéricas em mucosas. A infecção apresenta tropismo por queratinócitos e células epiteliais, com replicação viral ocorrendo inteiramente no citoplasma celular, resultando em hiperplasia epitelial e formação de nódulos ou placas diftéricas. Em aves silvestres, o diagnóstico de campo apresenta desafios significativos, uma vez que as manifestações clínicas da doença podem se assemelhar a outras afecções dermatológicas e respiratórias, o que pode comprometer a identificação precoce e a adoção de medidas de controle. Este estudo teve como objetivo sintetizar e analisar a literatura científica recente acerca das limitações e avanços no diagnóstico do poxvírus em aves silvestres, caracterizando o trabalho como uma revisão narrativa conduzida na base PubMed, com publicações dos últimos cinco anos nos idiomas português e inglês. Os resultados indicam que, embora a avaliação macroscópica seja útil em um momento inicial, a confirmação diagnóstica depende de métodos laboratoriais, como a histopatologia e as técnicas moleculares, especialmente a Reação em Cadeia de Polimerase (PCR), que permite a diferenciação de clados virais circulantes e o monitoramento filogenético. Por ser um vírus com grande diversidade genética e capacidade de persistência ambiental em crostas contaminadas, a complexidade epidemiológica torna-se ampla e reforça a necessidade de vigilância molecular contínua.

Palavras-chave: Poxvírus Aviário. *Avipoxvirus.* Aves Silvestres. Diagnóstico. PCR. Histopatologia.

RESUMEN

El poxvirus aviar, perteneciente a la familia Poxviridae y al género *Avipoxvirus* (APV), representa una importante enfermedad infecciosa en aves silvestres y domésticas, caracterizada por lesiones cutáneas proliferativas y manifestaciones diftéricas en las mucosas. La infección presenta tropismo por queratinocitos y células epiteliales, con replicación viral que ocurre completamente en el citoplasma celular, resultando en hiperplasia epitelial y formación de nódulos o placas diftéricas. En aves silvestres, el diagnóstico de campo presenta desafíos significativos, ya que las manifestaciones clínicas pueden asemejarse a otras afecciones dermatológicas y respiratorias, lo que puede comprometer la identificación temprana y la adopción de medidas de control. Este estudio tuvo como objetivo sintetizar y analizar la literatura científica reciente sobre las limitaciones y avances en el diagnóstico del poxvirus aviar en aves silvestres, caracterizándose como una revisión narrativa realizada en la base de datos PubMed, incluyendo publicaciones de los últimos cinco años en portugués e inglés. Los resultados indican que, aunque la evaluación macroscópica es útil en una etapa inicial, la confirmación diagnóstica depende de métodos de laboratorio como la histopatología y las técnicas moleculares, especialmente la Reacción en Cadena de la Polimerasa (PCR), que permite la diferenciación de clados virales circulantes y el monitoreo filogenético. Debido a su amplia diversidad genética y capacidad de persistencia ambiental en costras contaminadas, la complejidad epidemiológica del virus es elevada, reforzando la necesidad de una vigilancia molecular continua.

Palabras clave: Poxvirus Aviar. *Avipoxvirus*. Aves Silvestres. Diagnóstico. PCR. Histopatología.

1 INTRODUCTION

The *Poxviridae* family comprises enveloped viruses with double-stranded DNA genome, known to cause proliferative skin lesions in a wide range of vertebrate and invertebrate hosts (McInnes et al., 2023; Molteni et al., 2023). In birds, infection with the genus *Avipoxvirus* manifests predominantly in two clinical forms: cutaneous (or "dry"), characterized by nodules in areas devoid of feathers, and diphtheria (or "wet"), which affects the mucous membranes of the upper respiratory and digestive tract (Mohamed et al., 2024; Souci and Denesvre, 2024).

From the anatomopathological point of view, the cutaneous form is characterized by epidermal hyperplasia (acanthosis), ballooning degeneration of keratinocytes and formation of intracytoplasmic inclusions compatible with Bollinger bodies, these alterations directly reflect the intensity of viral replication in the cell cytoplasm. The lesions are most frequently observed in apteric regions, such as the eyelids and the base of the beak, and can indirectly compromise the bird's vision and feeding capacity, which, even in the absence of high direct lethality, directly impacts the individual's survival in nature due to the inability to see and feed (Souci and Denesvre, 2024).

The diphtheria form, also called the "wet" form, is characterized by the involvement of mucous membranes of the digestive and upper respiratory tract, including the oral cavity, oropharynx, esophagus, larynx, and trachea. In this presentation, the infection is usually associated with inhalation or ingestion of the virus. In addition to the mechanical impact of the lesions, the diphtheria form can predispose to secondary bacterial or fungal infections, aggravating the clinical picture, amplifying the local inflammatory response, and making diagnosis difficult. (Souci and Denesvre, 2024).

The cutaneous form generally has low mortality, while the diphtheria form may be associated with higher mortality rates due to breathing and feeding difficulties, and the severity of clinical signs varies according to the initial virulence and pathogenicity of the viral strain involved (Santos et al., 2023).

Field diagnosis for avian poxvirus is often challenging due to the similarity of macroscopic lesions with other pathologies, such as fungal infections or nutritional deficiencies (Mohamed et al., 2024). Although visual inspection provides an initial suspicion, confirmation requires laboratory approaches, such as histopathology for identification of intracytoplasmic inclusion corpuscles (Bollinger bodies) and molecular techniques (Santos et al., 2023). The genetic diversity of Avipoxviruses and the

emergence of variants that escape the immunity conferred by commercial vaccines make monitoring and accurate diagnosis essential for the conservation of wild birds and the protection of commercial poultry farming (Chacón et al., 2022; Santos et al., 2023).

In addition to the health impact, Avipoxvirus represents a relevant threat to the conservation of wild birds, especially due to its wide geographical distribution and ability to infect several species. Phylogenetic studies demonstrate the active circulation of the virus in natural populations, evidencing its epidemiological importance and the potential impact on the population dynamics of these species (Santos et al., 2023).

The virus also has the ability to persist in the environment, especially in contaminated crusts, which can remain infectious for long periods. This characteristic favors indirect transmission and makes it difficult to control the disease in wild populations, in addition to representing an additional challenge for field diagnosis, since the presence of crusts in the environment can indicate viral circulation even in the absence of birds with visible lesions at the time of evaluation (Mohamed et al., 2024).

Despite advances in knowledge about Avipoxvirus, there are still significant limitations in diagnosis under field conditions, especially in wild birds. Thus, it is essential to gather and analyze the available scientific evidence on the challenges related to the diagnosis of this infection, contributing to the improvement of strategies for identifying and controlling the disease.

2 METHODOLOGY

The present study is characterized as a narrative literature review, developed with the objective of synthesizing and analyzing the most recent scientific evidence related to the challenges in the field diagnosis of poxvirus in wild birds. The search was carried out in the PubMed database, using the descriptors "Poxvirus", "Birds", "Diagnosis" and "Treatment", combined through the Boolean operators AND and OR, according to the terminology of Medical Subject Headings (MeSH). Articles published in the last five years, available in full and written in Portuguese or English, that directly addressed the topic, were included. Studies that did not have a direct relationship with the central theme, duplicate publications, narrative reviews with low methodological rigor, and articles not indexed in the database used were excluded. The selection of studies was conducted in two stages: screening of titles and abstracts, followed by the evaluation of full texts to confirm relevance. The information extracted was organized in a descriptive way.

3 RESULTS AND DISCUSSION

The analysis of the studies shows that the field diagnosis of avian poxvirus is not limited to the observation of lesions, but depends on the integration between clinical, epidemiological and environmental aspects. The variability in clinical expression between different species and the possibility of subclinical infections hinder the early identification and standardization of diagnostic criteria based only on macroscopic evaluation.

Thus, the recognition of the disease in wild populations requires a broader approach, in which the initial clinical examination is associated with the interpretation of the ecological context and laboratory confirmation, contributing to safer and more effective health decisions.

Pathogenesis and Cutaneous Tropism: Poxviruses have an intrinsic affinity for keratinocytes and mucosal cells. Infection usually occurs through breaches in the skin barrier or via mechanical vectors, such as mosquitoes (Souci and Denesvre, 2024), but it can also involve aerosols, ingestion of contaminated water or food, being favored by ecological factors, such as the migration of wild birds and the introduction of new species into certain ecosystems (Santos et al., 2023; Souci and Denesvre, 2024). Viral replication occurs entirely in the cytoplasm of infected cells, in structures called viroplasms, and induces characteristic epithelial hyperplasia, resulting in nodules that, in severe cases, can lead to blindness or difficulty feeding (Mohamed et al., 2024; McInnes et al., 2023). The study of the genomic composition of these viruses shows significant variation in GC content and codon use, which reflects adaptation to different host spectra (Molteni et al., 2023).

In addition to the evident clinical manifestations, subclinical infections can also occur, in which infected birds do not have visible lesions, but still harbor the virus. This condition represents an additional challenge for diagnosis in the field, as it hinders the early identification of infected individuals and allows the silent maintenance of the virus in the population, acting as a reservoir of infection and favoring its dissemination. (Mohamed et al., 2024).

Diagnostic limitationsIn the field, the diagnosis is based on the presence of crusts and nodules, but the differentiation between the avian smallpox virus (FWPV) and the pigeon pox virus (PGPV) cannot be made by direct observation (Mohamed et al., 2024). In histopathological evaluation, it is possible to have definitive evidence through the visualization of large eosinophilic inclusion bodies,

also known as Bollinger bodies, which act as viral factories in the cell cytoplasm (Mohamed et al., 2024; McInnes et al., 2023).

The study of cutaneous pathogenesis has been significantly enhanced by the development of permissive culture systems used to replicate APV, primarily primary embryonic chicken fibroblasts, DF-1 cell line, and embryonated chicken eggs inoculated into their chorioallantoic membranes (CAM). Although current models have been developed using chickens *(Gallus gallus),* some could be adapted to other bird species (Souci; Denesvre, 2024). The use of chorioallantoic membrane (CAM) as a biological model proved to be effective in the pathogenic characterization of the samples. The observation of visible epithelial changes after inoculation not only confirms the viability of the virus, but also enables detailed histopathological analysis for the identification of Bollinger bodies (Mohamed et al., 2024). However, this technique requires tissue collection and laboratory processing, which limits its immediate application in large-scale field screenings.

In addition, the severity of lesions can vary significantly between infected individuals, including differences between males and females. A significantly greater severity was observed in males, who have deep necrotic lesions at the base of the beak (ramphottheca), leading to severe deformities and feeding incapacity. This finding suggests that physiological and hormonal factors may influence the response to infection and disease progression, contributing to variations in clinical presentation and making it difficult to standardize the diagnosis based exclusively on macroscopic observation under field conditions. This variability can lead to underestimation of the severity of infection or failure to identify milder cases, compromising health surveillance in wild populations (Chacón et al., 2022).

Advances in Molecular Diagnostics: The Polymerase Chain Reaction (PCR) technique has proven to be the most robust tool for the detection and differentiation of Avipoxvirus. The use of PCR targeting specific genes, such as envelope genes (P4b), allows the identification of different clades (A, B, C, E) circulating in wild and domestic bird populations (Santos et al., 2023; Chacón et al., 2022). Studies carried out in Portugal, for example, identified a predominance of Clade A in several bird species between 2017 and 2023. However, phylogenetic analysis of *Turdus merula isolates* revealed mutations that point to a continuous diversification of *avipoxvirus* in natural ecosystems. This phenomenon suggests that the interaction between multiple clades in wild birds favors

recombination events, challenging the specificity of conventional PCR primers, reinforcing the need for constant phylogenetic surveillance (Santos et al., 2023).

In addition, molecular analyses allow the evaluation of genetic relationships between different strains, contributing to the understanding of the epidemiology of the virus and enabling the monitoring of spread between different species and geographic regions (Santos et al., 2023).

Management and Treatment Challenges: Managing outbreaks in wild birds is complex due to the lack of specific antiviral treatments and the difficulty in reaching populations. Although vaccination is common in commercial poultry farming, outbreaks in vaccinated flocks—such as those caused by Clade E variants with exacerbated beak lesions—indicate that cross-protection is not always effective (Chacón et al., 2022). Rapid diagnosis in the field is therefore vital to implement isolation and vector control measures to prevent spread in vulnerable populations. (CAMILA SOARES RAMOS) Sanitary measures, such as arthropod control and proper management of water and food sources, are preventive strategies complementary to vaccination (Santos et al., 2023).

Added to this is the virus's ability to remain viable in crusts present in the environment, which can act as a source of infection for other birds. This characteristic makes it difficult to control the disease in natural environments and reinforces the importance of adopting adequate sanitary measures. (Mohamed et al., 2024).

In general, the studies analyzed show that the diagnosis of Avipoxvirus in wild birds still represents a significant challenge, especially in field conditions. Although clinical evaluation is an important tool for initial suspicion, its limitations highlight the need for complementary tests for definitive confirmation. In addition, factors such as viral genetic variability, the occurrence of subclinical infections, and the virus's ability to persist in the environment increase the complexity of diagnosis and hinder the implementation of effective disease control measures (Santos et al., 2023; Mohamed et al., 2024).

4 CONCLUSION

The growing genetic diversity of *Avipoxviruses*, associated with the simultaneous circulation of multiple clades and subclades and the occurrence of outbreaks in previously vaccinated birds, highlights the complexity of the current epidemiological scenario. These factors reinforce the need for continuous molecular surveillance integrated with clinical, histopathological, and phylogenetic diagnosis, ensuring faster and more accurate results.

In addition, the improvement of field monitoring strategies, combined with the development of accessible and rapid diagnostic tools, is essential to mitigate the impacts of the disease both in commercial poultry farming and in the conservation of wild birds. (Chacón et al., 2022; Santos et al., 2023).

Field diagnosis of avian poxvirus represents a challenge that goes beyond the simple observation of characteristic lesions. Although clinical signs may raise important suspicions, they are not always sufficient to guarantee a safe diagnosis, especially in view of the diversity of manifestations and similarity with other diseases. In this context, it becomes evident the need to associate clinical evaluation with laboratory methods, especially molecular techniques, which increase precision and allow a better understanding of viral circulation in populations.

Thus, understanding and improving the diagnosis of avian poxvirus is not only a technical issue, but also a commitment to animal health, biodiversity, and the sustainability of ecosystems.

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