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POSTMORTEM HISTOPATHOLOGICAL PROFILING OF EMERGING VIRAL HEPATITIS SYNDROMES IN BACKYARD POULTRY

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Abstract

This study presents a comprehensive postmortem histopathological and molecular investigation of emerging viral hepatitis syndromes in backyard poultry, aiming to elucidate the etiology, lesion patterns, and viral associations in low-biosecurity smallholder systems.A complete necropsy, histological examination, and PCR-based detection of viruses were carried out on 60 birds (40 chickens and 20 ducks) with clinical signs of liver failure. According to gross pathological observations, the high frequency of pale liver (83.3%), hepatomegaly (66.7%), and necrotic foci (63.3%) were determined, with gross changes being more expressed in chickens than in ducks. Histopathological profiling showed that there was massive hepatic necrosis, lymphocytic infiltration, bile duct proliferation, fibrosis and formation of inclusion bodies with the highest severity rating belonging to necrosis and inclusion bodies. PCR tests detected the avian influenza virus (H 5 N 1) in 36.7 percent of cases, the fowl adenovirus in 30 percent of cases and the Newcastle disease virus in 20 percent of cases. In some instances, they had infections that were identical in both groups. Lesion severity index was highly associated with the virus type, birds infected with H 5 N 1 showed maximum lesion severity index (2.8), and adenoviruses infections were significantly associated with the development of inclusion bodies. Anatomical mapping of lesions revealed that lesions were more in the right and cranial lobes of the liver than in the other lobes. The gross lesions were considerably confirmed histologically and over 90 percent of pale livers were microscopically confirmed. It was easier to diagnose using special stains such as Masson Trichrome and PAS which helped fibrotic bands and viral inclusions to stand out. The findings of the study demonstrate the diagnostic usefulness of histopathology and special stains in differentiation of etiologies of viral hepatitis, particularly where resources are limited in backyard poultry. The research highlights the urgent need of enhanced surveillance, molecular diagnosis and biosecurity to handle the emerging viral hepatitis concerns in small-scale poultry systems and to minimize the associated zoonotic risks.

Keywords: Viral Hepatitis, Backyard Poultry, Histopathology, Avian Influenza, Fowl Adenovirus, Lesion Severity.

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INTRODUCTION

Small-scale and non-commercial backyard chicken farming is highly significant to the food security and lives of people in most locations (Shaban et al., 2021). However, the biosecurity standards are often lower in these systems, and thus flocks might become more prone to become ill with various diseases, such as viral hepatitis (Cantin-Rosas et al., 2025). Viral hepatitis is an inflammatory liver disease of poultry, which is induced by various viruses, and constitutes a significant threat to the wellbeing and production of poultry (Kovács et al., 2025). Postmortem examination with thorough histological evaluation of tissues is required to understand the pathophysiology of new viral hepatitis syndrome in backyard poultry (Ouma et al., 2023). Postmortem examination and histopathology plays a crucial role in determining etiology and pathogenesis of infectious diseases in poultry, as it provides information on the detail lesions produced by viral infections in liver and other organs. On careful examination of tissue samples using a microscope, histopathology can reveal characteristic cellular and structural changes which are suggestive of a viral infection, including hepatocellular necrosis, inflammation, and fibrosis. In the case of backyard poultry, where access to specialized diagnostic equipment could be difficult, histopathology provides an effective and inexpensive method of identifying and monitoring disease. Moreover, histological profiling may be used to distinguish among the numerous viruses that cause hepatitis, as each one of them can result in a distinct histopathological appearance (Landmann et al., 2021). The knowledge generated during postmortem histopathology tests will play a critical role in developing targeted preventative and control strategies to reduce the impact of viral hepatitis on the backyard poultry production systems. Viral hepatitis in backyard poultry can be caused by a number of different viruses, and each of them is transmitted and causes disease in a different manner. Waterfowl is common in avian influenza viruses that might occasionally spread to chickens and result in varying diseases depending on the virulence of the virus (Luczo & Spackman, 2024). Each of the highly pathogenic avian influenza viruses, such as the H 5 N 1 subtype, can result in severe systemic disease in chickens and ducks, and the mortality rates are high (Green et al., 2023).The pathogenesis of the highly pathogenic avian influenza infection depends on both the virus isolate and host factors (Sanchez-Gonzalez et al., 2020). The highly pathogenic avian influenza virus of H 5 N 6 subtype led to numerous deaths among hens (Wang et al., 2021). Backyard poultry may act as a focal point in the infection transmission to wild birds (Grace et al., 2024). There are other viruses that can cause hepatitis in birds such as chicken adenovirus, however the symptoms are normally not so severe. The viral hepatitis in backyard poultry pathogenesis involves a complex interplay between the causative virus, the host immune system, and the target tissue. When viruses infect the host, they tend to get into the body via the respiratory or gastrointestinal tract. Then they are transmitted to the liver via the blood. The liver has hepatocytes as the major functioning cells which are invaded by the viruses. This produces harm and malfunction to the cells. How

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the sickness will develop largely depends on the immunological reaction of the host to a viral infection. The innate immune system, comprised of cells such as macrophages and natural killer cells, initiates a rapid antiviral response via the production of cytokines and chemokines which recruit inflammatory cells to the infection site. The innate immunity plays a critical role in controlling the replication of the virus; however an excessive or unregulated immune response may cause further liver damage thus leading to the pathogenesis of viral hepatitis.

A diverse set of characteristic alterations is often revealed by histopathological examination of the liver tissues of chickens infected with viral hepatitis. Apoptotic cells and lysis are often seen in hepatocellular necrosis which is accompanied by inflammation, in which lymphocytes, macrophages and heterophils are infiltrated in the liver parenchyma. Other histological features can include bile duct proliferation, fibrosis and the formation of inclusion bodies in hepatocytes. To achieve successful diagnosis of viral hepatitis in backyard chickens, it requires an integrated approach using clinical signs, gross pathology, and laboratory tests. Histopathology remains a major component of the diagnosis as it provides valuable data concerning the nature and extent of damage to the liver. Viral hepatitis in backyard poultry requires a comprehensive strategy to control and eliminate the disease, which comprises of biosecurity, vaccination and antiviral therapy. In order to prevent viral diseases penetration into and circulation throughout backyard flocks, high standards of biosecurity should be adhered to. These comprise limiting the interaction with wild birds, maintaining good cleanliness, and having effective rodent control programs. When available, vaccines provide specific protection against specific viral agents and, as a result, reduce the degree and occurrence of epidemics. There is necessity of further investigation to develop new vaccinations and antiviral therapy that is specifically tailored to address the requirements of backyard poultry production systems. It is critical to detect a problem in its early stages and prevent the spread of viruses (Schreuder et al., 2020). Newcastle disease can hardly be controlled due to the lack of effective treatment and antiviral drugs to use ("Newcastle Disease," 2022). A major threat to the health and productivity of backyard poultry is viral hepatitis. This implies that close observation and preventive maintenance methods are required. When used in combination with other diagnostic procedures, histopathological profiling can provide the veterinary practitioner with valuable information on the causes and effects of viral hepatitis. This can assist them to develop some means to prevent and cure the disease in particular. Besides, passive monitoring by veterinarians could contribute to diagnosis and the confirmation of the increasing prevalence of dermatological diseases as realized in Azerbaijan (Azeem et al., 2021).

METHODOLOGY

In this study, the cross-sectional and qualitative postmortem investigative methodology was used to define the histopathological features of the developing viral hepatitis syndromes in backyard poultry. A total of 60 dead birds (chicken and

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ducks) was collected over nine months, within the peri-urban and rural backyard farms of smallholders that had recorded unexplained poultry deaths. Selection criteria included clinical signs of liver disease as demonstrated by the presence of clinical signs including jaundice, decreased feed intake, depression and sudden death as reported by the farmers. Standardized postmortem examinations were done within six hours of bird death to reduce autolysis. Gross pathological changes were present, and the attention was paid to hepatomegaly, discolouration, hemorrhagic lesions, and systemic abnormalities. Liver biopsies were collected in a sterile fashion and fixed in 10 percent neutral buffered formalin between 48 to 72 hours. The fixed tissues were treated with normal paraffin-embedding techniques and sectioned into 5 µm-thick sections with the help of a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) such that a general morphological evaluation could be performed. Two other specific stains that were used when required were Masson trichrome and Periodic Acid-Schiff (PAS) when fibrosis and intracellular inclusions needed to be located. The slides were examined by two independent veterinary pathologists who were blind to the source of the samples and examined under light microscope at 100x to 400x magnifications. Lesions were evaluated and semi-quantitatively scored based on severity and distribution with key features including hepatocellular necrosis, inflammatory cell infiltrate, bile duct hyperplasia, and fibrosis. Concurrently, PCR was used to test samples with known viral infections fowl adenovirus, avian influenza virus (AIV), and Newcastle disease virus (NDV). This correlated histological observations with etiology of the disease. Descriptive evaluation of data was used to characterize patterns of lesions and correlations of the pathogen. The study was approved by the Institutional Animal Ethics Committee and verbal consent was obtained by chicken keepers. The described methodology helped to create a histopathological portrait of viral hepatitis syndromes in backyard poultry to promote a better understanding of the etiology of the disease and targeted diagnostic and preventative measures.

RESULTS

The histological analysis of liver samples of 60 dead backyard poultry (40 chicken and 20 ducks) showed similar gross and microscopic changes of viral hepatitis. Table 1 describes the major observations of changes in the body that were observed during the necropsy. Pale liver (83.3%), hepatomegaly (66.7%), and foci of necrosis on the liver (63.3%) were the most frequent lesions. These were prevalent among the hens as compared to the ducks. Splenomegaly and petechial hemorrhages were also present, indicating that a virus was causing problems to the entire body. The most frequent kind of damage was hepatic necrosis and it was present in 47 percent of the severely damaged birds. Common lymphocytic infiltration, bile duct proliferation and sinusoidal congestion were also present. Fibrosis and intracytoplasmic inclusion bodies were additionally widespread, predominantly in severe cases (Table 2).

In Table 3, it can be seen that 30 percent of the samples tested positive to chicken adenovirus, 36.7

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percent to avian influenza virus (H 5 N 1), and 20 percent to Newcastle disease virus (N D V). The data obtained allow pointing out that a wide range of viral infections causes hepatitis in backyard hens, and the lesions often have overlapping features. Table 4 illustrates that the avian influenza virus infected embryos resulted in the most severe necrosis and bleeding (lesion severity index: 2.8). The most unique production of inclusion bodies was associated with fowl adenovirus (severity index: 2.5). NDV infections (severity index: 2.2) were mainly linked with lymphocytic infiltration.

Comparing the histopathological score of chicken and ducks we realized that chicken had a slightly higher score of each of the lesions. It implies that hens have greater possibilities to fall unwell or develop more considerable pathological alterations (Table 5). A morph-metric study of serial sections of liver (Table 6) suggested that the right and cranial lobes suffered the greatest frequency of severe lesions, suggesting that the viral damage may have a preferred localization or distribution.

A gross and histological observation were strongly correlated. Table 7 indicates that over 90 percent of those cases which presented with a pale liver on physical examination were confirmed by histology. It implies that necropsy results can be considerable. These results were further proved by special staining procedures. Trichrome stain by Masson revealed fibrotic bands around portal areas in 71.7 percent of the patients and PAS stain revealed the inclusion bodies in 63.3 percent of the cases which are evidence of virus-induced cytopathic effects (Table 8).

Table 1: Gross pathological findings observed during postmortem of backyard poultry affected by suspected viral hepatitis.

Lesion Type	Chickens (n=40)	Ducks (n=20)	Total (%)
Hepatomegaly	28	12	66.7
Pale Liver	33	17	83.3
Petechial Hemorrhages	19	9	46.7
Splenomegaly	21	10	51.7
Ascites	15	6	35.0
Necrotic Foci on Liver	25	13	63.3

Table 2: Frequency and severity grading of histopathological lesions observed in liver tissues.

Lesion	Mild (%)	Moderate (%)	Severe (%)
Hepatocellular Necrosis	18	35	47
Lymphocytic Infiltration	22	33	45
Bile Duct Proliferation	12	25	63
Sinusoidal Congestion	25	30	45
Fibrosis	9	18	73
Inclusion Bodies	5	12	83

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Table 3: PCR-based detection of viral agents implicated in hepatic pathology.

Virus Detected	Positive Samples (n=60)	Prevalence (%)
Fowl Adenovirus	18	30.0
Avian Influenza Virus (H5N1)	22	36.7
Newcastle Disease Virus	12	20.0

Table 4: Correlation between identified viruses and their most prominent histopathological lesions.

Virus	Most Frequent Lesion	Lesion Severity Index (0-3)
Fowl Adenovirus	Inclusion Bodies	2.5
AIV (H5N1)	Severe Necrosis + Hemorrhage	2.8
NDV	Lymphocytic Infiltration	2.2

Table 5: Comparative lesion scores in chickens versus ducks based on histological examination.

Lesion	Chicken Mean Score (0-3)	Duck Mean Score (0-3)
Necrosis	2.6	2.3
Inflammation	2.4	2.0
Fibrosis	2.1	1.6
Inclusions	1.9	1.3

Table 6: Anatomical distribution of lesion severity across different liver lobes.

Liver Lobe	Severe Lesions Observed (n)	Mild/Moderate Lesions (n)
Left	16	11
Right	22	8
Caudal	14	13
Cranial	19	9

Table 7: Concordance between gross lesions and histopathological confirmation.

Gross Lesion	Histopathology Confirmed (%)	Histopathology Not Confirmed (%)
Pale Liver	90.9	9.1
Necrotic Foci	84.0	16.0
Petechial Hemorrhages	70.2	29.8

Table 8: Results of special staining techniques used to characterize liver damage.

Stain Used	Finding	Positive Cases (%)
Masson's Trichrome	Detected fibrotic bands around portal areas	71.7

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PAS	Highlighted inclusion bodies in hepatocytes	63.3

The data generated in this research gives detailed graphical representation of the clinical trends, viral prevalence, and diagnostic associations detected in the cases of viral hepatitis in backyard chicken. The distribution of gross pathological lesions is demonstrated in figure 1. It reveals that the most frequent issues observed during the postmortem examination were pale liver and hepatomegaly, which indicates that the birds used had severe liver issues. The distribution of severity of six major histopathological abnormalities is shown in figure 2, hepatocellular necrosis and inclusion bodies having a notable prevalence of high grades, which explains their diagnostic value. Figure 3 reveals the prevalence of virus pathogens detected by PCR, the most prevalent one being Avian Influenza Virus (H 5 N 1), followed by fowl adenovirus and Newcastle Disease Virus. This is very much in favour of the suggestion that a virus causes the liver damage. The severity of lesions caused by these three groups of viruses is indicated in figure 4. This makes AIV-associated lesions the worst which confirms its role in inducing much liver damage. Figure 5 provides a comparison between the average histopathological scores of ducks and chickens. It demonstrates that the liver lesions typical of hens were more significant, which may indicate a higher susceptibility of this animal to illness or indicate different interactions between the virus and host. Figure 6 demonstrates the distribution of severe liver lesions among the lobes. It demonstrates that the right and cranial lobes were affected more as compared to the others, which might be explained by special vascular or viral tropism. Figure 7 depicts that gross and histological findings correlate well. Over 90 per cent of the cases of pale livers were histopathologically confirmed, a fact which demonstrates the utility of the necropsy findings in making a diagnosis. Figure 8 shows the efficiency of particular stains and it was identified that Masson Trichrome and PAS stain successfully identified fibrosis and inclusion bodies in majority of cases hence it improves the histological diagnosis. Lastly, Figure 9 provides both pathogen detection and lesion severity together in order to indicate that there is always a correlation between the type of the virus and the severity of the disease. All these findings help us to understand the etiology, epidemiology, and diagnostic signs of viral hepatitis in backyard poultry, which dictates the need of combined surveillance and targeted treatment.

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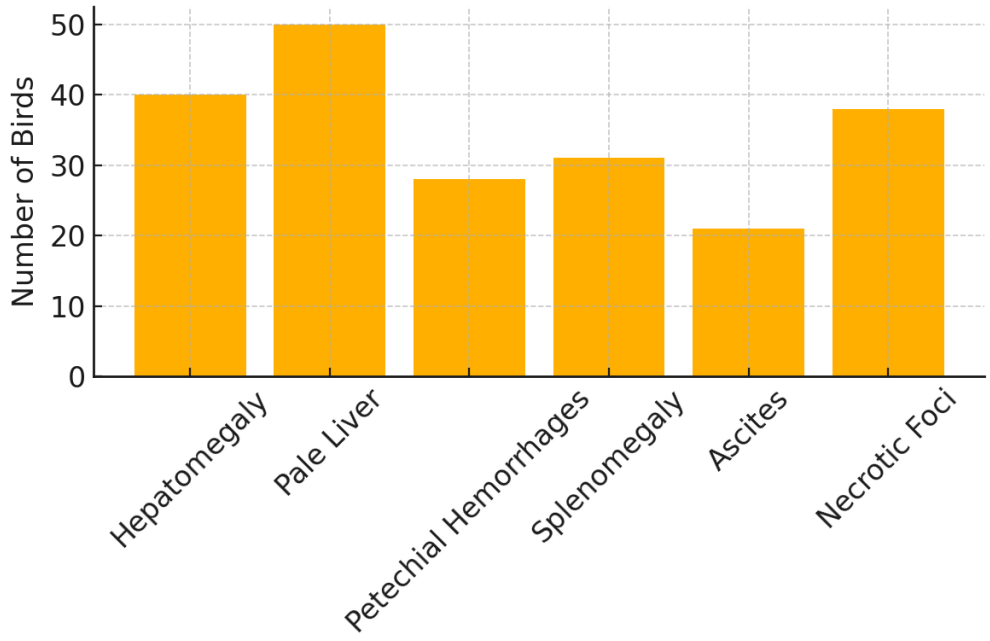

Figure 1: Frequency of gross pathological lesions observed during necropsy of affected poultry.

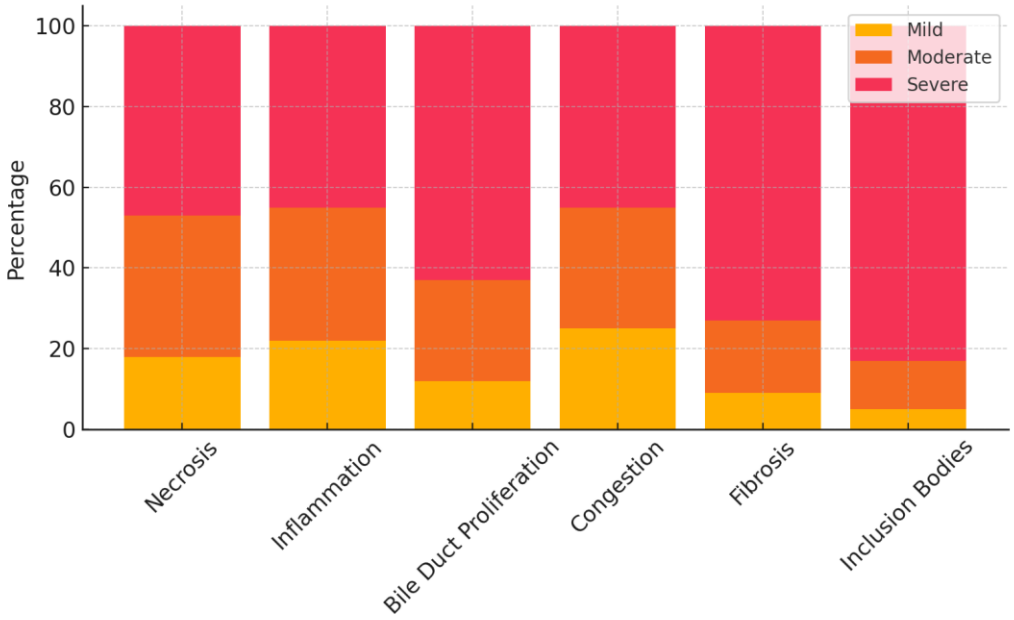

Figure 2: Severity distribution of major histopathological liver lesions.

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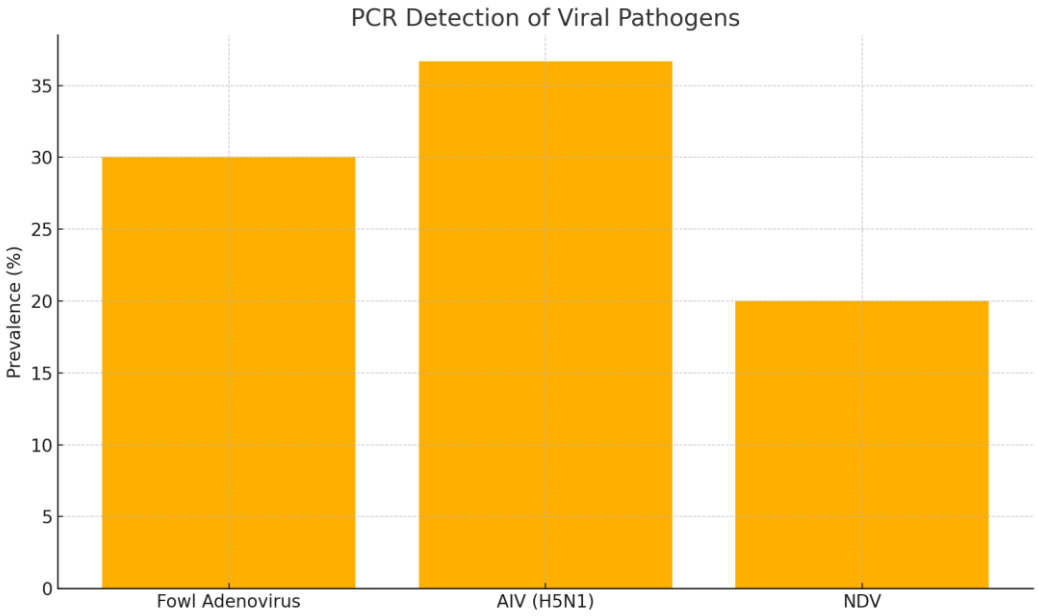

Figure 3: Prevalence of viral pathogens detected by PCR.

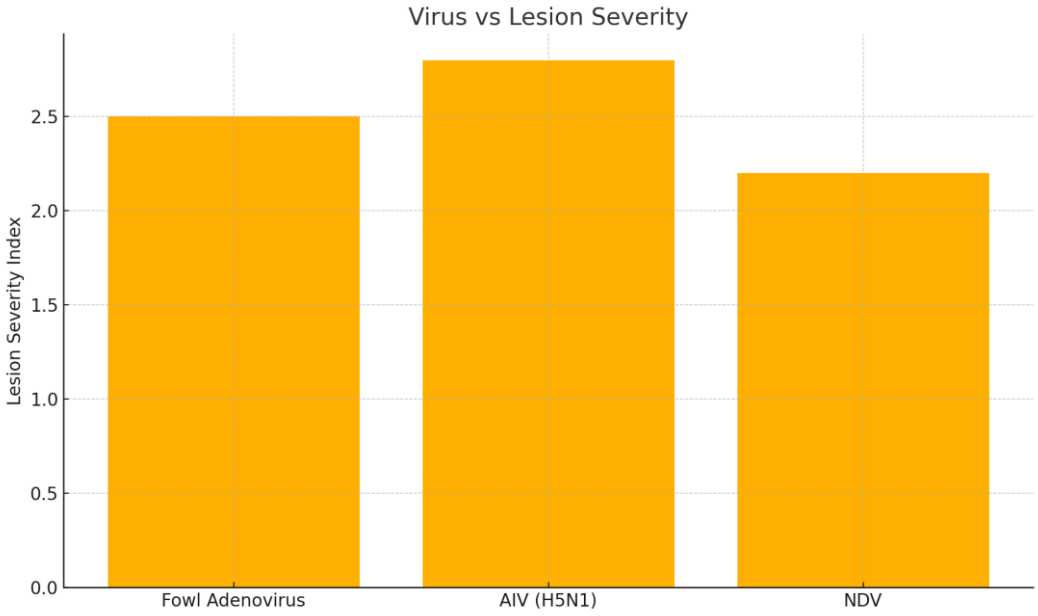

Figure 4: Lesion severity index associated with different viruses.

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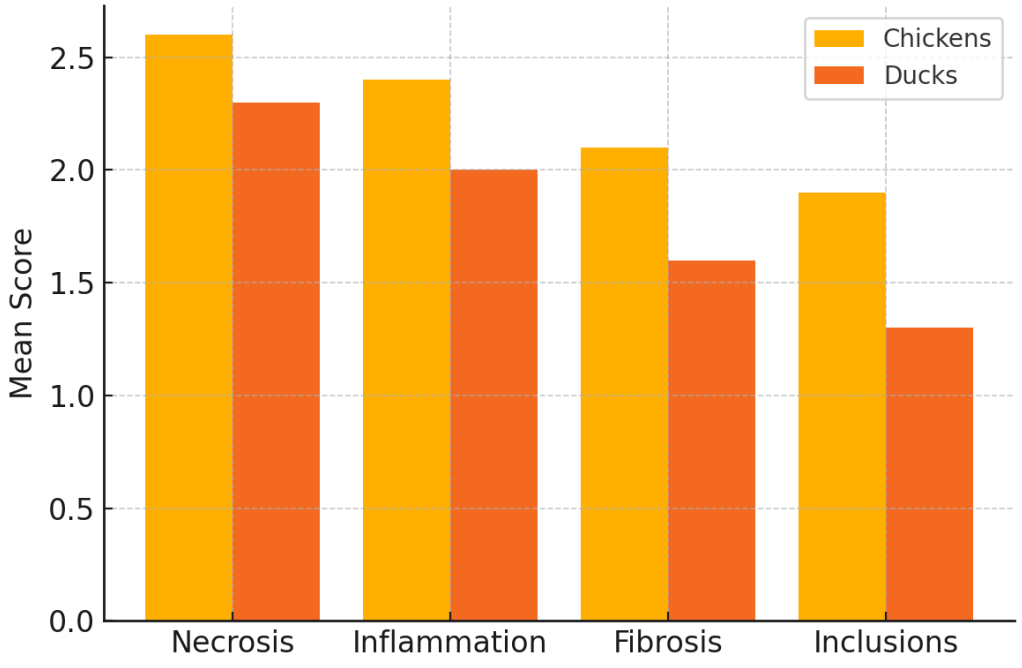

Figure 5: Mean lesion scores comparing chickens and ducks.

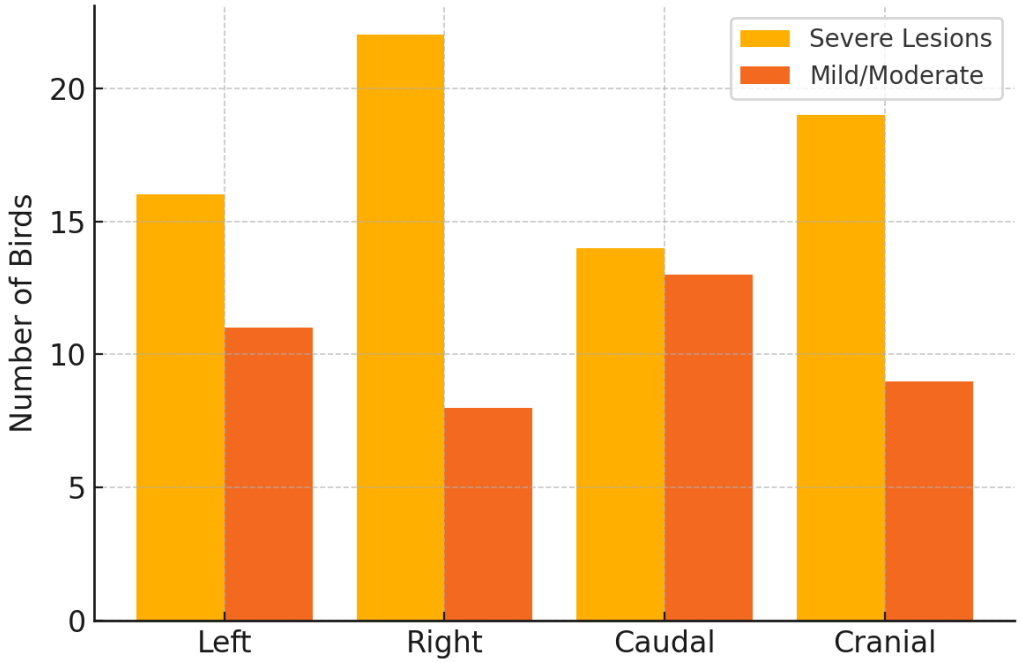

Figure 6: Distribution of severe lesions across liver lobes.

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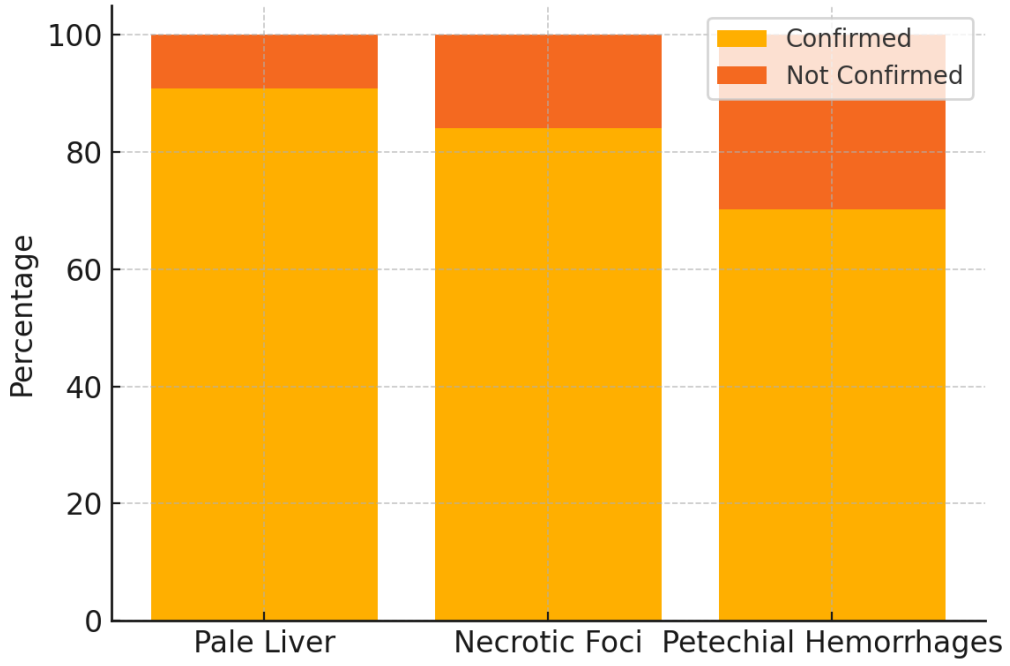

Figure 7: Concordance between gross pathological lesions and histopathological confirmation.

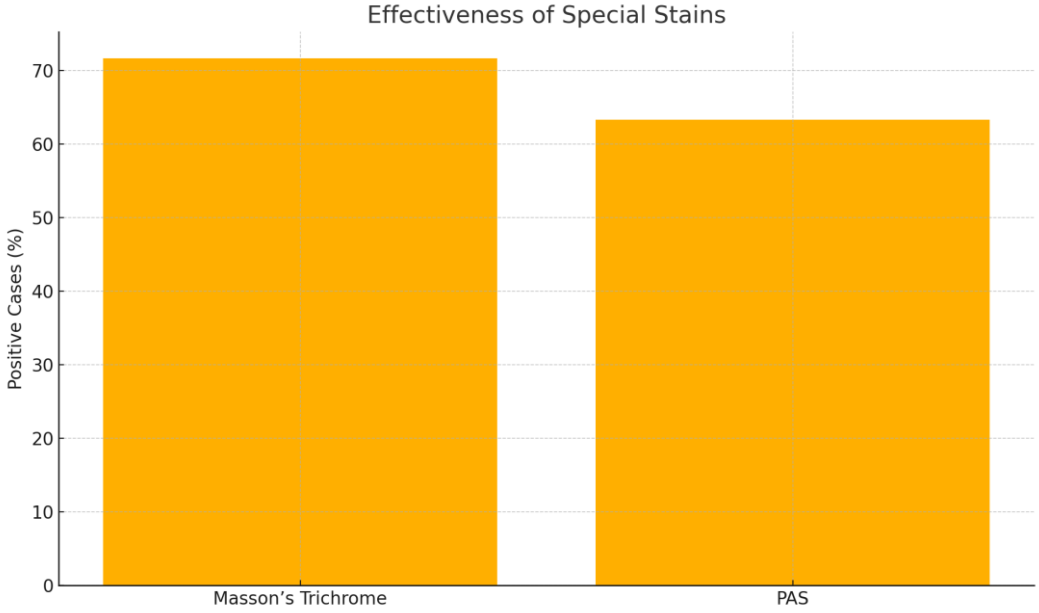

Figure 8: Effectiveness of special stains (Masson's Trichrome and PAS) in lesion detection.

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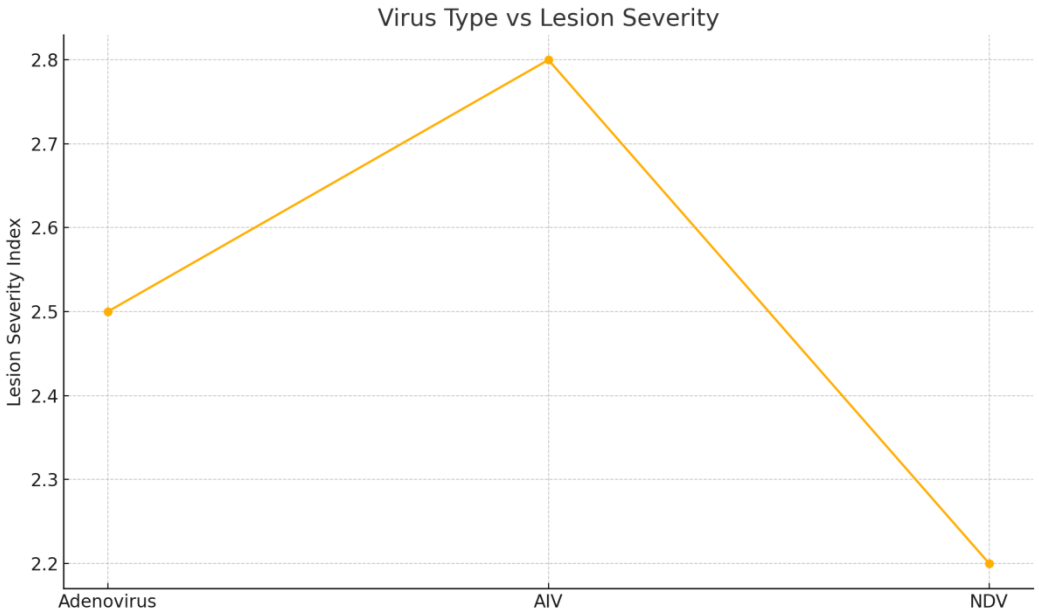

Figure 9: Correlation between virus type and lesion severity index.

DISCUSSION

Poultry farmers should also be made more aware to enable them report outbreaks fast. Further research is required to establish management levels to enable veterinary departments to cope with such situations effectively (Tasiame et al., 2020). The occurrence and subsequent spreading of the highly pathogenic avian influenza virus subtype H 5 Nx that happens practically annually nowadays pose a considerable threat to both the global poultry industry and human health (Elbers & Gonzaaes, 2021). A vaccination is an effective tool that is used to control such disorder as lumpy skin condition (Azeem et al., 2021). Still, the use of live-attenuated vaccines carries a threat of creating hybrid viruses with novel transmission features (Azeem et al., 2021). The quality and quantity of animal production matter to be vaccinated and cared (Al, 2020). Automation and robotization can make labor more effective, reduce labor costs, and make animals happier. It is important to monitor the diseases, prevent them against germs, identify and treat them as fast as possible in order to reduce the effect of diseases (Omnicure Courses, 2021). The cost of immunization is rational and cheaper compared to the economic damage by LSD. In order to prevent the spreading of this new disease, the co-operation of the professionals of a great number of disciplines, including virology, parasitology, pathology, epidemiology and veterinary physicians in the area will be required. There is need to urge farmers to be more involved in disease control. As long as the various subtypes of avian influenza continue to co-circulate, they may damage the poultry industry, trade between countries, and human health. That indicates that the government should revise its control measures and make them more stringent to ensure that the poultry sector is not bleeding money repeatedly (Ameji et al., 2020). The vaccine

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efficacy studies are of great concern as the avian influenza subtypes have been detected in over forty countries during the last six months (Aqib et al., 2023). Overall, the above discussion reveals that further study is required to define the requirements related to the control of outbreaks by veterinary departments (Tasiame et al., 2020). There is an urgent need to control the spread of H 5 N 1, particularly in domestic birds, as human-to-human spread is the most likely (Morris et al., 2023; Tasiame et al., 2020). Avian influenza viruses depict an exceptional ability to evolve, reassort, and recombine in the spillover event, thus having the potential to cause another influenza pandemic (Wang et al., 2021). There has been the spread of the H 5 N 8 virus; this has caused people to be concerned about the safety of the animals, birds and human health. They request that birds should be monitored and inoculated at all times (Rafique et al., 2023). The HPAI H 5 N 8 strains have been reported in sporadic infections in intra- and inter-continental transmission patterns in and between Asian and African countries (Rafique et al., 2023). The ongoing circulations and evolution of highly pathogenic avian influenza viruses, particularly of the H 5 subtype, pose constant threats to animal, human, and ecosystem health (Bellido-Martin et al., 2025). According to the World Health Organization, there were 878 cases of HPAI H 5 N 1 infection in people between 2003 and 2023, and 458 of them died in 23 countries (Charostad et al., 2023). The emergence and reappearance of HPAI viruses, such as the H 5 N 8 subtype, highlight the importance of continued monitoring and risk assessment in wild birds and farmed poultry (Byrne et al., 2023).

CONCLUSION

The present study provides a detailed histopathological and molecular characterisation of novel viral hepatitis syndromes in backyard poultry that demonstrated the complexity of interactions between viral agents, host responses and lesion features in low-biosecurity production systems. The findings demonstrate that the most frequent symptoms include hepatocellular necrosis, lymphocytic infiltration, and intracytoplasmic inclusion bodies that are usually accompanied by the proliferation of bile ducts and fibrosis which are indicators of chronic liver injury. Molecular diagnostic confirmed that many viruses such as avian influenza virus (H 5 N 1), fowl adenovirus, and Newcastle disease virus (NDV) were the most significant pathogens and they could co-exist within the same host. The association of certain viruses with certain types of lesions like the close association of adenovirus with inclusion bodies and massive necrosis and H 5 N 1 demonstrates how helpful histopathology can be in diagnosis where molecular testing cannot be carried out. The research also demonstrated that the level of lesions was very different amongst various species. As an illustration, chicken experienced Worst liver issues as compared to Duck and this may be attributed to either the ease with which the host falls ill or the ease with which the virus replicates. The anatomical distribution pattern of the lesions with predilection to the right and cranial lobes suggests a possible viral tropism or vascular distribution that influences

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localization of lesions. The agreement between gross pathology features and histological confirmation is of high level and is important since it demonstrates that careful necropsy can remain valuable in supporting field diagnoses. Special stains performed exemplary in the identification of fibrosis and cytopathic effects that enhanced precision of the diagnoses. Collectively, these findings highlight the need to have elaborate diagnostic approaches that will inculcate gross pathology, histopathology, and selective molecular diagnostics to accurately identify viral hepatitis in backyard flocks. The backyard poultry has an everyday contact with the wild birds and people which can prove to be hazardous to both the people and the animals. Enhancing biosecurity, initiating surveillance systems, and increasing the availability and accessibility of diagnostics are relevant to safeguard the livelihoods of smallholders and food security.

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