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Effect of IgY Treatment on The Histopathological Finding in Tissue Sections of Ducks Naturally Infected with AI-H5N1

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Abstract

A NATURAL outbreak of Avian Influenza H5N1 (AI-H5N1) in Muscovy ducks, with nervous signs, eye opacity, high mortality as well as necrosis, and hemorrhage in pancreas. The infection was confirmed by RT-PCR. Injection of IgY revealed stopped mortality and signs with improvement in general health conditions in birds injected with dilution 1/5 and 1/10 as compared with noninjected. Examined tissue sections from AI-H5N1 naturally infected ducks showed area of hemorrhages in the brain and hemorrhage and necrosis in the liver—pancreas showing areas of hemorrhages and focal area of coagulative necrosis characterized by lymphocytic infiltration. Severe depletion of the splenic lymphoid follicle. The trachea is suffering from moderate to severe submucosal hemorrhages. IgY-injected ducks showed milder lesions than infected non-treated. Birds injected with 1/5 diluted IgY showed milder lesions than those injected with 1/10.

The results suggest that administering IgY antibodies against the HPAI H5N1 virus can mitigate the severe signs, lesions, mortality, and histopathological changes observed in ducks naturally infected with the virus. The IgY antibodies may neutralize the virus and reduce the viral load, thereby limiting the extent of tissue damage and inflammatory response, indicating its potential as a therapeutic approach for managing avian influenza outbreaks. The usage of IgY appears to confer protection against signs, mortality, and pathological changes induced by the AI-H5N1 infection.

Keywords: Ducks, HPAI-H5N1, signs, lesions, Histopathology, IgY injection.

Introduction

Domestic ducks serve as a reservoir for several AIV subtypes and their reassortments, aiding in the creation of new AIV genotypes and virus pathogenicity [1,2]. Pathological lesions in domestic ducks infected with HPAIVs vary according to regional circumstances, whereas the extent of the disease varies based on the infected animal, viral pathogenicity and virulence, and secondary infections [3,4]. Domestic ducks intranasal (i.n) inoculated with H5N1 HPAI viruses frequently showed corneal opacity more than neurologic signs and mortality [5,6]. Pancreatic hemorrhages with necrosis are frequently seen [7,8].

The histopathological characteristics of infected meat-type ducks included necrotic lesions of heart and brain, are contributed to the high mortality rate in infected young (meat-type) and older (breeder) ducks that were naturally infected with the highly pathogenic avian influenza (HPAI) H5N1 virus [9].

Histopathological finding in naturally infected ducks with AI H5N8 showed inflammatory cells infiltration of the intestinal mucosa and congestion of the submucosa, necrosis of the mucosa with leucocytic and mononuclear cells infiltration and submucosal congestion. Liver showing congestion of the sinusoids with severe congestion of central vein. Spleen showing congestion of red pulp [6]. Thymus showing medullary haemorrhages and inter

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follicular haemorrhages and depletion of the lymphoid follicles. Pancreatic acinar showing Area of haemorrhages with inflammatory cells infiltration and, pancreatic acinar showing acute coagulative with necrosis [6]. Ducks exhibited lymphohistiocytic meningoencephalitis with multifocal haemorrhages, multifocal necrotic pancreatitis, and severe necrotic myocarditis with mineralization [10]. In H5N8 infected ducks severe congestion and haemorrhages in whole organs, proliferation of glia cells and neuronal degeneration in brain, proliferative reaction with leucocytes infiltration in lungs and liver with degenerated and necrosis in pancreatic acini were seen [11]. Additionally, acute Inflammatory, necrotic and vascular reactions that involve different tissues were detected [12].

Many studies have proven that the use of IgY as a type of therapeutic and preventive intervention was effective against many viral infectious diseases [13-15], including AI in the ducks [14] and Newcastle disease (ND) in chickens [15]. The supplementation of purified IgY, combined with probiotics, was shown to significantly improve the overall activity of broilers with immune stress in a study conducted by Rehan et al. [16]. Administration of IgY was reported to reduce the clinical signs and mortality as well as severity of tissue lesions in infected treated birds [15,17]. Also, it was found that IgY was shown to improve drug efficacy by reducing ulcer lesions [18].

This study aimed to evaluate the histopathological changes in tissues of ducks naturally infected with HPAI H5N1 and the effect of IgY treatment to control the infection on these changes.

Material and Methods

Egg yolk antibodies (IgY) injection:

It was separated, purified and standardized for injection by Radwan et al. [14].

Duck flock:

Muscovy ducks 900 33-day old male replacement herd for semen for artificial insemination reared on deep litter. After field investigation and collection of flock history. clinical examination, postmortem (p.m), and collection of liver, spleen, and pancreas for molecular diagnosis of H5N1 by RT-PCR [19].

Bird grouping and Administration of IgY:

After 36 hs from start of signs, ducks with mild signs or apparent healthy were divided into 3 groups (1-3). Group 1 and 2 each had 135 ducks to be 3 successive injected with 1/5 or 1/10 diluted IgY while group 3 (260 birds) were kept as non injected control. IgY was injection in thigh muscles for 3 successive days. All groups were kept under daily observation for 10 days from the 1st injection with recording of signs, mortality, lesions and collection of samples for histopathology.

Histopathological examination:

Three ducks from nontreated, treated with 1/10 and 1/5 injected ducks were slaughtered at day 6th from last IgY injection (45 days of age) for histopathological examination, organs including the liver, spleen, and brain were collected and fixed on 10% formol saline, sectioned, stained with H&E and subjected for histopathological examination by light microscope [20].

Results and Discussion

Avian influenza virus specially HPAI-H5N1 affect mostly chicken, turkey and domestic ducks with serious losses [1,2,4]. Naturally infected ducks showed clinical signs including off-food nervous signs (Torticollis), respiratory signs, recumbency, incoordination of movement, corneal opacity, and increased mortality. Mortality reaches 35% within 36 h from the start. The recorded p.m lesions were congestion of the liver, spleen, and lungs that appear after more than 48 h from appear clinical signs. Pancreatic hemorrhage with necrosis was detected. The recorded signs indicating a progression of the disease and a systemic inflammatory response [21], and suggesting that the AI H5N1virus affects the central nervous system (CNS) [5,22], mortality that emphasizes the rapid onset and lethality of H5N1 in ducks [14,23,24], and lesions including eye obesity and pancreatic hemorrhage [6,14,22,24].

The extracted viral RNA from liver, spleen, and pancreas for detection of H5N1 by RT-PCR was revealed positive results at CT 20. This result proved infection with HPAI H5N1 [6, 14].

After the 3rd injection injected diseased ducks restored their health and become apparent normal. The mortality was 3.6, 8.0 and 92.22 % in 1/5, 1/10 and non-injected birds. By the end of the 10th day of observation (7th day after last injection) the total mortality was 23.7%, 45.18% and 98.08% in 1/5,1/10 and non-injected birds, respectively. This result is indicative for the efficacy of IgY injection in control of signs and mortality [14,15,17]. In case of AI IgY had inhibit virus hemagglutination [25], of the homologous as well as in some cases heterologous clades and homologous strains [14,15, 26, 27].

Avian influenza (AI) virus infection is associated by vascular endothelial cells in chicken, which leads to altered permeability of blood vessels, lead to hemorrhage, oedema, and micro- thrombosis, and result in death by multi-organ failure because of endothelial tropism [22]. One potential treatment approach is the use of IgY antibodies derived from egg yolks of hens immunized with the HPAI H5N1 virus [13,14, 25].

The most recorded lesions are area of hemorrhages in brain (fig 1 a), focal area of coagulative in liver (Fig 1 b), area of hemorrhages and focal area of coaglutive necrosis characterized by lymphocytic infiltration in pancreas (Fig 1 c), spleen sections showed associated with severe congestion of the splenic blood vessels in the lymphoid follicle. (Fig 1 d). while trachea was suffering from severe submucosal hemorrhages (Fig 1 e) to severe congestions of the blood vessels with submucosal hemorrhages (Fig 1 f). Now, lesions of vascular damage in the pathogenesis HPAI in ducks were rarely observed [28,29]. Multiple virus-associated necrotic lesions in one or more organs (pancreas, heart, brain) were responsible for death, and tissue tropism has also been observed in avian species such as mute swans, ruddy shelducks and mandarin ducks [30]. The brain tissue shows areas of hemorrhage. Viral infection can lead to disruption of the vascular system, resulting in bleeding and hemorrhage within the brain parenchyma [31].

The liver tissue exhibits hemorrhage and necrosis. Avian influenza H5N1 virus can induce hepatocellular degeneration and necrosis, which is accompanied by hemorrhage due to the virus's tropism for the vascular endothelium [31,32].

Pancreatic and splenic necrosis were the common pathological findings due to HPAIV H5N1 infection in both Galliformes and Anseriformes, also increased detection of pathological changes in domestic ducks [7]. The virus can target the pancreatic tissue, leading to focal necrosis and an inflammatory response characterized by lymphocytic infiltration [31,32]. Pancreas: The pancreatic tissue shows areas of hemorrhage and focal coagulative necrosis with lymphocytic infiltration. The spleen exhibits severe depletion of the lymphoid follicles. Avian influenza H5N1 virus can induce apoptosis and necrosis of lymphoid cells, leading to the depletion of the splenic lymphoid tissue [31,32].

The tracheal tissue shows moderate to severe submucosal hemorrhages. The virus can disrupt the vascular integrity of the tracheal mucosa, resulting in bleeding and hemorrhage within the submucosal layers [31,32].

AI H5N1 infected ducks and injected with 1/10 diluted IgY showed moderate congestion of the blood vessels (Fig2 A). This clearly indicated that infection could lead to resulting in increased blood flow and congestion of the cerebral vasculature [33]. The liver (fig 2b) and spleen (Fig 2d) tissues exhibit severe congestion of the portal vein and red pub; respectively [33]. The liver tissue sections shows congestion of the sinusoids (Fig 2 c) . Indicating that the inflammatory response to the virus infection and subsequent activation of hepatic stellate cells, leading to the deposition of extracellular matrix components and the development of liver fibrosis [32,33]. The spleen displays severe congestion of the red pulp (Fig 2 d). The viral infection can cause vascular disturbances within the splenic resulting in engorgement of the splenic sinuses and red pulp with blood [33]. The focal areas of necrosis detected in the pancreatic tissue (Fig 2 e). The virus targeting the pancreatic tissue, leading to localized cell death and tissue damage [32,33]. The tracheal tissue exhibits moderate submucosal hemorrhages (Fig 2 f). The viral infection can disrupt the vascular integrity of the tracheal submucosa, resulting in bleeding and the extravasation of red blood cells [6, 14,15, 33].

The histopathological lesions in tissue sections from Avian influenza H5N1 virus naturally infected ducks after injection with 1/5 IgY revealed that brain tissue shows mild congestion of the blood vessels (Fig 3 a). The liver tissue exhibits severe congestion of the portal veins (Fig3 b) to moderate congestion (Fig3 c) in central veins. The pancreatic tissue suffers from areas of necrosis (Fig 3d). The spleen shows depletion of the lymphoid follicles (Fig3 e). The tracheal tissue exhibits mild submucosal hemorrhages (Fig3 f). The viral infection can disrupt the vascular integrity of the tracheal submucosa, leading to bleeding and the extravasation of red blood cells, though the extent of the hemorrhage is less severe compared to higher doses of IgY [33].

Comparing the recorded lesion of the treated, those showed milder lesions than the non-treated birds. The result clearly shows that the administration of IgY has been shown to result in low tissue damage and histopathological lesions in avian influenza virus-infected chickens and ducks. Several studies have demonstrated the protective effects of IgY against the pathological consequences of avian influenza virus infection in poultry [14,15,33]. Also, Tian et al [34] evaluated the efficacy of IgY against H9N2 avian influenza virus infection in chickens. They reported that chickens receiving IgY displayed significantly lower tissue damage and fewer histopathological changes in the respiratory and digestive systems, including the trachea, lungs, and intestines, compared to infected chickens without IgY treatment. Zi-Feng et al [35] demonstrated the protective effects of IgY in the

chickens challenged with the H7N9 AIV. Chickens administered IgY showed reduced histological lesions in the trachea, lung, and other organs, with decreased inflammation, necrosis, and hemorrhage, compared to infected control chickens. In the other hand increased IgY concentration had resulted in more milder lesions in AI-H5N1 naturally infected ducks [13-15].

In the IgY treatment group, the histopathological changes were less severe compared to the control group. The trachea showed mild to moderate epithelial necrosis and inflammation, with reduced hemorrhage and edema. The other organs, such as

liver, spleen, and kidneys, also exhibited less severe pathological changes in the IgY treatment group [14,15, 25,36,37]. The IgY antibodies may neutralize the virus and reduce the viral load, thereby limiting the extent of tissue damage and inflammatory response [38,39]. (Adachi et al., 2008, Eriksson et al., 2024).

Conclusion

These studies suggest that the administration of IgY can effectively mitigate the severity of signs, lesions, mortality as well as histopathological lesions associated with avian influenza virus infection in both chickens and ducks. The passive immunization provided by IgY appears to confer protection against the tissue damage and pathological changes induced by the viral infection.

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Authors' contributions

Fatma M. Radwan and Ahmed Ali El-Shemy collected samples and carried out laboratory and field work. Mohamed Bosila carried out histopathology. Aziza M. Amer collects data and writes the draft. Mohamed M. Amer supervised the manuscript. All members revised the original draft and approved the final manuscript.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Competing interests:

The authors declare that they have no competing interests.

Ethical of approval

The study was carried out according to guidelines for animal welfare and was approved for inclusion of the hens and ducks by the Institutional Animal Care and Use Committee, Vet. Cu. Iacuc. 2009: 2022525.

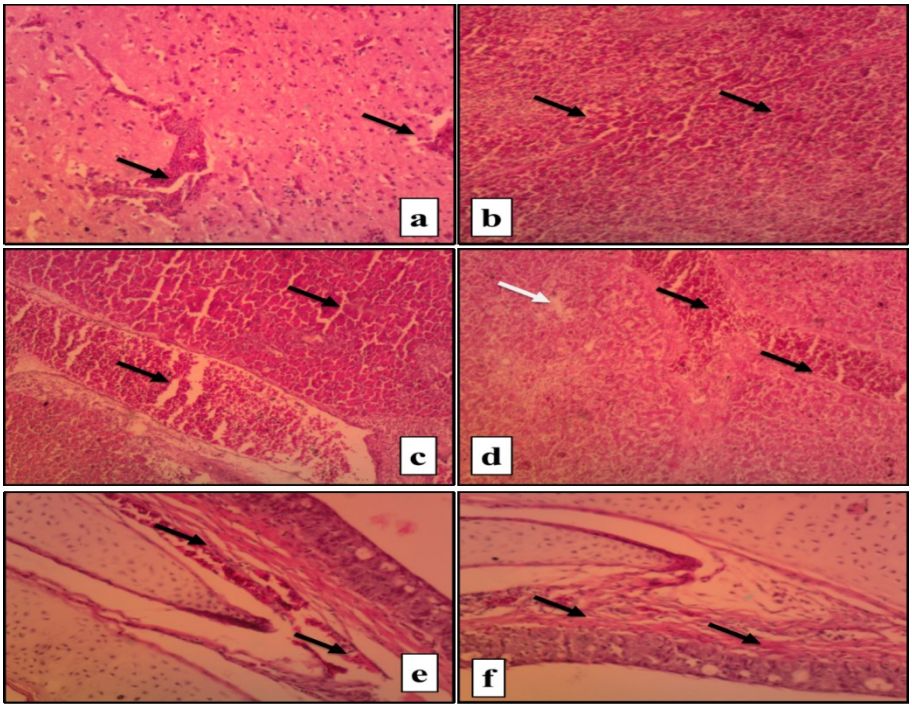

Fig 1. Histopathological lesions in tissue sections (H&E, X 100) from naturally infected ducks with AI-H5N1 virus showing:

a: Brain showing area of hemorrhages (arrows). b: Liver showing focal area of coagulative necrosis (arrows).c: Pancreas showing area of hemorrhages and focal area of coaglutive necrosis characterized by lymphocytic infiltration (arrows). d: Spleen: sever depletion of the lymphoid follicle associated with severe congestion of the splenic blood vessels . (arrows). e: Trachea suffering from severe submucosal hemorrhages (arrows). f. Trachea with submucosal congestions of the blood vessels (arrows).

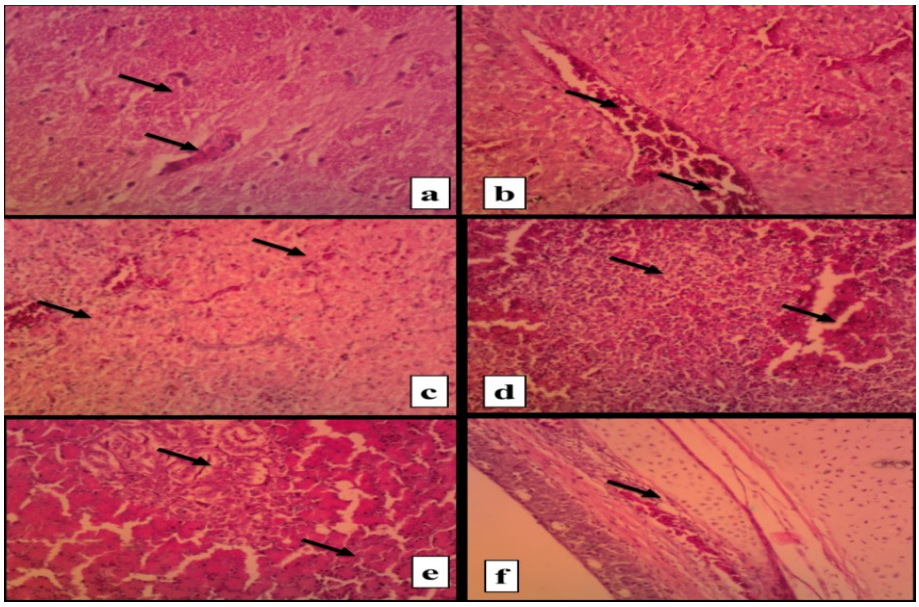

Fig 2. Histopathological lesions in tissue sections (H&E, X 200) from AI- H5N1 virus naturally infected ducks after injection with 1/10 IgY showing:

a: Brain showing moderate congestion of the blood vessels (arrows). b. Liver showing severe congested portal vein. (arrows). c. liver congestion of the sinusoids (arrows). d. spleen showing severe congestion of the red bulb(arrows). e. Pancreas showing focal necrosis (arrows). f. Trachea moderate submucosal hemorrhages (arrows).

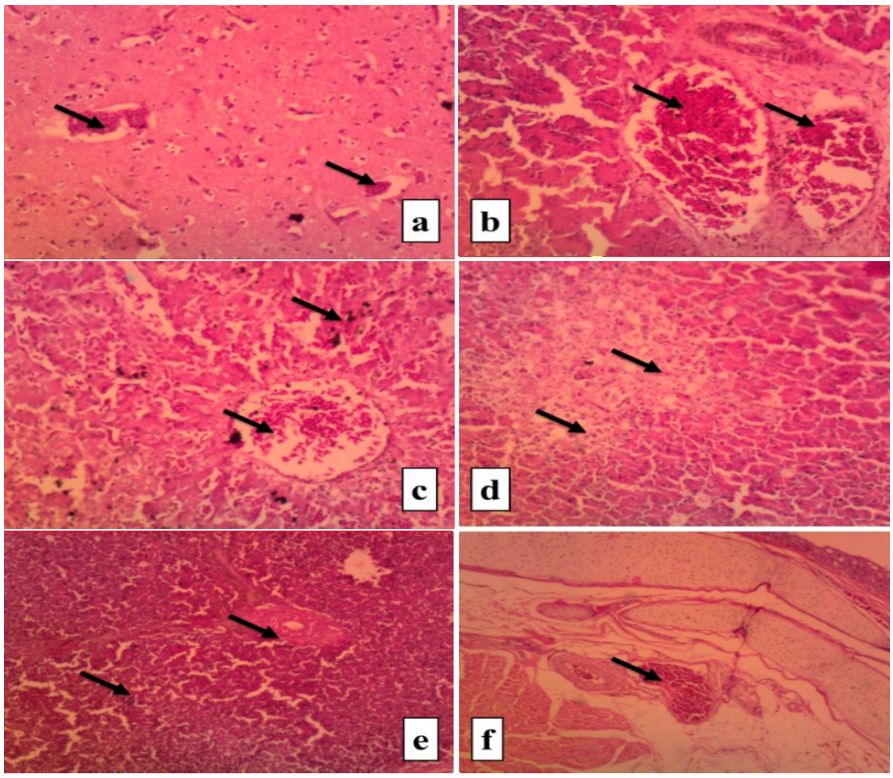

Fig 3. Histopathological lesions in tissue sections (H&E) from AI- H5N1 virus naturally infected ducks after injection with 1/5 IgY showing:

a: Brain shows mild congestion of the blood vessels (arrows) (X 200). b: Liver shows severe congestion of the portal veins (arrows)(X 100) . c: Liver shows moderate congestion of the central vein (arrows)(X200). d: Pancreas suffering from area of necrosis. (arrows)(X 100). e: Spleen showing depletion of the lymphoid follicles (arrows)(X 100). f. Trachea with mild submucosal hemorrhages (arrows)(X 100).

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تأثير العلاج بـ IgY على النتائج النسيجية المرضية في انسجة البط المصاب طبيعياً بفيروس انفلونزا الطيور H5N1

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الملخص

عدوي طبيعيه لأنفلونزا الطيور بفيروس الانفلونزا فى البط المسكوفي(AI-H5N1) ، مع ظهور اعراض عصبية وعتامه العين وارتفاع معدل النفوق وكذلك النخر والنزيف في البنكرياس. وتم تأكيد الإصابة بواسطة RT-PCR . أظهر حقن IgY وجود توقف النفوق و تحسن في الحالة الصحية العامة في الطيور المحقونة بتخفيف 5/1 و 10/1 مقارنة مع غير المحقونة. أظهرت المقاطع الأنسجة المجهزه من البط المصاب طبيعياً بفيروس AI-H5N1 وجود منطقة نزيف في الدماغ ونزيف ونخر في الكبد. يُظهر البنكرياس منطقة النزف والمنطقة البؤرية للنخر التخثري الذي يتميز بالارتشاح اللمفاوي. استنزاف شديد للجريب اللمفاوي الطحالي. القصبة الهوائية تعاني من نزيف معتدل إلى شديد تحت المخاطية. أظهرت البط المحقونة بالـ IgY آفة أخف من تلك المصابة غير المعالجة. أظهرت الطيور التي تم حقنها بـ 5/1 من IgY المخفف آفة أخف من تلك التي تم حقنها بـ 10/1 .

تشير النتائج إلى أن إعطاء الأجسام المضادة IgY ضد فيروس HPAI H5N1 يمكن أن يخفف من التغيرات النسيجية الشديدة التي لوحظت في أنسجة البط المصابة بالفيروس بشكل طبيعي. قد تعمل الأجسام المضادة IgY على تحييد الفيروس وتقليل الحمل الفيروسي، مما يحد من مدى تلف الأنسجة والاستجابة الالتهابية. كان علاج IgY فعالاً في الحد من شدة التغيرات النسيجية المرضية في البط المصاب بشكل طبيعي بفيروسHPAI H5N1 ، مما يشير إلى إمكاناته كنهج علاجي لإدارة تفشي أنفلونزا الطيور. يبدو أن التحصين السلبي الذي يقدمه IgY يمنح الحماية ضد الاعراص والنفوق وتلف الأنسجة والتغيرات المرضية الناجمة عن العدوى الفيروسية.

الكلمات الدالة: البط، فيروس الانفلونزا شديد الضراوه (HPAI-H5N1) ،الاعراص ، التشريح ، التغيرات النسيجيه، حقن الجلوبيولن.