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Correlation incidence between infectious bursal disease and aflatoxicosis in broilers chicken farms in Nineveh province, Iraq

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Abstract

This study aimed to investigate the incidence of infectious bursal disease IBD and aflatoxicosis with the correlation between them in broilers of six different areas in Nineveh Province, Iraq, which include: Mosul, Talafer, Hamdanya, Bartella, Baaj, and Gayara for six months (October 2021-March 2022). The necropsy of the suspected infected birds and blood sampling were conducted on 25 birds for each area to diagnose IBD. Samples of broiler feed were collected from the same farms to diagnose aflatoxin B1. The detection of both IBD and aflatoxicosis occurred by the ELISA technique. The necropsy results showed hydropericardium, paleness or congestion, friable and swelling of the liver, kidneys, and bursa of Fabricius, with more severe lesions in a few sporadic cases as gelatinous fluid surrounding the heart, intense paleness of the liver, kidney, and enlarged bursa of Fabricius. The detection of IBD revealed that the positive results were 142, and the negative results are 8 birds out of a total of 150 suspected broiler attributed to the subclinical infection according to the vaccine index equation. Also, positive aflatoxin B1 concentrations were recorded in all broiler feed in the 6 areas as the highest concentration mean was 0.23 ppb and the lowest concentration mean was 0.186 ppb in Mosul and Baaj, respectively. This study indicated a positive correlation between IBD and aflatoxin B1 but was not statistically significant.

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Introduction

Infectious bursal disease IBD or Gumboro disease as a synonym is an acute, highly contagious viral infection of young chickens 3 weeks of age and older that has lymphoid tissue, especially the bursa of Fabricious, as its primary target, and it is referred to as avian nephrosis because of the extreme kidney damage found in birds that succumbed to infection (1-3). The economic importance of this disease is the high mortality rate for some of its viral strains, which may reach 60% first. The second more critical manifestation is severe prolonged immunosuppression of chickens infected early or may not be clinically detectable (subclinical) (4,5). Sequelae related to this immunosuppression include hydropericardium hepatitis syndrome, anemic syndrome, *E. coli* infection, and vaccination failure. This is usually accomplished by combining maternal antibody transfer and active immunization of the newly hatched chick. The pathological changes in the bursa of Fabricious are enlargement, hemorrhage, and serous transudate. Also, hemorrhages and necrosis may be seen in the spleen, muscles, kidney, and intestines (6,7). Among the identified mycotoxins, aflatoxins primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* have received global concern because of their high toxicity and carcinogenicity (8,9). Predominately, aflatoxin B1 has potent carcinogenesis characteristics to the liver and other toxic effects, including immunosuppression, reduced protein synthesis, teratogenesis, and mutagenesis (10,11). The metabolism of aflatoxins is in the liver, so it exposes to injuries mostly.

Aflatoxins also cause decreased egg production, hatchability, male and female fertility, and feed conversion efficiency (12,13). The pathological changes in the liver are redness due to congestion and hemorrhages with necrosis or yellowish due to fatty changes in addition to hemorrhages in other tissues (14). If the birds are fed aflatoxin with IBD virus infection, it reveals extensive symptoms and increased mortality (15). Aflatoxicosis increases the effects of IBD; therefore, the combination of both diseases offers a valuable potential model for understanding the correspondence between aflatoxicosis and infectious agents (16,17).

This study aims to detect the infectious bursal disease and aflatoxicosis in the broiler farms of six different areas in Nineveh province with the correlation incidence between the two diseases.

Materials and methods

The study was designed to investigate and diagnose the infectious bursal disease and aflatoxicosis in broilers (3-5 weeks old) of six different areas in Nineveh Province, Iraq, consisting of Mosul, Talafer, Hamdanya, Bartella, Baaj, and Gayara for six months (October 2021-March 2022).

Ethical approve OR data collection permit

The samples of the infected broiler with IBD and aflatoxicosis were obtained from the private broiler chicken farms located in the Mosul, Talafer, Hamdanya, Bartella, Baaj, and Gayara for six months in in Nineveh province, Iraq from October 2021to March 2022. It depends upon the submission of the College of Veterinary Medicine at the University of Mosul on form UM.VET.2021.44.

Necropsy examination

Necropsy examination of the recently dead chicks with clinical signs of IBD and aflatoxicosis was carried out, and the gross pathological findings were recorded. Representative tissue samples such as heart and liver were collected (18).

Blood samples:

One hundred fifty blood samples from infected chicken were collected from 6 broiler farms as 25 blood samples from each area, and the serum was separated from blood by centrifugation and stored at -20ºC to diagnose IBD by ELISA technique (19).

Feed samples

Twenty-four feed samples were collected from that 6 areas in Nineveh province as 2 feed samples from each farm to diagnose aflatoxicosis by ELISA technique.

Diagnosis of IBD by ELISA technique

It was measured by an ELISA kit manufactured by Bio check Co., Netherlands. Two plates were used to test 150 chicken serum samples**.** The technique principle depends on the detection of IBD virus antibodies in the serum by binding to the kit antigen, and the intensity of the color is measured by the amount of IBD antibody present in the test sample. The kit results suggest revealing the clinical infection, sub-clinical infection, or vaccination, which is measured by calculations and the following equation (vaccine index): SD (standard deviation)*100 /mean)*2.

Diagnosis of aflatoxicosis by ELISA technique

The technique principle is competitive ELISA for the diagnosis of aflatoxin B1. That aflatoxin antigen competes with the antigen in samples. The optical density OD value of the samples and the AFB1 concentration have a negative relationship, and the AFB1 concentration in the samples can be estimated by comparing the OD of the samples with the standard curve. The result is measured by calculations and equations attached to the kit.

Correlation between IBD and aflatoxicosis

A statistical program (SPSS software) was used to conduct the correlation analysis. Data were presented and analyzed using the Pearson correlation test with a significant level set on $P<0.05$.

Results

Necropsy examination

The results of necropsied birds showed the presence of serous transudate or fluid surrounding the heart (hydropericardium) with pale or congestion as mosaic appearance, friable and swelling of the liver (Figures 1 and 2). Moreover, there was swelling, paleness or necrosis, and congestion of the kidneys and bursa of Fabricious (Figures 3 and 4). There were more severe gross pathological changes in some sporadic birds' cases of some broiler farms as intense paleness or fatty change of the liver with gelatinous fluid surrounding the heart (Figure 5) and severe swelling, paleness, icteric kidney and enlarged bursa of Fabricious surrounding by yellowish transudate and hemorrhage (Figure 6).

Diagnosis of IBD by ELISA technique

The result revealed the titers of IBD antibody in the 6 areas of Nineveh Province, including Mosul, Talafer, Hamdanya, Bartella, Baaj, and Gayara as 2 broiler farms for each area with calculating the mean of each area, the positive results are 142 and the negative results are 8 of total 150 suspected broiler chicken. In order to find out the positive results, whether the result of a clinical or subclinical infection or from the vaccine, we applied the equation of the vaccine index. SD (standard deviation) *100 / mean)2) =

(vaccine index) V.I. According to this equation and the kit's instructions, if the vaccination index is more than 300, it results from clinical infection. If it is less, it is either the result of a subclinical infection or the IBD vaccine. Since there are no vaccination programs for IBD in poultry farms in the six study areas, the positive results are attributed to the presence of subclinical infection or a natural attack of the IBD virus (Table 1).

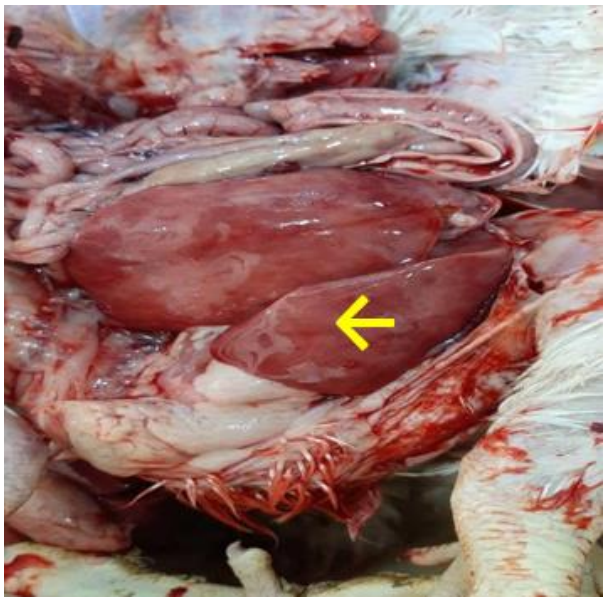

Figure 1: Cross section of broiler (3-5 weeks age) showing paleness and congestion as mosaic appearance and friable liver (arrow).

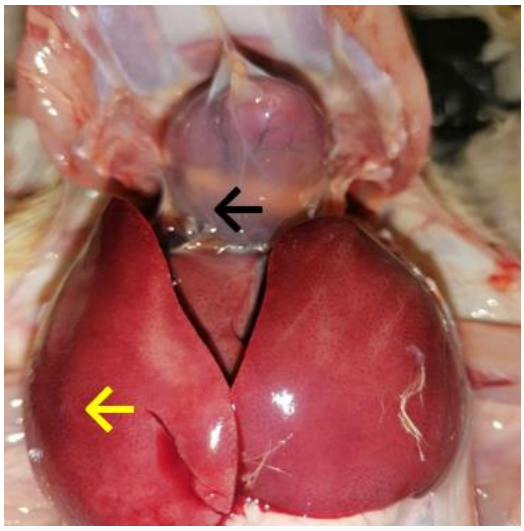

Figure 2: Cross section of broiler (3-5 weeks age) showing serous transudate or fluid surrounding the heart (black arrow) with congestion and swollen of the liver (yellow arrow).

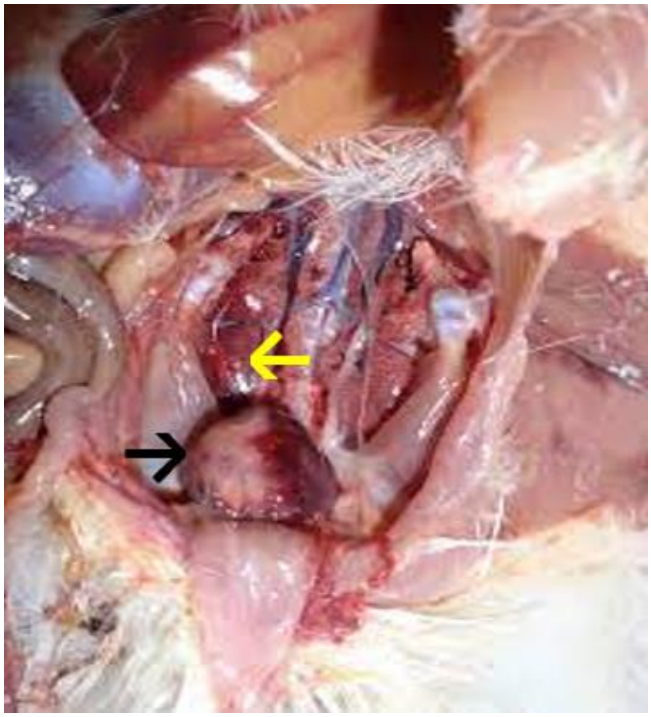

Figure 3: Cross section of broiler (3-5 weeks age) showing swelling, paleness and congestion of the kidneys (yellow arrow) and bursa of Fabricious (black arrow).

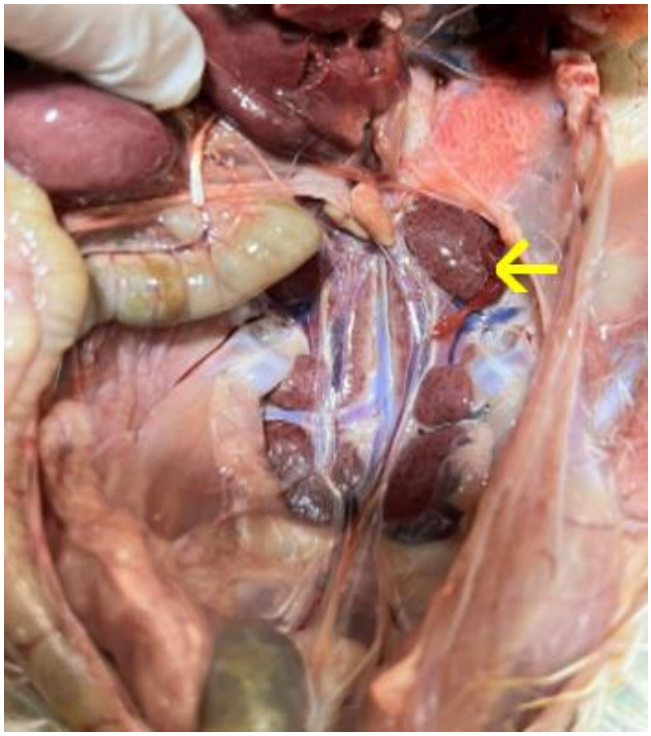

Figure 4: Cross section of broiler (3-5 weeks age) showing mild enlarged and congestion of the kidneys (arrow).

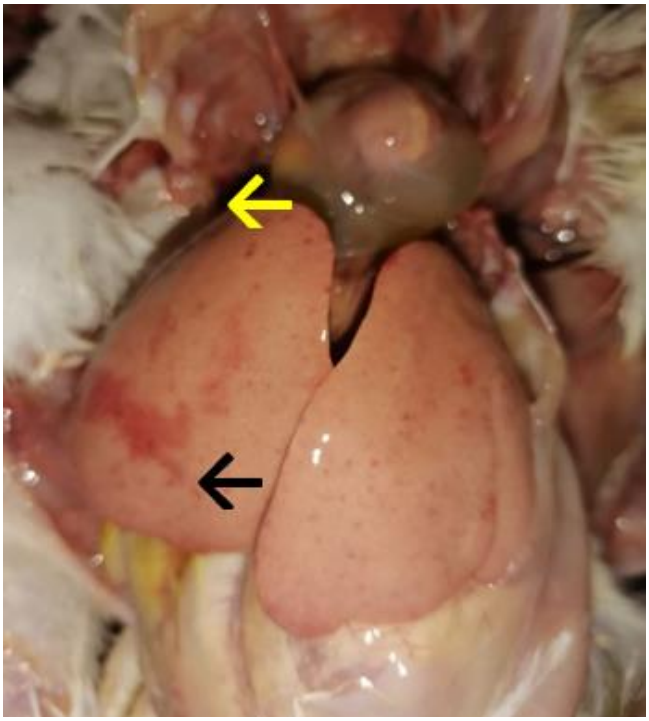

Figure 5: Cross section of broiler (3-5 weeks age) showing severe paleness or fatty change of the liver (black arrow) and gelatinous fluid surrounding the heart (yellow arrow).

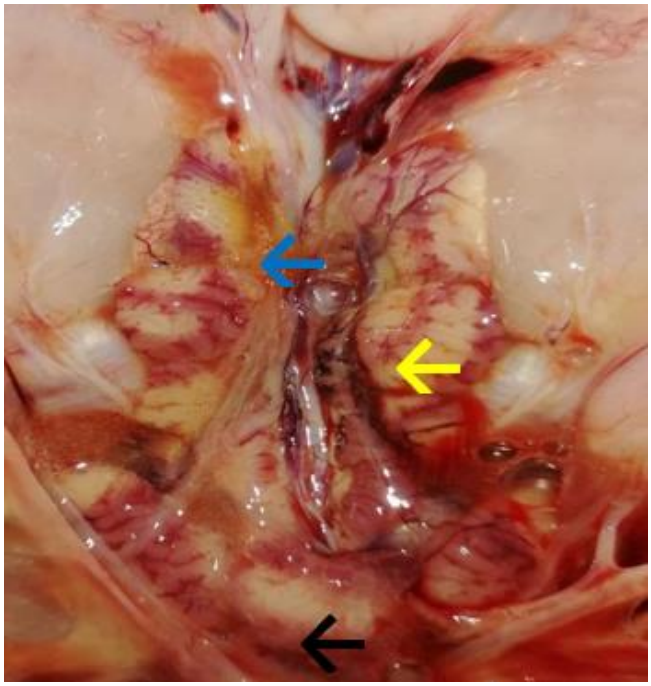

Figure 6: Cross section of broiler (3-5 weeks age) showing severe swelling, paleness, icteric kidney (yellow arrow) and enlarged bursa of Fabricious (black arrow) surrounding by yellowish transudate and hemorrhage (blue arrow).

Table 1: The results of IBD diagnosis in the broiler chicken of 6 areas of Nineveh province by ELISA

Areas	Farm (n)	Samples (n)	Farm (mean)	Area (mean)	Result of farms		Infection type		
					-	+	Clinical	Sub-clinical	vaccination
Mosul	1	14	4156.786	3518	1	13	-	66.01	-
	2	11	2705		2	9			
Talafer	1	13	3886.76	4201.8	3	10	-	81.09	-
	2	12	4543.08		Zero	12			
Hamdanya	1	12	6255.917	3198.920	1	11	-	63.57	-
	2	13	5543.538		Zero	13			
Bartella	1	10	3185.4	4051.240	Zero	10	-	65.46	-
	2	15	3207.933		Zero	15			
Baaj	1	14	3291.429	5885.480	Zero	14	-	244.78	-
	2	11	5018.237		Zero	11			
Gayara	1	15	3172.386	2956.56	Zero	15	-	62.5	-
	2	10	2682		1	9			

Diagnosis of the aflatoxicosis by ELISA technique

The result showed the positive results of aflatoxin B1 titers but less than the permissible limit of 20 ppb in all broiler feed samples of the broiler farms in the 6 areas as 2 feed samples for each farm and 2 farms for each area with a total of 24 feed samples. Mosul's highest mean titer of aflatoxin B1 was 0.23 ppb, and the lowest mean titer was 0.186 ppb in Baaj. Although no clinical signs and pathological changes of aflatoxicosis appeared generally, it appeared in some individual birds (Table 2).

Correlation between IBD and aflatoxicosis

The correlation between the IBD and aflatoxicosis in the broiler farms of the 6 areas in Nineveh Province is an incremental positive correlation but not significant in the Person correlation test (Figure 7).

Table 2: The results of diagnosing aflatoxicosis in the broiler chicken of 6 areas of Nineveh Province by ELISA

Areas	Broiler farms (n)	Broiler feed samples (n)	titer concentration (ppb)	Result	permissible limit >or< from 20 ppb
Mosul	1 2	2 2	0.23	+	less
Hamdanya	1 2	2 2	0.207	+	less
Talafer	1 2	2 2	0.220	+	less
Bartella	1 2	2 2	0.201	+	less
Baaj	1 2	2 2	0.186	+	less
Gayara	1 2	2 2	0.207	+	less

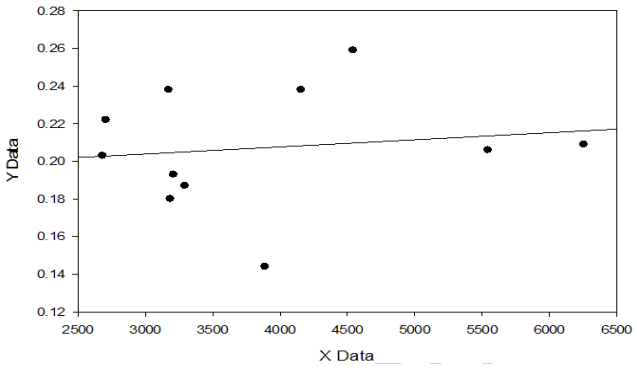

Figure 7: The Pearson correlation test between IBD and aflatoxin B1 in the broiler chicken of 6 areas of Nineveh Province (October 2021-March 2022) with a significant level set on $P<0.05$.

Discussion

The current study investigated the prevalence of infectious bursal disease IBD and aflatoxicosis with their correlation in broilers chicken in six different areas of Nineveh Province, Iraq, namely Talafer, Hamdanya, Bartella, Baaj, and Gayara for six months (October 2021-March 2022) through diagnosis by ELISA technique. The necropsy results showed transudate or gelatinous fluid and hydropericardium in varying severity with congestion, friable, and swelling of the liver. This may be attributed to the hydropericardium hepatitis syndrome or inclusion body hepatitis caused by fowl adenoviruses or aflatoxicosis (20). In addition to the swelling, necrosis of the kidneys, and enlarged bursa of Fabricious in varying severity, which may be attributed to infected by IBD with low-rate severity (21) in addition to the few bird's cases showed extreme paleness or fatty change of the liver which attributed to aflatoxicosis (22). These macroscopic lesions may result from simultaneous infections of these diseases, in association with other causes, or may also be attributed to other diseases and syndromes due to immunosuppression of broilers (23).

The results revealed the presence of a subclinical infection or a natural attack of the IBD virus according to the vaccine index equation attached with the measurement kit, where low titers of antibodies to the virus appeared. It is caused either by a subclinical infection or vaccination, and since most of the broiler farms in the current study areas did not have the vaccination for IBD, according to what was inquired from the owners of these farms, it was considered a subclinical infection or a natural attack of the IBD virus (24,25).

As for the results of diagnosing aflatoxin poisoning, they were positive values for aflatoxin B1 with a range of 0.186-0.23 ppb, and they are consistent with the levels of aflatoxin in broiler feed specified in the Middle East (26-28), and it did not cross the permissible rate of 20 parts per billion ppb (29). Natural contamination of chicken feeds with aflatoxicosis was recorded in many countries, such as the United States, the United Kingdom, Australia, Poland, Indonesia, Malaysia, India, Nigeria, Morocco, Sudan, and Iraq (30). Aflatoxin B1 is a toxic product for the growth of fungi, mainly produced by *Aspergillus flavus* and *A. parasiticus* in cereals, especially corn, which contains its spores and germinates during storage (22). Studies have shown that aflatoxin is immunosuppressive, and taking it with feed decreases birds' immunity. The levels of Aflatoxin B1 causing aflatoxicosis depend on the strain of the fungi and chicken susceptibility (31).

The effect of mycotoxins in poultry farming is very complex and varies significantly according to their toxicological mechanisms affecting several organs, which may lead to animal mortality in case of high contamination levels. When mycotoxins in feed combine simultaneously, they may have a synergistic or additive effect. Even when mycotoxins are low in feed during the sensitive period of the production cycle or upon prolonged exposure, they can

weaken the immune system, leading to immunosuppression (32,33).

The immunosuppressive effect of aflatoxin may be related to direct inhibition of protein synthesis, including specific functions such as immunoglobulins IgG and IgA, inhibition of migration of immune cells, interference with the activity of proteolytic enzymes, reduction in the number of lymphocytes through its toxic effect on the follicle of Fabricius and impaired cytokine synthesis by lymphocytes. The rate of immunosuppression is inevitably linked to exposure to aflatoxin B1 (34,35). Our results agree with one of the studies conducted to evaluate the effects of aflatoxin-contaminated feed on the immune response of flocks of broilers to live viral attenuated bursa disease (IBD) vaccines (36). In other studies, it has been shown that aflatoxins in feed negatively affect immune responses and thus increase the susceptibility of poultry and mammals to infectious diseases. Several studies have shown that aflatoxins significantly reduce the initial immune responses and lower the IgA level, leading to IBD in poultry (37). The metabolic pathway of aflatoxin is different. Aflatoxin B1 may enter the cell and be metabolized using mono-oxygenase enzymes in the endoplasmic reticulum to hydroxylated metabolites that are also metabolized to glucuronide and conjugated sulfate, or it may be oxidized to a functional epoxide that undergoes spontaneous hydrolysis to aflatoxin B1-8,9-dehydrodihydrofol. It binds with proteins, causing cytotoxicity. The epoxide release can be reacted with DNA or protein or detoxed by inducing glutathione sulfide transporters to convert to conjugated glutathione (38).

One study showed that exposure of chickens to subclinical levels of aflatoxin in feed leads to some effects on the immune system. This can be especially dangerous when the exposure is concurrent with exposure to an infectious virus such as lymphotropic, and during field infection in chickens, it can cause severe lesions of the Fabricius follicle (39). The reason for the emergence of the weak relationship between contamination with aflatoxin and IBD may explain the fact that the season of collecting samples was in periods of low temperatures, the reasons for the increased production of mycotoxins, especially aflatoxins, or because of the relatively good biosecurity and the broiler feed storage conditions for poultry farms in our study (40). This study indicated a positive correlation between IBD and aflatoxicosis but was insignificant. It appears that bird immunity has essential roles in such cases, that aflatoxin is one of the immunosuppressants, and the use of more cost-effective solutions to prevent aflatoxin from reaching the food chain contributed reduce its harmful effects.

Conclusion

The detection of both IBD and aflatoxicosis occurred by the ELISA technique. The necropsy results showed hydropericardium, paleness or congestion, friable and swelling of the liver, kidneys, and bursa of Fabricius, with more severe lesions in a few sporadic cases as gelatinous fluid surrounding the heart, intense paleness of the liver, kidney, and enlarged bursa of Fabricius. The detection of IBD by ELISA revealed that the positive results were 142, and the negative results are 8 birds out of a total of 150 suspected broiler attributed to the subclinical infection according to the vaccine index equation. Also, positive aflatoxin B1 concentrations by ELISA were recorded in all broiler feed in the 6 areas as the highest concentration mean was 0.23 ppb and the lowest concentration mean was 0.186 ppb in Mosul and Baaj, respectively. This study indicated a positive correlation between IBD and aflatoxin B1 but was not statistically significant.

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Conflict of Interest

There is not any conflict of interest.

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ترابط حدوثية مرض التهاب جراب فايبريشيا المعدي مع التسمم الفطري بسموم الأفلا في حقول فروج اللحم في محافظة نينوى، العراق

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الخلاصة

استهدفت الدراسة الحالية التحري عن مرض التهاب جراب فايبريشيا المعدي (جمبورو) والتسمم الفطري بسموم الأفلا في أعلاف فروج اللحم وتحديد مدى العلاقة بينهما في ستة مناطق مختلفة من محافظة نينوى، العراق وهي الموصل وتلعفر والحمدانية وبرطلة والبعاج والكيارة لفترة ٦ أشهر (تشرين الأول ٢٠٢١-اذار ٢٠٢٢). أجريت الصفة التشريحية للطيور المشكوك بإصابتها بواقع ٢٥ طير لكل منطقة وجمع عينات الدم منها لغرض تشخيص مرض التهاب غدة فايبريشيا بتقنية الاليزا، كما تم

جمع عينات من أعلاف فروج اللحم لنفس الحقول بواقع عينتين من كل حقل تربية و ٤ عينات لكل منطقة من مناطق الدراسة الستة لتشخيص التسمم بالافلاتوكسين ب١ بتقنية الاليزا أيضا. أظهرت نتائج الآفات العيانية موه القلب وشحوب أو احتقان وتورم الكبد والكلى وجراب فايبريشيا، مع آفات أكثر شدة في حالات قليلة متفرقة مثل وجود سائل جيلاتيني حول القلب وشحوب شديد في الكبد والكلى وتضخم جراب فايبرشيا. بلغت نتائج تشخيص مرض التهاب جراب فايبريشيا المعدي الإيجابية ١٤٢ طير والنتائج السلبية ٨ طيور من إجمالي ١٥٠ فروج مشتبه به وعزيت إلى الإصابة تحت السريرية أو الإصابة الطبيعية حسب معادلة مؤشر اللقاح، وظهرت نتائج إيجابية لتراكيز الأفلاتوكسين ب١ ولكن أقل من الحد المسموح به وهو ٢٠ جزء بالبليون في جميع عينات العلف في مناطق الدراسة حيث كان أعلى معدل هو ٠,٢٣ جزء بالبليون في منطقة الموصل وأدنى معدل ٠,١٨٦ جزء بالبليون في منطقة البعاج. استنتجت هذه الدراسة إلى وجود علاقة إيجابية بين مرض التهاب جراب فايبريشيا المعدي والأفلاتوكسين ب١ ولكن ليس معنويًا من الناحية الإحصائية.