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The Effect of Mycotoxins in Naturally Contaminated Diet on the Pathogenicity of *Escherichia coli* in Broiler Chickens

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ABSTRACT

Contamination of feedstuffs with mycotoxins is considered a huge issue plaguing the poultry sector of Egypt. Therefore, the current study was conducted to examine the effect of the neglected natural mycotoxin contamination of ration on the clinicopathological picture of *Escherichia coli* infection in broiler chickens. A total of 240 one-day-old chicks were divided into 5 groups. The first 3 groups (1, 2, and 3) were further equally subdivided into 2 subgroups (A and B). Those in group A were fed on commercial ration treated with antimycotoxin while group B was fed on ration contaminated with mycotoxins. Groups 1, 2, and 3 were inoculated at one-day-old with *E. coli* O_{78}, O_{128}, and O_{157}, respectively while group 4 was kept as a negative control and fed on a ration treated with antimycotoxin. Finally, group 5 was kept as positive controls and fed on a mycotoxin contaminated ration and inoculated with physiological saline. All groups contained 30 chicks. Results showed that the challenged groups fed on a ration containing mycotoxin had high mortality (23.3%) and also a high feed conversion ratio. Moreover, severe clinical symptoms, postmortem lesions, histopathological pictures, and a high rate of bacterial re-isolation were recorded. The pathological lesions were congestion of the liver, spleen, and kidneys in addition to severe pericarditis, perihepatitis, air sacullitis, ascites, and unabsorbed yolk sac. The histopathological changes included degeneration, necrosis, and liver inflammation with lymphoid depletion in the spleen and enteritis associated with sloughing of the mucosal epithelium. The obtained results were less severe in the challenged groups fed on a ration treated with antimycotoxin indicating combined action of both *Escherichia coli* and mycotoxins. In conclusion, the obtained results revealed that the mycotoxins even at low concentrations can augment the pathogenisity of *Echerichia coli* in broiler chickens.

Keywords: Broiler chicken, Clinicopathological finding, *Escherichia coli*, Histopathology, Mycotoxin, Organ colonization

INTRODUCTION

Mycotoxins are biologically active, toxic metabolites produced by toxigenic fungi that invade crops in the farm fields and may grow on feed during storage in case of desired temperature and humidity conditions (Shamsudeen et al., 2013). There are about 200 species of fungi that produce mycotoxins, the majority of which form mycotoxins in three genera of Aspergillus, Penicillium, and Fusarium. Even though these fungi produce over 500 mycotoxins, only a subset of these mycotoxins (mainly Aflatoxin, Ochratoxin, Zeralenone, Fuminisin) are pathogenic (Filazi et al., 2017). Natural mycotoxin contamination of feedstuffs is regarded as a major issue affecting the poultry industry of Egypt (El Nabarawy et al., 2020).

Different mycotoxin types had a synergistic highly toxic and fatal impact even at low concentrations (Boermans and Leung, 2007; Streit et al., 2012). The most significant mycotoxins affecting poultry through naturally contaminated feeds are aflatoxin (AF), ochratoxin A (OTA), zearalenone, T-2 toxin, vomitoxin, and fumonisin, which have serious toxic effects and possibly synergistic properties (Njobeh et al., 2012).

Naturally contaminated broiler diets with aflatoxin, ochratoxin (OTA), and zearalenone at permissible levels lead to a significantly decreased body weight (El Nabarawy et al., 2020). Mycotoxin-related clinical signs and pathological lesions are closely related to poultry species, mycotoxin type, dose consumed, and duration of exposure. Chickens are exposed to multiple mycotoxins and subjected to a broader range of stressors in the field. Accordingly, even with apparently low levels of mycotoxins in the feed, chickens may exhibit signs and lesions of mycotoxicosis (Rodrigues and Naehrer, 2012).

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Colibacillosis, on the other hand, is one of the most common infectious diseases in farmed poultry and one of the leading causes of morbidity and mortality in young chickens (Stordeur and Manil, 2002). The link between *Escherichia coli* (*E. coli*) strains and disease conditions in avian species was discovered over a century ago (Sojka and Carnaghan, 1961). Some mycotoxins interfere with host defenses against *E. coli*, resulting in bacterial clearance failure and promoting mucosal colonization, invasion, and inflammatory responses. Consequently, mycotoxins can make hosts more susceptible (Park et al., 2015).

Ochratoxin (80 mg/kg, in vivo) exacerbates *E. coli* infection-mediated diseases in broiler chickens, such as perihepatitis and pericarditis, and increases the thickness of the fibrin layer that covers the heart and liver after *E. coli* infection (Kumar et al., 2004). Thus, the goal of this study was to investigate the effect of natural contamination of mycotoxins (Aflatoxin and Ochratoxin) on the clinicopathological picture of day-old CUBB broiler chicks infected with different pathogenic *E. coli* serotypes.

MATERIAL AND METHODS

Ethical approval

Chicken handling procedures during the experiment were performed and approved by the institutional animal care and use committee of the Faculty of Veterinary Medicine, Cairo University, Egypt (VetCU02122019108).

Experimental chickens

One-day-old CUBB chicks (n = 240) were purchased from AL-WATANIA company (Egypt). All used chicks were from the same breeding stock. Feed and water were delivered to the broiler chickens *ad libitum*. All the chickens in the experiment were floor reared in separate pens at a density of 10 broiler chicks/m2 on a concrete floor with fresh wood shavings as bedding and housed in environmentally controlled rooms. At 7 and 10 days of age, chickens in all groups were vaccinated against NDV + IBV with Hitchner B1 + H_{120} vaccine and avian influenza inactivated H_5N_2 vaccine via intraocular and subcutaneous routes, respectively. The La Sota vaccination and the IBDV vaccine were administered intra-ocularly on days 14 and 18 of life, respectively. The broiler chicks were maintained on broiler mash from day 1 to day 28.

Chicken diet

A balanced commercial ration for broiler chickens in the form of a mash diet was purchased from a commercial factory (Alshimaa factory in Kafr El-Shiekh Governorate, Egypt) without any additives. Mycotoxins analysis in the ration was performed for Aflatoxin, Ochratoxin, and Zeralenone using high-performance liquid chromatography (HPLC) (El Nabarawy et al., 2020)

Antimycotoxin medication

Antimycotoxin medication (containing cell wall extract derived from hydrolyzed yeast and hydrated sodium calcium aluminosilicate with a dose of 250gram/tone) was used in the groups fed ration without antimycotoxins (the B groups in addition to the positive control group) during the 4 weeks of the experiment.

Experimental design

The study was extended from one day of age up to 28 days. On day 1, chicks were randomly assigned into five groups (1-5). The first three groups contained 60 chicks each, which were further divided into two subgroups (A and B) each one with 30 chicks while groups 4 and 5 contained 30 chicks each and were kept as a negative (-ve) and a positive (+ve) controls, respectively. The A groups (from the first 3 groups) fed on antimycotoxin-treated ration, while the B groups (from the first 3 groups) fed on a diet containing mycotoxin within permissible limits of the Egyptian regulations (10 ppb Aflatoxin, and 20 ppb Ochratoxin) with their amounts measured using HPLC (aflatoxin was 3 PPB and ochratoxin was 6 PPB while Zearalenone mycotoxin was undetected) according to El Nabarawy et al. (2020). Chicks in group 4 (-ve controls) fed on antimycotoxin-treated ration, while those in group 5 (+ve controls) fed on mycotoxins contaminated ration. The first three groups (1, 2, 3) with their two subgroups (A and B) were inoculated with *E. coli* serotype O_{78}, O_{128}, and O_{157}, respectively, at a dose of 1.5×10^8 CFU/ chick via oral route using a feeding tube (Ibrahiem et al., 2016), while groups 4 and 5 were inoculated with physiological saline. The broiler chicks were housed in separated units under similar management and biosecurity parameters for four weeks (Table 1).

The clinical signs, mortality rate, post mortem lesions were recorded daily. Chicken performance response was determined after North (1985). All broiler chicks were individually weighed on the first day and weekly for body weight and body weight gain along with the duration of the experiment. Feed consumption was measured on the same days as the weights of the broiler chicks were done. FCR was calculated (g feed/g live body weight) following Timmerman et al. (2006). Organ samples were collected weekly for histopathological examination and bacterial re-isolation.

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Table 1. Experimental design of different pathogenic *E. coli* serotypes in broiler chickens in the presence of low concentrations of afla and ochratoxins mycotoxins

	Group 1		Group 2		Group 3		Group 4	Group 5
	***Escherichia Coli* challenged groups**						**Control negative**	**Control positive**
Total no. of chicks/group	60		60		60		30	30
Sub-grouping	A	B	A	B	A	B	-	-
No. of chicks/ subgroup	30	30	30	30	30	30	30	30
E. coli serotype 1.5×10^8 CFU/Chick	O_{78}	O_{78}	O_{128}	O_{128}	O_{157}	O_{157}	-	-
Mycotoxin contaminated ration	-	+	-	+	-	+	-	+
Mycotoxin treated ration	+	-	+	-	+	-	+	-

Aflatoxin (3PPB), Ochratoxin (6 PPB)

Histopathological examination

Specimens from the liver, kidney, and intestine were collected weekly by sacrificing 2 chickens from each group for histopathological investigation. Samples were collected in jars containing 10% formol saline then tissue specimens were washed, dehydrated, embedded in paraffin, sectioned at 4-5 µm thickness, and then stained with Hematoxylin and Eosin (H&E) as a routine work for histopathological studies (Bancroft and Stevens, 1990). The lesion was recorded as a cumulative lesion scoring (Hepatic lesions received 9 scores (3 degeneration, 3 necrosis, and 3 inflammation) and lymphoid depletion in the spleen received 3 scores while intestinal lesions in the form of enteritis received 3 scores) based on Hegazy (1991).

***Escherichia coli* re-isolation**

The organ colonization was conducted using liver, spleen, and heart samples from all groups collected weekly during the experiment. A swab from these organs was immersed in MacConkey broth (Oxoid, UK), which incubated at 37ºC for 24 hours, then a loopful was streaked onto MacConkey agar (Oxoid, UK) and Eosin methylene blue (EMB) agar (Oxoid, UK) and incubated at 37ºC for 24 hours and the *E. coli* suspected colonies were stored on agar slant in the refrigerator at 4ºC for further investigation (Azza et al., 2018).

Statistical analysis

The data were statistically analyzed using the general linear model procedure of the Statistical Analysis System software (SAS, 1999). Overall data were analyzed using one-way ANOVA test through Tukey and a significant level was defined as $p \leq 0.05$ (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Clinical signs and post-mortem lesions

Clinical signs appeared at day 3 post *E. coli* infection in the experimental groups. Dullness, anxiety, wing drooping, anorexia, ruffled feathers, inability to stand, gasping, and white diarrhea that progressed to brownish diarrhea were among the observed clinical signs. Later, sneezing and coughing were recorded. Upon necropsy of died or sacrificed chickens, the postmortem picture indicated congestion of the liver, lung, spleen, and kidneys, severe pericarditis, perihepatitis, airsaculitis, ascites, and unabsorbed yolk sac (Figure 1). The previously mentioned clinical signs and PM lesions were more severe in the B groups that challenged with *E. coli* and feed on ration contaminated with low concentrations of Afla and Ochratoxin than the A groups that challenged with *E. coli* and feed on mycotoxin treated diet. This may be due to the effect of mycotoxins on various organs, such as the gastrointestinal tract and liver (Murugesan et al., 2015). Furthermore, the robust clinical signs and pathological pictures in the mycotoxicated challenged groups may be related to the immunosuppressive effect of mycotoxins on the chicken immune system as mentioned by Murugesan et al. (2015). In other words, mycotoxins can affect activated and proliferating cells by decreasing lymphocyte proliferation, impairing macrophage phagocytic function, and suppressing cytokine production, damaging epithelial tissue, increasing intestinal permeability, and thus resulting in a weakened immune system (Park et al., 2015). Accordingly, when a pathogen enters, an appropriate and efficient immune response cannot be mounted which worsens clinical signs and PM lesions. Similarly, Park et al. (2015) mentioned that mycotoxin could suppress the immune response and increase the inflammatory response. Moon et al. (1999) also explained that mycotoxins can intervene the innate immunity of macrophages by suppressing tumor necrosis factor-α (TNF- α), interleukin (IL)-1, and IL-6, resulting in the disruption of pulmonary and systemic host defenses, and consequently increase susceptibility to bacterial infections. However, on day 21 post-infection, the clinical signs were insignificant in all challenging groups.

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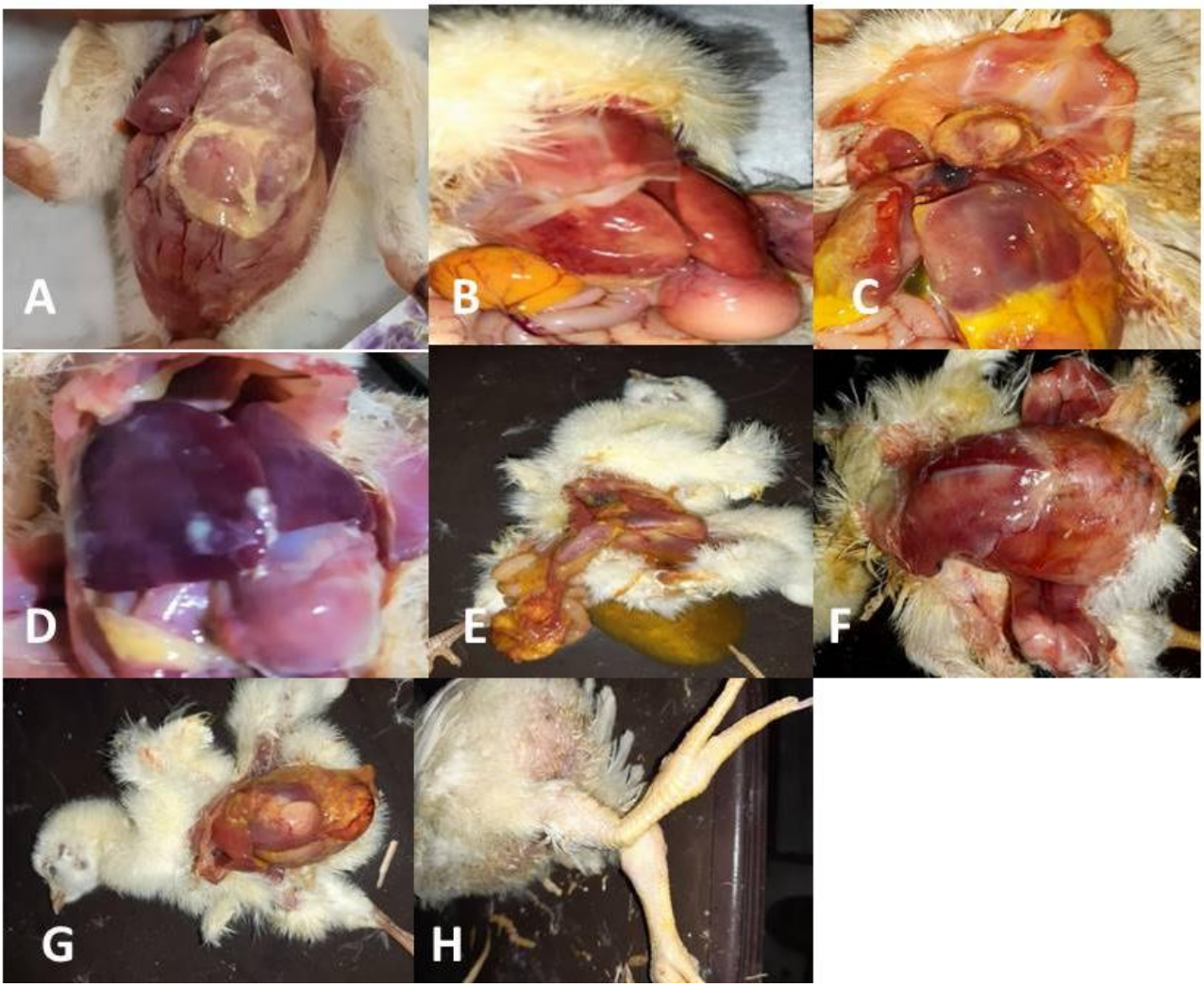

Figure 1. Post mortem lesions observed in the broiler chickens challenged with *E. coli* O78, *E. coli* O128, and *E. coli* O157 during 4 weeks of the experiment. **A, B,** and **C:** Unabsorbed yolk sac, congested liver, pericarditis, and perihepatitis in mycotoxicated challenged *Escheichia coli* O_{78} group. **D** and **E:** Unabsorbed yolk sac and pericarditis in *E. coli* O_{128} groups **F, G,** and **H:** Ascites and congested carcass unabsorbed yolk sac, and Greenstick fracture bones in *E. coli* inoculated and mycotoxicated groups

Mortality

The mortality pattern began at the 5 DPI. The *E. coli* O_{78} group had the highest mortality rate, followed by the *E. coli* O_{157} inoculated group, and finally the *E. coli* O_{128} infected group. The highest death rate was found in the *E. coli* O_{78} group, followed by the *E. coli* O_{157} and the lowest in the *E. coli* O_{128} group. However, no mortalities were recorded in the controls as mentioned in Table 2. In the present study, the mortality rate of *E. coli* $O_{\mathbf{78}}$ (16.7%) was higher than that reported by Tawab et al. (2015) as 10% in *E. coli* $O_{\mathbf{78}}$ groups. Likewise, Shen-Orr et al. (2002) recorded a mortality rate of 8% induced by *E. coli* infection in broiler chickens, on the other hand, the mortality rate in the mycotoxin treated group was lower than that reported by Abd El-Ghany and Madian, (2011) who recorded a mortality rate of 38% (19 out of 50) in broiler chickens infected with *E. coli* O_{78}. The current study recorded a mortality rate of 8.3% in *E. coli* $O_{\mathbf{128}}$ and according to the available literature, there was no data on *E. coli* $O_{\mathbf{128}}$ experimental infection in poultry. Finally, the mortality in the groups inoculated with *E. coli* $O_{\mathbf{157}}$ was 13.3% and this rate was higher than that reported by Azza et al. (2018) who recorded a mortality rate of 6.7%(2 out of 30) due to *E. coli* $O_{\mathbf{157}}$ infection in broilers. Among *E. coli* infected groups, the mortality rate varied with higher mortality recorded in *E. coli* $O_{\mathbf{78}}$ than the other inoculated two serotypes which may be related to the difference in the virulence genes of *E. coli* serotypes. This interpretation was assessed by Kemmett et al. (2013) who mentioned the contribution of virulence genes present in *E. coli* serotypes to early broiler deaths. Additionally, the mortalities in the challenged groups that fed on ration containing mycotoxins even with low levels were higher than the *E. coli* challenged groups and feed on diet treated with antimycotoxin and this indicated the effect of mycotoxin on the immune defense mechanism of broiler chickens as mentioned by Park et al. (2015).

Table 2. The mortality rate among all broiler chicken challenged with *E. coli* O78, *E. coli* O128, and *E. coli* O157 during 4 weeks of the experiment

	Group 1		Group2		Group 3		Control (-ve)	Control (+ve)
	1A	1B	2A	2B	3A	3B		
Total	3	7	2	3	3	5	0	0
%	10%	23.3%	6.66%	10%	10%	16.6%	0	0

A: Fed on ration treated with antimycotoxin, B: Fed on ration containing measured mycotoxin. Group 1, Group 2, and Group 3: Inoculated with *E. coli* O 78, *E. coli* O_{128} and *E. coli* O_{157}, respectively.

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Feed conversion ratio

Table 3 and Figure 2 represent the performance parameter (cumulative feed conversion) during the experiment. The *E. coli* infection in poultry is one of the principal causes of retardation and decreased feed conversion rate and this may be due to lower absorption of nutrients from the inflamed intestinal tract (Abd Elatiff et al., 2019). The performance parameters were the best in the control -ve group followed by control +ve then in the *E. coli* O_{128}, *E. coli* $O_{157,}$ and *E. coli* O_{78} groups. It was found that there was a significant decrease in body weight gain with bad FCR in groups inoculated with *E. coli* O_{157} and these results agreed with Abd Elatiff et al. (2019), also the increase in FCR in the group inoculated with *E. coli* O_{78} agreed with Tawab et al. (2015), Elmenawey et al. (2019), and Hassanen et al. (2021). According to Table 3, there was an increase in the FCR in the mycotoxicated challenged groups even with permissible limits of mycotoxin contamination than the challenged groups that feed on a diet treated with antimycotoxin. In the same way, Murugesan et al. (2015) and Abdelnaser et al. (2017) mentioned that mycotoxins have an effect on the broiler performance due to their destructive effects on nutrient digestion and absorption. Furthermore, the malabsorption that occurred due to aflatoxin may have a role in the reduction of body weight as mentioned by Osborne and Hamilton (1981) or may be due to the reduction of the pancreatic enzyme due to the mycotoxin as reported by Richardson and Hamilton (1987). Correspondingly, these results agreed with El Nabarawy et al. (2020) who reported that the naturally contaminated broiler diet by aflatoxin, ochratoxin, and zearalenone at permissible levels resulted in a significant reduction in body weight with a significant increase in FCR.

Table 3. Mean feed conversion ratio of the different broiler chicken groups during the 4 weeks of the experiment

Week	Group 1		Group2		Group 3		Control	
	1 A (mean ± SE)	1 B (mean ± SE)	2A (mean ± SE)	2B (mean ± SE)	3 A (mean ± SE)	3 B (mean ± SE)	-ve (mean ± SE)	+ve (mean ± SE)
1	1.5 ± 0.06^{ab}	1.6 ± 0.006^{bc}	1.4 ± 0.06^{ab}	1.7 ± 0.06^{c}	1.5 ± 0.06^{abc}	1.7 ± 0.06^{c}	1.3 ± 0^{a}	1.6 ± 0.06^{bc}
2	1.7 ± 0^{a}	2.5 ± 0.09^{c}	1.7 ± 0.06^{a}	2 ± 0.06^{ab}	1.9 ± 0.09^{ab}	2.2 ± 0.06^{b}	1.7 ± 0^{a}	2.2 ± 0.09^{b}
3	2.1 ± 0.06^{d}	2.5 ± 0.06^{e}	1.6 ± 0.03^{ab}	1.9 ± 0.03^{cd}	1.6 ± 0.06^{bc}	2 ± 0.03^{d}	1.4 ± 0.03^{a}	1.9 ± 0.06^{cd}
4	2.8 ± 0.06^{bcd}	3.1 ± 0.09^{d}	2.6 ± 0.09^{bc}	2.9 ± 0.06^{cd}	2.7 ± 0.06^{bc}	3 ± 0.03^{d}	2.1 ± 0.03^{ab}	2.5 ± 0.03^{bc}

A: Fed on antimycotoxin treated ration, B: Fed on ration containing Afla and Ochratoxins, Mean ± SE: Mean ± standard error, ^{a,b,c,d} Means different letters in the same row are significantly different ($p \leq 0.05$). Group 1, Group 2, and Group 3: Inoculated with *E. coli* O 78, *E. coli* O_{128}, and *E. coli* O_{157}, respectively.

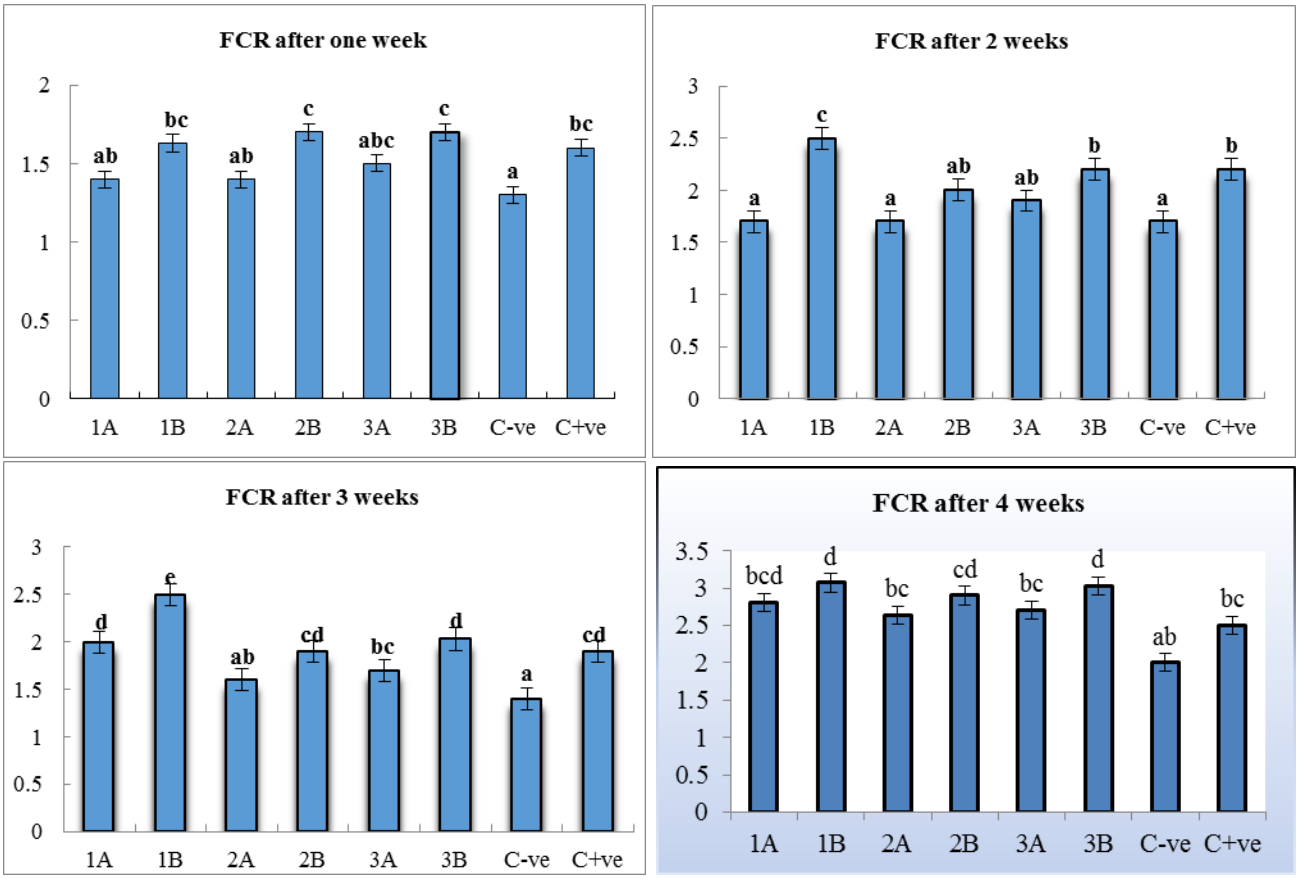

Figure 2. FCR of broiler chickens challenged with *E. coli* O78, *E. coli* O128, and *E. coli* O157 during four weeks of the experimentation. **A:** Fed on antimycotoxin treated ration, **B:** Fed on ration containing Afla and Ochratoxins, ^{a,b,c,d} means different letters in the same week are significantly different ($p \leq 0.05$). Group 1, Group 2, and Group 3: Inoculated with E. coli O 78, E. coli O128, and E. coli O157, respectively

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Re-isolation of *Escherichia coli* in the groups under experiment (organ colonization)

In the present study, the challenged groups with *E. coli* O_{78} showed the re-isolation rate of 77.7% from organs (66.6% from liver, 100% from spleen, and 66.6% from heart) while these percentages increased in mycotoxicated challenged groups to reach an average of 88.8% (100% from liver, 100% from spleen, and 66.6% from heart) at the first-week post-inoculation. These percentages declined weekly to reach 44% and 55.5% in challenged and mycotoxicated challenged groups at the second-week post-inoculation, while at the third-week post-inoculation, the organ colonization was reduced to be 11% in both subgroups. Similarly, these percentages of re-isolation in groups inoculated with *E. coli* O_{128} were 88.8% in the first week post-inoculation in both subgroups A and B and decreased to be 55.5% in the second week, and at the third week post-inoculation no isolates were detected in the challenged group, whilst at the mycotoxicated challenged groups, the re-isolation rate was 22.2%. On the other hand, the re-isolation rate from organs in subgroup A inoculated with *E. coli* O_{157} was 77.7%, while this percentage increased to 100% in subgroup B at the first-week post-inoculation. These percentages reduced at the second week to be 44.4% and 77.7% in subgroup A and subgroup B, respectively, and at the third week post-inoculation the re-isolation rates were 11% and 22% in the subgroups A and B, respectively (Table 4)

From these results, the mycotoxicated groups even the acceptable range of mycotoxins were showing *E. coli* re-isolation rate higher than the antimycotoxin treated groups. These results were in line with the explanation of Park et al. (2015) indicating that some mycotoxins interfere with host defenses against *E. coli*, resulting in bacterial clearance failure and promoting mucosal colonization, invasion, and inflammatory responses. Similarly, Devriendt et al. (2009) found that dietary fumonisin B1 (0.5 mg/kg, *in vivo*) significantly increases *E. coli* colonization in the small and large intestines, as well as subsequent bacterial translocation to extraintestinal organs such as the mesenteric lymph nodes, lung, liver, and spleen, resulting in longer *E. coli* shedding after infection.

Histopathology

At the first-week post-infection, the control -ve group showed no histopathological alterations with the normal histological structure of liver hepatocytes (Figure 3G)., normal white and red pulp of the spleen, as well as the normal length of intestinal villi. On the other hand, in the control +ve group, the liver showed periportal fatty degeneration with lymphoid depletion in the spleen (Figure 3H) with a decrease of intestinal villi length. In the *E. coli* O_{78} challenged group, the liver of broiler chicks revealed congestion of portal blood vessels associated with marked vasculitis, severe degree of lymphoid depletion as well as reticular fibers within the white pulp of the spleen. Intestine showed necrotic enteritis associated with sloughing of the mucosal epithelium (Figure 3A). Moreover, the liver of the mycotoxicated *E. coli* O_{78} challenged group showed severe vasculitis associated with hemorrhage and a severe degree of fatty changes (Figure 3B), meanwhile the spleen showed a severe degree of lymphoid necrosis. Intestine showed a severe degree of necrotic enteritis associated with sloughing of the mucosal epithelium (Figure 3A). These observations were like those detected by Sahar and El-shazly (2002), Riaz et al. (2016), and Awaad et al. (2021) who found that *E. coli* O_{78} caused perihepatitis, hepatocyte vascular degeneration, and infiltration of mononuclear leucocytes, inflammatory cells, and portal vein dilatation. Liver of chickens challenged with *E. coli* O_{157} indicated hepatic vacuolar degeneration with a marked degree of lymphoid depletion in the spleen (Figure 3E), while intestine showed mild to moderate degree of necrotic enteritis associated with sloughing of the mucosal epithelium. In mycotoxicated chicken group challenged with *E. coli* (O_{157}), the liver showed hepatitis associated with vasculitis and severe degree of fatty changes. Moreover, spleen showed a severe degree of lymphoid necrosis (Figure 3F) and the intestine showed a severe degree of necrotic enteritis. These observations were similar to those detected by Dutta et al. (2013), and Azza et al. (2018).

In the chickens challenged with *E. coli* (O_{128}), the liver showed marked fatty degenerative changes within hepatocytes (Figure 3C), lymphoid depletion within the white pulp in the spleen, and a moderate degree of catarrhal enteritis in the intestine. On the other hand, the liver of mycotoxicated chickens challenged with *E. coli* (O_{128}) indicated a focal area of coagulative necrosis associated with mononuclear cells infiltration (Figure 3D), marked degree of lymphoid depletion in the spleen, and a moderate degree of necrotic enteritis in the intestine. These lesions were similar to those detected by Nataro and Kaper (1998). The histopathological changes decreased gradually from the second week (Figure 4), then a third week (Figure 5) till the fourth week of age (Figure 6) at the *E. coli* challenged groups with the absence of mycotoxins effect, in contrary with the mycotoxicated *E. coli* challenged groups which indicates that the presence of mycotoxin aggravate the condition and prolong the time needed for tissue repair. These results were in line with these recorded by Kubena et al. (1985), and Elaroussi et al. (2006). Likewise, Kumar et al. (2004) reported severe histopathological changes in poultry infected with *E. coli* and fed with mycotoxin, compared to those infected with *E. coli* only. Moreover, the histopathological changes increased weekly in the control +ve group, which was in line with a study by Bakeer et al. (2013) indicating that the pathological changes increased in correlation with prolonged administration of mycotoxins (Table 5).

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Table 4. Re-isolation of the inoculated *Escherichia coli* from different organs in broiler chickens challenged with *E. coli* O_{78}, *E. coli* O_{128}, and *E. coli* O_{157} during the weeks of experimentation

WPI Group	W1			W2			W3			W4			Total organs isolation			Total
	Liver	Spleen	Heart	Liver	spleen	Heart	liver	spleen	Heart	liver	Spleen	Heart	liver	spleen	Heart	
1A	66%	100%	66 %	33 %	33 %	66 %	0	33.3%	0	0	33%	0	25%	50%	33%	36%
1B	100%	100%	66 %	33 %	66 %	33 %	33%	0	0	33%	0	0	50	41.5	25	39%
2A	66 %	100%	100%	0	100%	66 %	0	0	0	0	0	0	16.5	50	41.5	27%
2B	100%	66 %	100%	33 %	66 %	66 %	0	33%	33%	0	0	33%	33.5	41.5	50	31%
3A	100%	66 %	66 %	66 %	33 %	33 %	0	33%	0	0	33%	0	41.5	41.5	25	27%
3B	100%	100%	100%	66 %	100%	66 %	33%	33%	0	0	0	0	50	58.5	41.5	50%
C -ve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C+ve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A: Fed on ration treated with antimycotoxin, B: Fed on a ration containing measured mycotoxins, W: Week, C+ve: Control positive, C-ve: Control negative, WPI: Week post-infection

Table 5. Cumulative histopathological changes for broiler chicken challenged with *E. coli* O_{78}, *E. coli* O_{128}, and *E. coli* O_{157} during the 4 weeks of the experiment

	Group 1 (*Escherichia coli* O_{78})		Group 2 (*Escherichia coli* O_{128})		Group 3 (*Escherichia coli* O_{157})		Negative ontrol	Positive control
	1 A	1 B	2 A	2 B	3 A	3 B		
Week 1	11	15	11	13	12	15	0	3
Week 2	9	14	8	10	9	12	0	4
Week 3	6	9	6	7	7	9	0	7
Week 4	5	8	5	6	6	8	0	9

The lesion scores were determined as follow, 1: Hepatic lesions 9 scores (3 points degeneration, 3 necrosis, and 3 inflammation) 2: Lymphoid depletion in spleen 3 scores 3: Enteritis 3 scores

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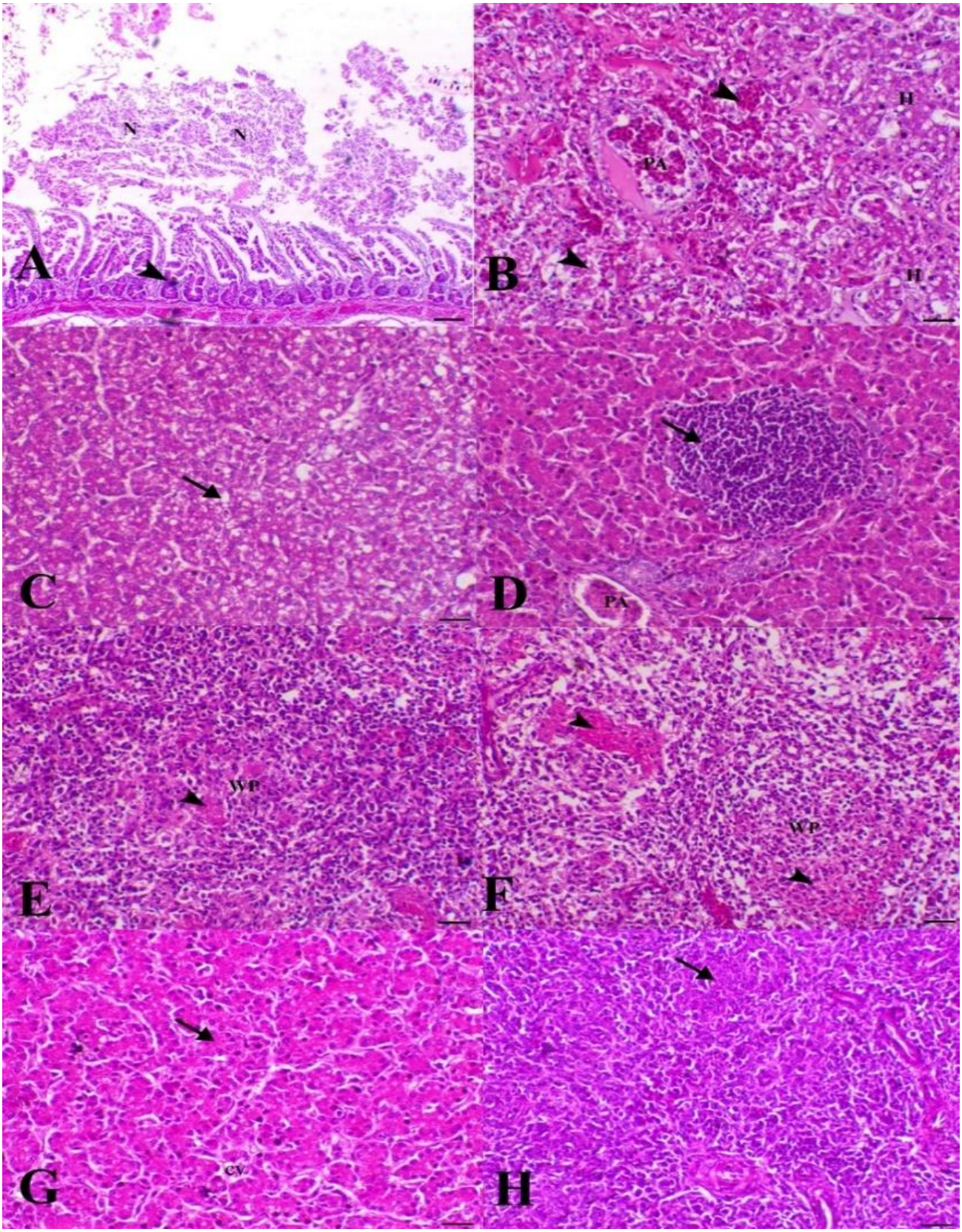

Figure 3. Histopathological lesions of broiler chickens at first week post-infection with different *E. coli* serotypes**. A:** Intestine of broiler chickens challenged with *E. coli* (O_{78}) showing necrotic enteritis associated with sloughing of the mucosal epithelium, **B:** Liver of mycotoxicated chicken challenged with *E. coli* (O_{78}) showing severe vasculitis associated with haemorrhage (arrowhead) associated with severe degree of fatty changes, **C:** Liver of chicken challenged with *E. coli* (O_{128}) showing marked fatty degenerative changes within hepatocytes, **D:** Liver of mycotoxicated chicken challenged with *E. coli* (O_{128}) showing focal area of coagulative necrosis associated with mononuclear cells infiltration, **E:** Spleen of chicken challenged with *E. coli* (O_{157}) showing marked degree of lymphoid depletion associated with deposition of fibrin (arrowhead) within the white pulp, **F:** Spleen of mycotoxicated chicken challenged with *E. coli* (O_{157}) showing severe degree of lymphoid necrosis associated with marked deposition of fibrin exudate within white pulp, **G:** Liver of control negative chicken showing normal hepatocytes around the central vein and **H:** Spleen of control positive chickens showing lymphoid depletion associated with marked histiocytic cells proliferation**.**

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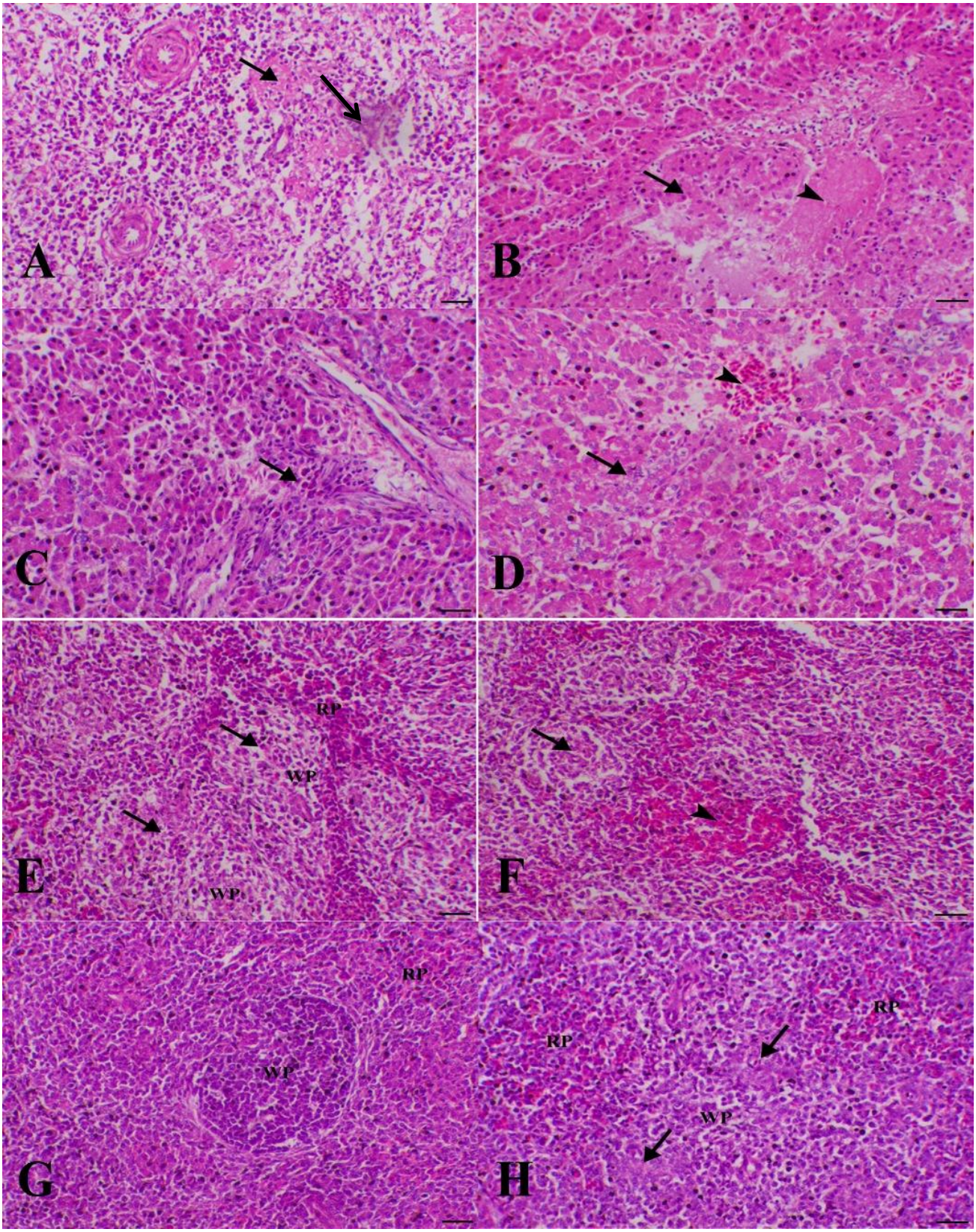

Figure 4. Histopathological lesions of broiler chickens at second week post infection with different *E. coli* serotypes. **A:** Spleen of chicken challenged with *E. coli* (O_{78}) showing severe degree of lymphoid necrosis associated with fibrin deposition, **B:** Liver of mycotoxicated chicken challenged with *E. coli* (O_{78}) showing severe coagulative necrosis (arrow) associated with marked deposition of fibrinoid-like materials, **C:** Liver of chicken challenged with *E. coli* (O_{128}) showing periportal hepatic necrosis associated with heterophilic cells infiltration, **D:** Liver of mycotoxicatedchicken challenged with *E. coli* (O_{128}) showing hepatic degeneration (arrow) and focal haemorrhage (arrowhead), **E:** Spleen of chicken challenged with *E. coli* (O_{157}) showing marked degree of lymphoid depletion which replaced with marked histocytic cells infiltration (arrows) within the white pulp, **F:** Spleen of mycotoxicated chicken challenged with *E. coli* (O_{157}) showing severe congestion of the red pulp (arrowhead) accompanied with lymphoid necrosis, **G:** Spleen of control chicken showing normal white pulp and red pulp and **H:** Spleen of control positive chickenshowing congestion of the red pulp and lymphoid depletion of the white pulp.

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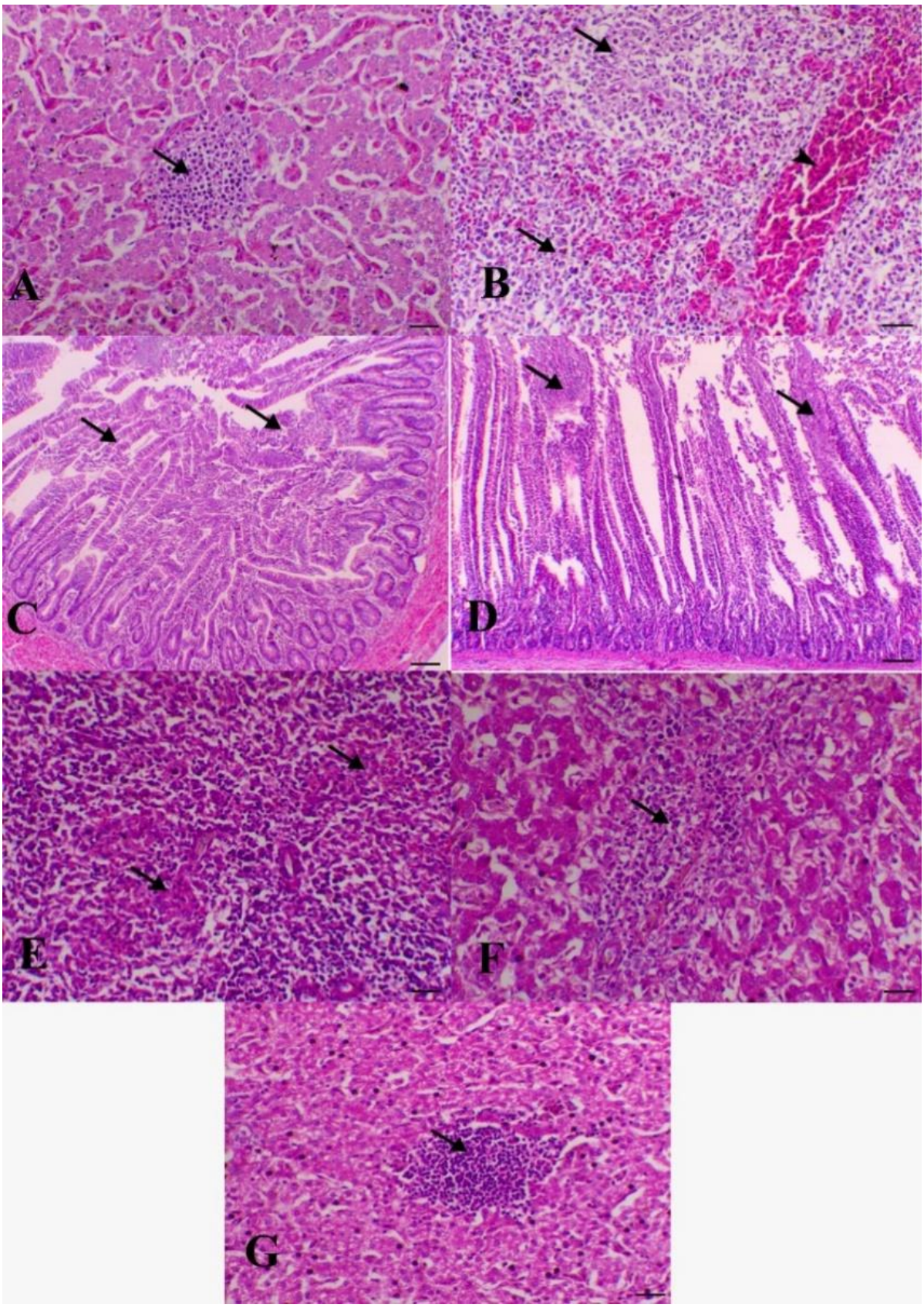

Figure 5. Histopathological lesions of the broiler chickens at third week post-infection with different *E. coli* serotypes. **A:** Liver of broiler chickens challenged with *E. coli* (O_{78}) showing marked sinusoidal congestion and focal coagulative necrosis associated with mononuclear cells infiltration, **B:** Spleen of mycotoxicated chicken challenged with *E. coli* (O_{78}) showing marked congestion and haemorrhage of the red pulp (arrowhead) and marked degree of lymphoid necrosis replaced with histocytes, **C:** Intestine of chicken challenged with *E. coli* (O_{128}) showing necrosis and sloughing of the intestinal villi, **D:** Intestine of mycotoxicated chicken challenged with *E. coli* (O_{128}) showing marked degree of necrotic enteritis, **E:** Spleen of chicken challenged with *E. coli* (O_{157}) showing marked degree of lymphoid depletion with marked histiocytic cells proliferation, **F:** Liver of mycotoxicated broiler chickens challenged with *E. coli* (O_{157}) showing marked vasculitis, periportal hepatic necrosis and marked mononuclear cells infiltration and **G:** Liver of control positive chicken showing focal necrosis associated with mononuclear inflammatory cells infiltration mostly lymphocytes and macrophages.

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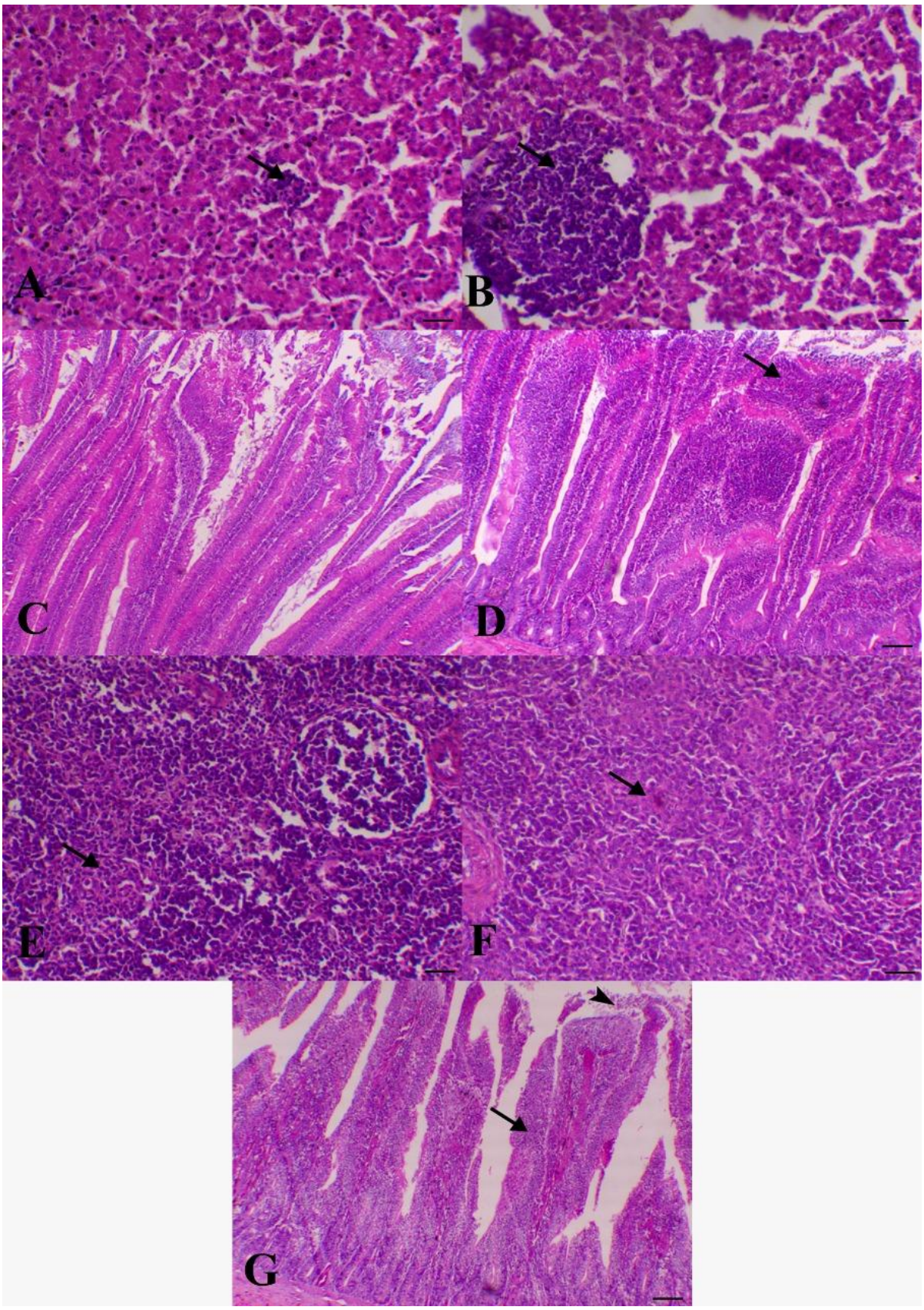

Figure 6. Histopathological lesions of broiler chickens at fourth week post-infection with different *E. coli* serotypes**. A:** Liver of chicken challenged with *E. coli* (O_{78}) showing small necrotic foci associated with mononuclear cells infiltration **B:** Liver of mycotoxicated broiler chickens challenged with *E. coli* (O_{78}) showing large necrotic foci associated with mononuclear cells infiltration **C:** Intestine of chicken challenged with *E. coli* (O_{128}) showing necrosis and sloughing of the intestinal most upper portions of the villi **D:** Intestine of mycotoxicated broiler chickens challenged with *E. coli* (O_{128}) showing shortening and blunting of the villi **E:** Spleen of chicken challenged with *E. coli* (O_{157}) showing moderate degree of lymphoid depletion with marked histiocytic cells proliferation **F:** Spleen of mycotoxicated chicken challenged with *E. coli* (O_{157}) showing marked lymphoid necrosis associated with perivascular histocytes proliferation **G:** Intestine of control positive chicken showing desqumative changes within the intestinal epithelium (arrowheads) associated with marked goblet cells hyperplasia.

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CONCLUSION

In conclusion, the previous results indicated that mycotoxins even at low levels worsen the clinical signs, mortalities, body weight, and feed conversion rate of the grown chickens when get infected with pathogenic strains as avian pathogenic *E. coli*. Thus, every endeavor should be adopted to reduce feed contamination with these mycotoxins.

DECLARATION

Ethical considerations

Ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors.

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions

Anwaar Mettwally El-nabarawy and designed and supervised the experiment. Mohamed Abdel-Salam Shakal supervised the experiment. Mohamed Bateekh, Abdel-Haleem Mohamed Hegazy, and Eman Anter Morsy performed the experiment and wrote the manuscript. Mohamed Bateekh performed the statistical analysis. All authors read and approve the manuscript.

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