



The Effect of Infectious Bronchitis Virus Vaccine on Pathogenicity of Avian Pathogenic *E. coli* in Broiler Chickens



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Abstract

OUR study was conducted to describe the pathogenicity of avian pathogenic *E. coli* (APEC) O78 in infectious bronchitis (IB) virus (IBV) vaccinated broiler chickens. Broiler chickens vaccinated at 1 day old and 14 days old with H120 and IB var2 vaccines respectively were challenged with APEC O78 strain 4 days later. This group was compared with vaccinated/unchallenged, unvaccinated/challenged, and unvaccinated/unchallenged broiler chicken groups. Clinical signs, postmortem findings, body weight, body-weight uniformity, pathological findings, and bacterial count from different tissues which measured 3 and 7 days post challenge (DPC) were used as an evaluation parameters. In our study, IBV live attenuated vaccines (IBV H120 and IB var2) followed by APEC O78 challenge improved colibacillosis clinical signs, pathological findings, and bacterial count in chicken tissues 3 DPC than 7 DPC comparing to unvaccinated/challenged group. However, body-weight uniformity was negatively affected 10 DPC in vaccinated/challenged group comparing to other chicken groups. From an applied perspective, prevention of APEC infection of turkey meat.

Keywords: Avian pathogenic *E. coli*, Colibacillosis, IBV, Vaccines, Broilers.

Introduction

Infectious bronchitis (IB) is a highly contagious respiratory and urogenital disease of chickens worldwide [1, 2]. High economic losses are caused by IB due to poor weight gain and feed efficiency, egg-production and egg-quality declines, and high mortalities in young chickens caused by nephropathogenic IB virus (IBV) strains [3]. In Egypt, different IBV genotypes have been detected from different poultry farms [4-8]. IBV variants belonged to IBV genotype I (G I -23) lineage were the most frequently identified [9]. Despite intensive vaccination program, IBV variants belonging to G I -23 lineage continue to detect frequently from vaccinated chickens in different Egyptian governorates causing significant economic losses [10-13].

IBV is a predisposing factor for several bacterial infections [14], especially colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) which causing a high broiler condemnations at processing plants [15]. APEC is a gram-negative bacterium, which causes a variable avian localized and systemic

infection [16, 17]. It had been reported that more than 80% of colibacillosis outbreaks was associated with three serogroups (O78, O2, and O1) [18]. Additionally, APEC serogroup O78 was mostly isolated from colibacillosis cases in Egypt [19-22] and worldwide [23-26].

The effect of IBV vaccine and/or IBV virulent stains on colibacillosis susceptibility have been studied on specific pathogen free (SPF) chickens [27] and broiler chickens [14]. In Egypt, different IBV live attenuated vaccines belonged to classic Massachusetts type (H120, M41, and Ma5) and variant (D274, CR88, and 4/91) strains are used to prevent IB. additionally, a live attenuated vaccine (IB var2) derived from IBV Eg/1212B/2012 strain (accession number: JQ839287). 1317-67-7-1427. Zanaty *et al.*, [28] had been developed and its efficacy to protect chickens against IBV variant stains related to G I -23 lineage had been evaluated [29]. The homologous live attenuated vaccine is commercially used in combination with other live attenuated IBV vaccines to control IB in Egypt. Thus, our study was conducted to describe the

pathogenicity of APEC O78 in broiler chickens vaccinated with H120 and IB var2 vaccines at 1 day old and 14 days old respectively.

Material and Methods

Chickens

One day old 80 broiler chickens (Cobb) were obtained from commercial company in Egypt.

Bacterial strain and IBV vaccines

E. coli O78 isolate was kindly obtained from Dr. Nagwa Rabie, Poultry Diseases Department, National Research centre, Egypt. The stock of bacterial strain was prepared as described previously [14]. And was diluted to 10^8 bacteria per ml for intra crop inoculation. We used a lyophilized live attenuated H120 and IB var2 vaccines (MEVAC, Egypt) according to the manufacture recommendations.

Experimental design

Four chicken groups ($n = 20$) were separately housed in cages. Chicks were reared under optimal temperature, humidity, and ventilation. The feed added to each chicken group was monitored daily. As shown in Table 1, two chicken groups were vaccinated at one day old and 14 days old with IB H120 and IB var2 vaccines, respectively. In the vaccinated groups, one group was challenged with *E. coli* O78 while one group remained unchallenged. Furthermore, we had unvaccinated/challenged group and unvaccinated/unchallenged group.

Body weight, body weight uniformity, and mean lesion score (MLS)

Chickens in all groups were individually weighted at 7, 14, 21, and 28 days old. Moreover, body weight uniformity was calculated for each group at 28 days old. Body weight uniformity is the percentage of chickens with a body weight between mean body weight plus 10% and mean body weight minus 10%. MLS was calculated at 3 and 7 days post challenge (DPC) as previously described [14].

Bacterial count in chicken tissues

Bacterial counts measured by colony-forming units (CFU), were determined by plating ten-fold serial dilutions of the inoculum on MacConkey agar to confirm counting of the colony [30].

Histopathological examination

Tissue specimens from liver, heart, air sac and lung, and trachea were taken and fixed in 10% neutral buffered formalin. Then, the specimens were processed in paraffin embedding method, sectioned and stained with Hematoxylin and Eosin (H&E) for light microscope examination [31].

Statistical analysis

Mean lesion score (MLS) differences per time point were non-parametrically analyzed using Kruskal-Wallis's test and Dunns test (p value <

0.05). Moreover, the transforming data of bacterial count in different tissues were also analyzed by nonparametric Kruskal-Wallis's test and Dunns test at p value < 0.05. Additionally, one way ANOVA was used to compare body weight between different groups. Statistical analysis was performed by GraphPad Prism 5.

Results

Clinical signs and pathological findings

No mortalities were detected in any groups. Chickens in challenged groups showed variable degree of ruffled feathers and lethargy beginning 3 DPC. Severe airsacculitis, perihepatitis, pericarditis, and tracheitis were detected in non-vaccinated/challenged group 3 DPC, while airsacculitis and tracheitis were detected in vaccinated/challenged group (Figure 1). The mean lesion score (MLS) was calculated 3 and 7 DPC (Table 2). No pathological lesions were observed in unchallenged groups. The pathological findings 7 DPC became mild in both unvaccinated/challenged chicken and vaccinated/challenged chickens.

Body weight and body-weight uniformity

As shown in Fig. 2, body weight was measured 7, 14, 21, and 28 days old. There was not a significant difference between chicken groups before and after challenge. We measured the body-weight uniformity at 21 days old (3 DPC) and 28 days old (10 DPC) (Fig. 3). The body-weight uniformity in vaccinated/unchallenged chicken group was 80% and 88% in 3 DPC and 10 DPC respectively. However, it was decreased from 85.7% (3 DPC) to 62.5% (10 DPC) in vaccinated/ challenged chicken group. The unvaccinated/challenged chicken group showed an increase in body-weight uniformity percent from 60% (3 DPC) to 84.6% (10 DPC) comparing to 71.5% (3 DPC) to 77.8% (10 DPC) in unvaccinated/unchallenged chicken group. There was no change between groups in feed intake after *E. coli* challenge except vaccinated/challenged chicken group decreased 14% feed intake from *E. coli* challenge to 10 DPC.

Histopathological findings

Different histopathological findings were determined in trachea, airsac, lung, liver, and heart 3 DPC. As shown in Fig. 4, (A) Trachea of chicken showed congestion of the mucosa accompanied with severe hyperplasia of the goblet cells with lymphocytic infiltration (H&E 100 \times). (B) Trachea of chicken showed lymphocytic infiltration of the mucosa accompanied with submucosal edema and presence of the mucous mixed with desquamated cells in the tracheal lumen (H&E 100 \times). (C) Chicken air sac suffered from severe airsacculitis characterized by severe lymphocytic infiltration and thickening of the air sac (H&E 200 \times). (D) Chicken air sac suffered from caseous airsacculitis characterized

by severe lymphocytic infiltration and massive thickening of the air sac by caseous exudate and dead neutrophils (H&E 200×). (E) Chicken lungs showed suppurative inflammation characterized by localized area of dead neutrophils and lung cells (H&E 200×). (F) Chicken lungs showed severe congestion of the pulmonary artery with perivascular edema (H&E 100×). (G) Chicken liver showed multifocal area of coagulative necrosis infiltrated with lymphocytes (H&E 100×). (H) Chicken liver showed multifocal area of coagulative necrosis infiltrated with lymphocytes (H&E 200×). (I) Chicken heart showed severe pericarditis characterized by thickening of the pericardium with lymphocytic infiltration (H&E 100×). (J) Chicken heart showed severe pericarditis characterized by thickening of the pericardium with lymphocytic infiltration (H&E 200×).

Bacterial count in different tissues 3 and 7 DPC

As shown in Fig. 5, the APEC counts (log₁₀ CFU/ml) from airsacs & lung, heart, and trachea were high in unvaccinated/challenged chickens 3 DPC comparing to vaccinated/challenged chickens. Furthermore, the bacterial count was significantly high in liver from unvaccinated/challenged chickens comparing to vaccinated/challenged chickens 3 DPC. Interestingly, the bacterial count in trachea 7 DPC in unvaccinated/challenged chickens decreased comparing to vaccinated/challenged chickens which was increased. No APEC was detected in other tissues (airsacs & lung, heart, and liver) 7 DPC.

Discussion

Colibacillosis is one of avian diseases which causes high economic losses due to increased mortality, decreased weight gain, decline in the egg production, high broiler condemnations at processing plants, decreased hatching rates, and extra cost for treatment and prophylaxis [32]. This disease which caused by APEC initiates as a primary infection or secondary to viral infections especially IB, mycoplasma infections, immunosuppressive diseases, or environmental stresses [18]. The ability of IBV virulent strains (D387 and M41) or IBV vaccine strains (H120 and H52) to induce susceptibility to colibacillosis in commercial broilers has been evaluated 7 days post *E. coli* strain 506 inoculation. They reported that both IBV vaccine strain induced colibacillosis as severe as that produced by IBV virulent strain [14]. The live attenuated vaccine (IB var2) derived from IBV Eg/1212B/2012 strain (accession number: JQ839287) [28] is widely used in combination with classic live attenuated IBV vaccines to prevent IB in Egypt. Thus, our study was conducted to describe the pathogenicity of APEC O78 in broiler chickens vaccinated with H120 and IB var2 vaccines at 1 day old and 14 days old respectively.

In our study, we reported severe pathological findings and high bacterial count in air sacs and lung,

heart, liver (significantly high), and trachea in unvaccinated/challenged chickens 3 DPC comparing to vaccinated/challenged chickens (Figure 5.A and B). It has been reported that numbers of T cells (CD4 and CD8) and macrophages were highly detected in trachea and air sacs at the time of *E. coli* infection of broilers previously vaccinated with H120 or challenged with IBV M41 strain. Moreover, a high number of CD4 and macrophages were also detected in lung [33]. Furthermore, IBV H120 vaccination at one day old commercial broilers significantly reduced the severity of *E. coli* air sacculitis after challenge with IBV M41 at 29 days of age and *E. coli* 506 at 35 days of age [34]. The host immune response to IBV vaccines may decrease the severity of pathological findings and the bacterial count at 3 DPC in vaccinated/challenged chickens, however immunopathological studies are required for further explanation of this point.

Despite the clinical signs and pathological findings were mild 7 DPC in our study in both vaccinated/challenged chickens and unvaccinated/challenged chickens, the vaccinated/challenged chickens showed respiratory signs and high bacterial count in trachea (Figure 5. B). It was reported that, the persistence of lesions in broiler chickens for at least 8 days was reported after inoculation of chickens with APEC preceded by IBV, NDV, or aMPV [35]. Moreover, the delayed recovery in broilers from *E. coli* challenge 5 days later inoculation with IBV vaccine strain or IBV virulent strain was associated with lower bactericidal activity of peripheral blood mononuclear cells (PBMC) and splenocytes 7DPC [36].

In this study, no change in mean body weight between chicken groups at 7, 14, 21, and 28 days old (Figure 2). This result was agreed with others [35] who did not report significant body weight differences at day of virus inoculation, at the day of APEC infection (6 days later), or 8 days after APEC infections. Body-weight uniformity in our study increased from 3 DPC to 10 DPC in all groups except vaccinated/challenged group in which it declined from 85.7% to 62.5% (Figure 3). This decrease in body-weight uniformity may be related to the reduction of feed intake in this group which was reported as 14% from challenge to 10 DPC.

Conclusion

IBV live attenuated vaccines (IBV H120 and IB var2) followed by APEC O78 challenge in our study improved colibacillosis clinical signs, pathological findings, and bacterial count in chicken tissues 3 DPC than 7 DPC comparing to unvaccinated/challenged group. However, inoculation of IBV vaccinated broiler chickens with APEC O78 negatively affected the body-weight uniformity 10 DPC.

Authors' contributions

All authors contributed to the study conception and design. Aly Ghetas and Mohamed Abdelbaki performed the laboratory and experimental work. Aly Ghetas analyzed and interpreted the data. Mohamed Bosila performed the histopathological work. Aly Ghetas wrote the draft manuscript. Nagwa Rabie revised the draft manuscript. All authors read and approved the final manuscript.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Ethics Committee of the National Research Centre, Egypt approved this study under number 1475062022.

TABLE 1. Experimental design

Group	1 st IB vaccination (IB H120)	2 nd IB vaccination (IB var2)	Challenge (E. Coli O78)
1	One day old	14 days old	18 days old
2	One day old	14 days old	Non challenged
3	Unvaccinated	Unvaccinated	18 days old
4	Unvaccinated	Unvaccinated	Non challenged

TABLE 2. Mean lesion score (MLS) between different chicken groups

	Vacc/Unchall	Vacc/Chall	Unvacc/Chall	Unvacc/Unchall
3 DPC	0.0 ± 0.0 ^a	2.0 ± 0.0 ^{ac}	7.3 ± 4.2 ^{bcd}	0.0 ± 0.0 ^{ac}
7 DPC	0.0 ± 0.0 ^a	2.6 ± 1.2 ^{bc}	1.3 ± 1.2 ^{ac}	0.0 ± 0.0 ^a

Different letters indicated significant difference ($p < 0.05\%$). 3 DPC and 7 DPC. Chickens vaccinated against IBV twice: H120 (one day old) and Var2 (14 day old). Chickens challenged with E. coli (18 day old). Vacc/Unchall = vaccinated/Unchallenged. Vacc/Chall= vaccinated/challenged 4 day post IBV var2 vaccination. Unvacc/chall = Unvaccinated/challenged. Unvacc/Unchall= Unvaccinated/ challenged.

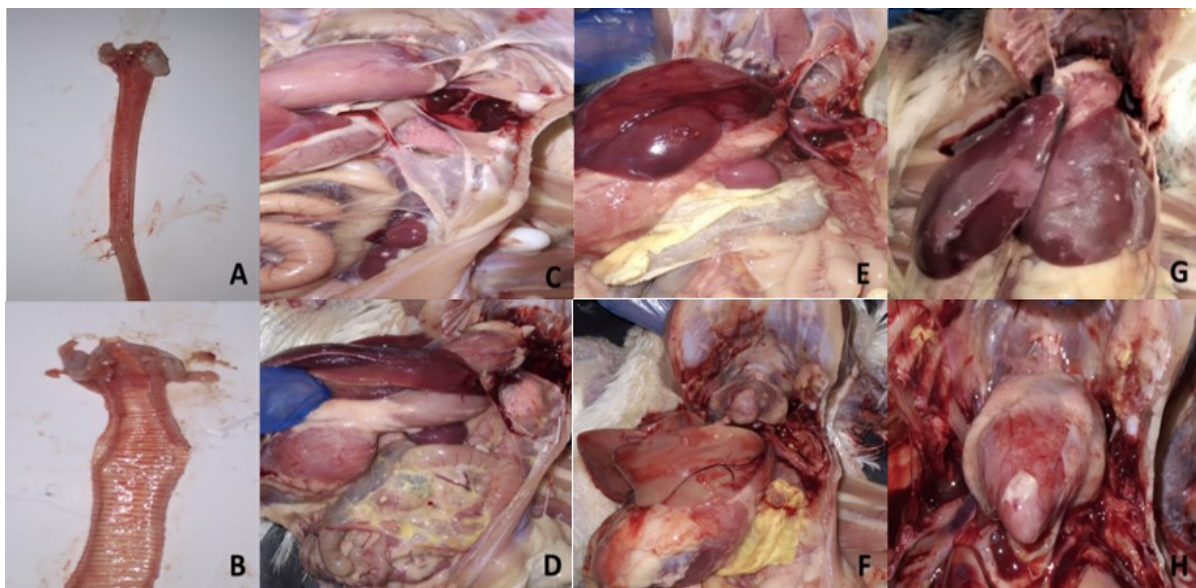


Fig. 1. Pathological findings recorded in chickens challenged with APEC (3 DPC). (A & B): Trachea shows mild hemorrhage, (C, D, E, and F): severe airsacculitis, perihepatitis, and pericarditis, (G): Severe fibrinous perihepatitis, (H): Fibrinous pericarditis.

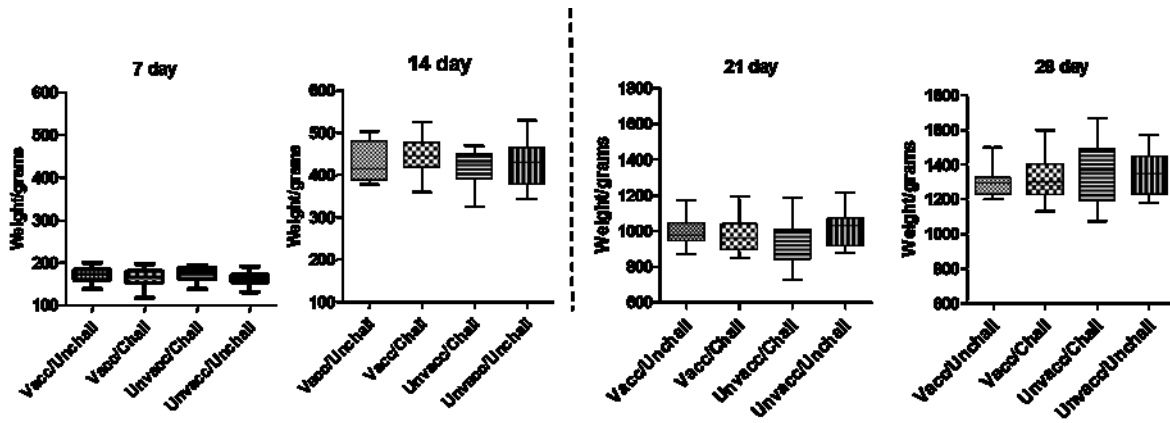


Fig. 2. Body weight of chickens in different groups (weight/grams). Chickens vaccinated against IBV twice: H120 (one day old) and Var2 (14 days old). Chickens challenged with *E. coli* (18 day old). Vacc/Unchall = vaccinated /unchallenged. Vacc/Chall= vaccinated/challenged 4 days post IBV var2 vaccination. Unvacc/chall = unvaccinated/challenged. Unvacc/Unchall= unvaccinated/challenged. No significant difference between groups. Statistical analysis by one way ANOVA.

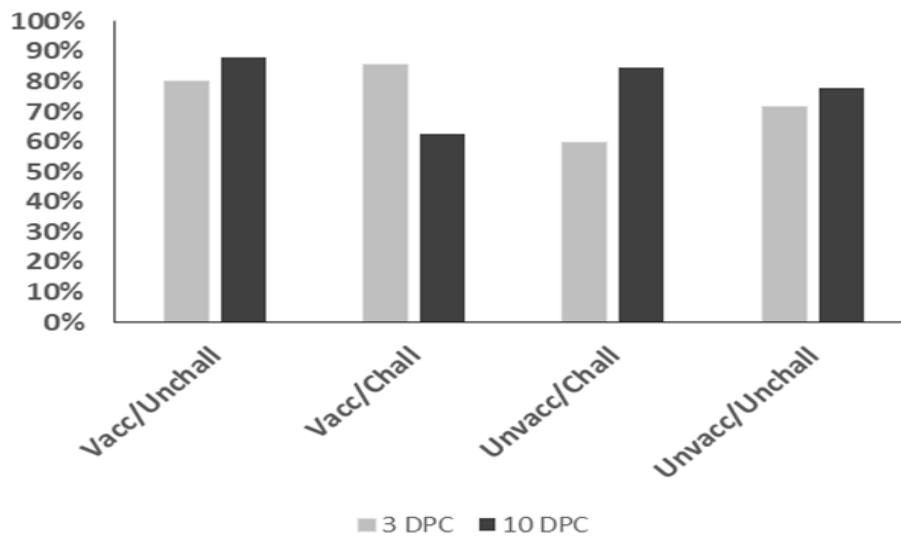


Fig. 3. Body-weight uniformity 3 DPC and 10 DPC. Chickens vaccinated against IBV twice: H120 (one day old) and Var2 (14 days old). Chickens challenged with *E. coli* (18 days old). Vacc/Unchall= vaccinated/Unchallenged chickens. Vacc/Chall= vaccinated/challenged chickens. Unvacc/Chall= unvaccinated/challenged chickens. Unvacc/Unchall= unvaccinated/unchallenged chickens

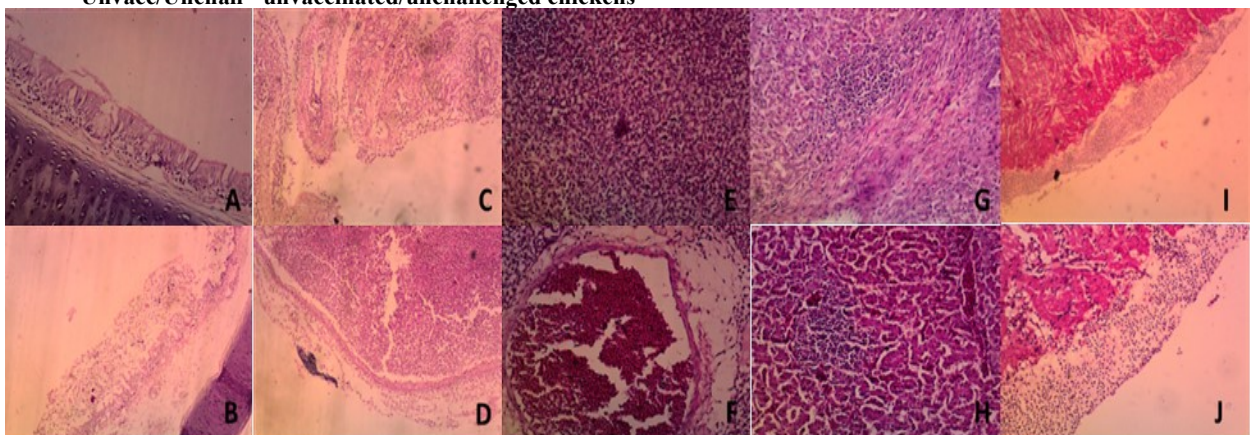


Fig. 4. Histopathological findings of IBV chickens challenged with APEC (3 DPC). (A & B): Tracheitis (100×), (C): Severe airsacculitis, (D): Caseous airsacculitis, (E): Suppurative inflammation in lung, (F): Severe congestion in lung and perivascular edema (100×), (G & H): Liver necrosis, (I & J): Pericarditis in which I (100×).

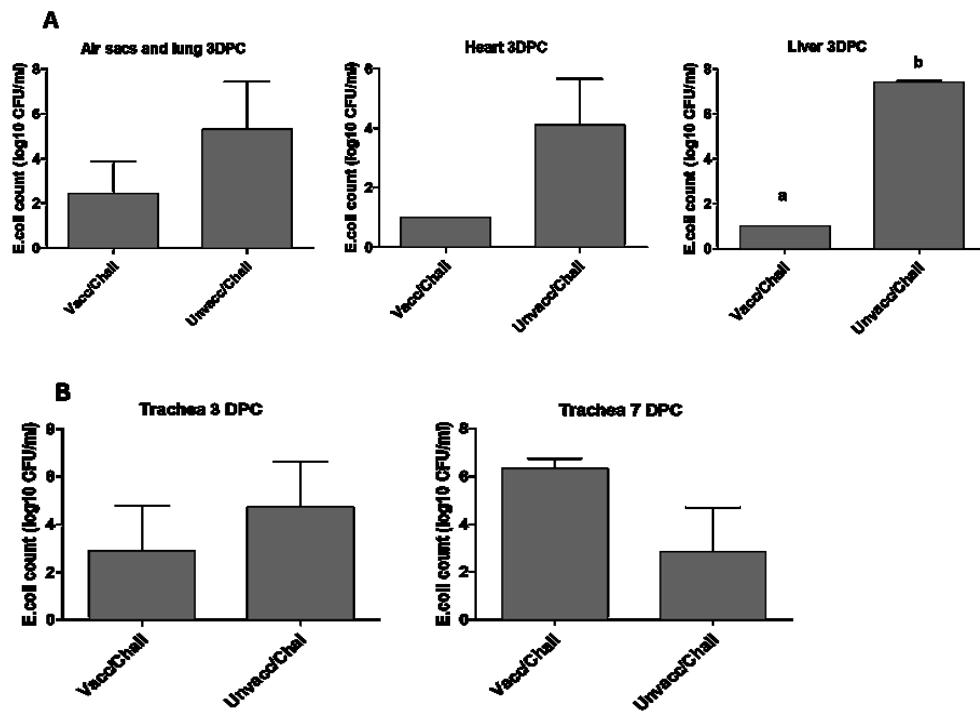


Fig. 5. Bacterial count 3 DPC from air sacs and lung, heart, liver (A), and 3 & 7 DPC Trachea (B). Different letters indicated a significant difference between groups. Transformed data were analyzed by nonparametric Kruskal-Wallis test and Dunns test (p value < 0.05).

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تأثير لقاح الالتهاب الشعبي الفيروسي المعدي على قدره المرضيه للعدوى ببكتريا الأيشريشيا كولاي في دجاج التسمين

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المستخلص

جريت دراستنا لوصف القدرة المرضية لبكتيريا الأيشريشيا كولاي الممرضة O78 المسببة للأمراض في الطيور في دجاج التسمين الملقح بفيروس التهاب الشعب الهوائية الفيروسي المعدي. تم تحصين دجاج التسمين عند عمر يوم واحد وعمر 14 يوماً بلقاحي الالتهاب الشعبي لل عترة OH12 وال عترة IB var2 على التوالي، وتم اجراء عدوى بمعزولة ببكتريا الأيشريشيا كولاي الممرضة O78 بعد 4 أيام من التحصين. تمت مقارنة هذه المجموعة مع مجموعات الدجاج اللاحم المحصنة/غير معديه، وغير المحصنة/المعدية، وغير المحصنة/غير المعدية. تم استخدام الاعراض المرضية، ونتائج ما بعد النفوق، ووزن الجسم، وعدم تماثلية الأوزان، ونتائج الفحص الباثولوجي، والعد البكتيري من الأنسجة المختلفة التي تم قياسها بعد 3 و 7 أيام من التحدي كمعايير للتقييم. في دراستنا، لقاحات الالتهاب الشعبي الفيروسي المعدي الحية المضعفة (IBV H120) و (B var2) تلبها العدوى ببكتريا الأيشريشيا كولاي تسبب في زيادة الاعراض المرضية، والصفات التشريحية، والنتائج الباثولوجيا وعدد البكتيريا في أنسجة الدجاج بعد العدوى ب 3 و 7 أيام مقارنة بالمجموعة غير المحصنة/المعدية. ومع ذلك، فقد تأثر تجانس أوزان الطيور سلبا عند اليوم العاشر بعد العدوى في المجموعة المحصنة/المعدية مقارنة بمجموعات الدجاج الأخرى. من الناحية التطبيقية، الوقاية من عدوى الأيشريشيا كولاي الممرضة مطلوبة لتجنب التأثير السلبي على تجانس أوزان الطيور بعد التطعيم بلقاحات الالتهاب الشعبي الفيروسي الحية المضعف. **الكلمات الدالة:** الأيشريشيا كولاي، لقاحات الالتهاب الشعبي الفيروسي الحية المضعفة، تجانس الأوزان، دجاج التسمين.