



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 is required to avoid the adverse effect on body-weight uniformity after vaccination with IBV live attenuated vaccines.

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 air sacculitis, 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×). (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×). (C) Chicken air sac suffered from severe airsacculitis

characterized by severe lymphocytic infiltration and thickening of the air sac (H&E 200×). (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.

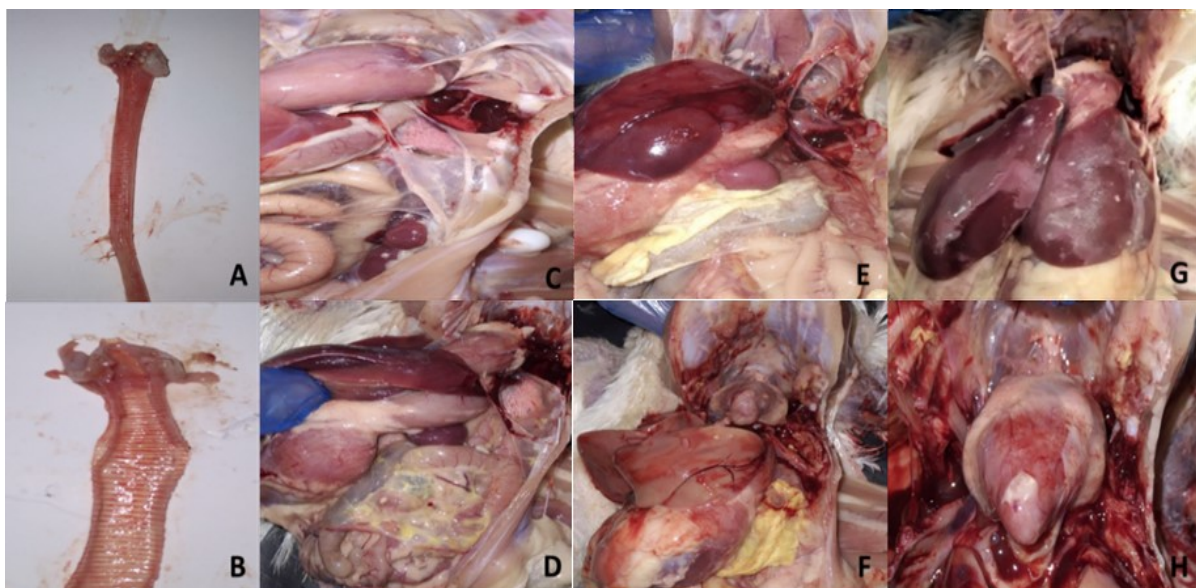


Fig. 1. Pathological findings recorded in chickens challenged with APEC (3 DPC). (A & B): Trachea shows mild hemorrhage, (C, D, E, and F): severe airsacculitis, perihepatitis, and pericarditis, (G): Severe fibrinous perihepatitis, (H): Fibrinous pericarditis.

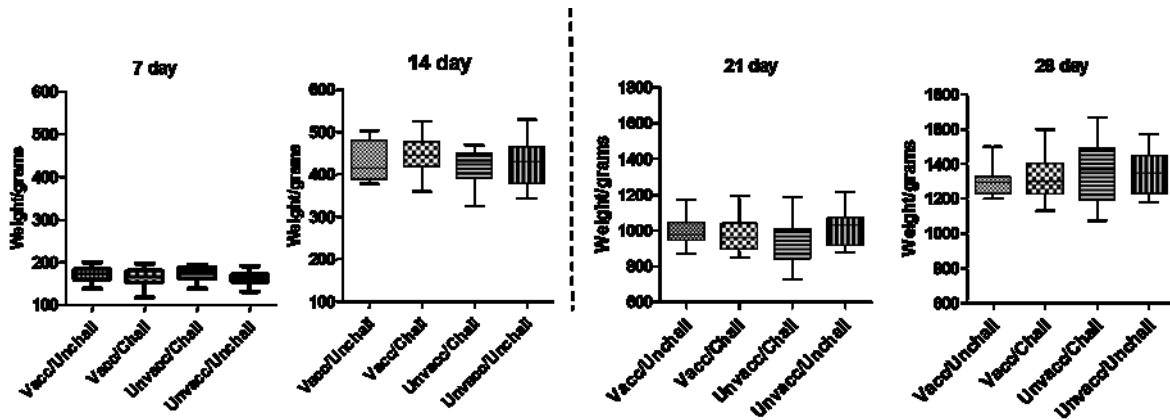


Fig. 2. Body weight of chickens in different groups (weight/grams). Chickens vaccinated against IBV twice: H120 (one day old) and Var2 (14 days old). Chickens challenged with *E. coli* (18 day old). Vacc/Unchall = vaccinated /unchallenged. Vacc/Chall= vaccinated/challenged 4 days post IBV var2 vaccination.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

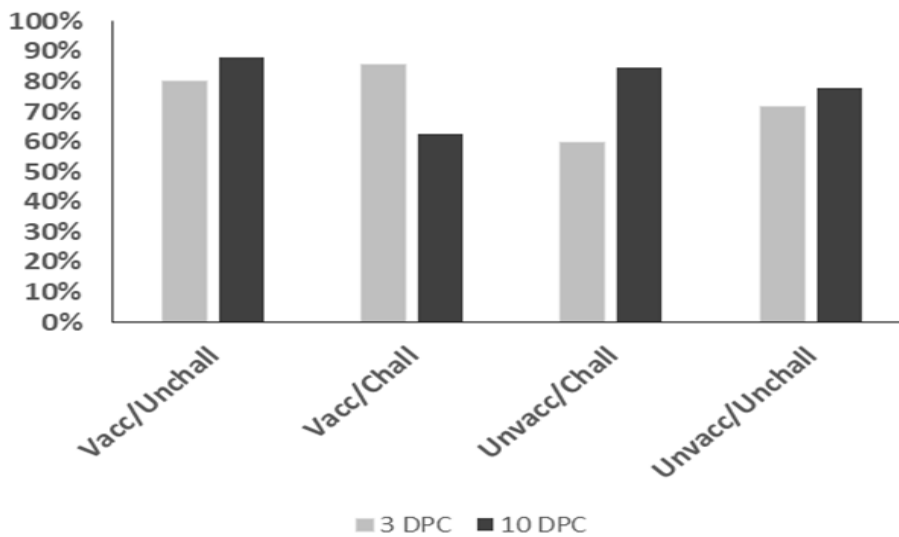


Fig. 3. Body-weight uniformity 3 DPC and 10 DPC. Chickens vaccinated against IBV twice: H120 (one day old) and Var2 (14 days old). Chickens challenged with *E. coli* (18 days old). Vacc/Unchall= vaccinated/Unchallenged chickens. Vacc/Chall= vaccinated/challenged chickens. Unvacc/Chall= unvaccinated/challenged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

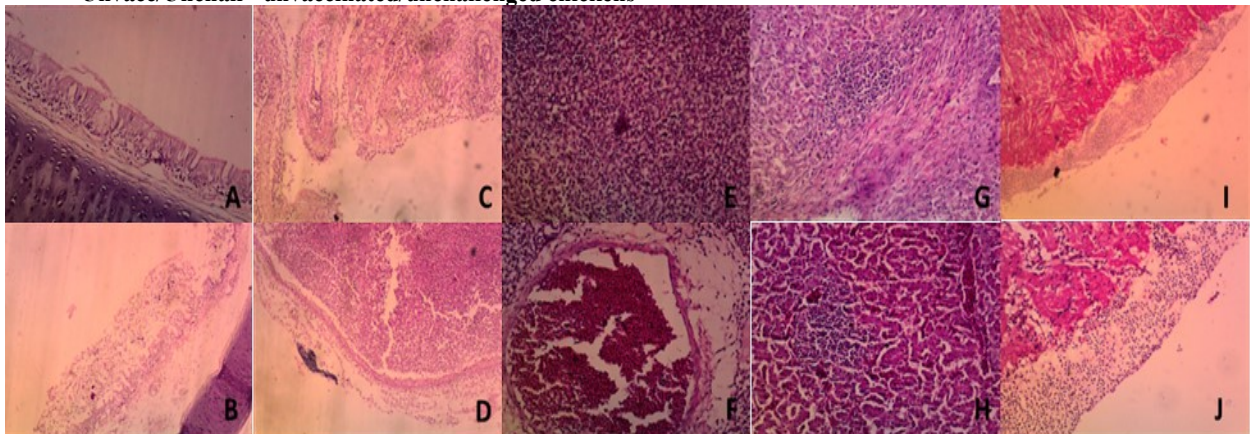


Fig. 4. Histopathological findings of IBV chickens challenged with APEC (3 DPC). (A & B): Tracheitis (100×), (C): Severe airsaccutitis, (D): Caseous airsaccutitis, (E): Suppurative inflammation in lung, (F): Severe congestion in lung and perivascular edema (100×), (G & H): Liver necrosis, (I & J): Pericarditis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

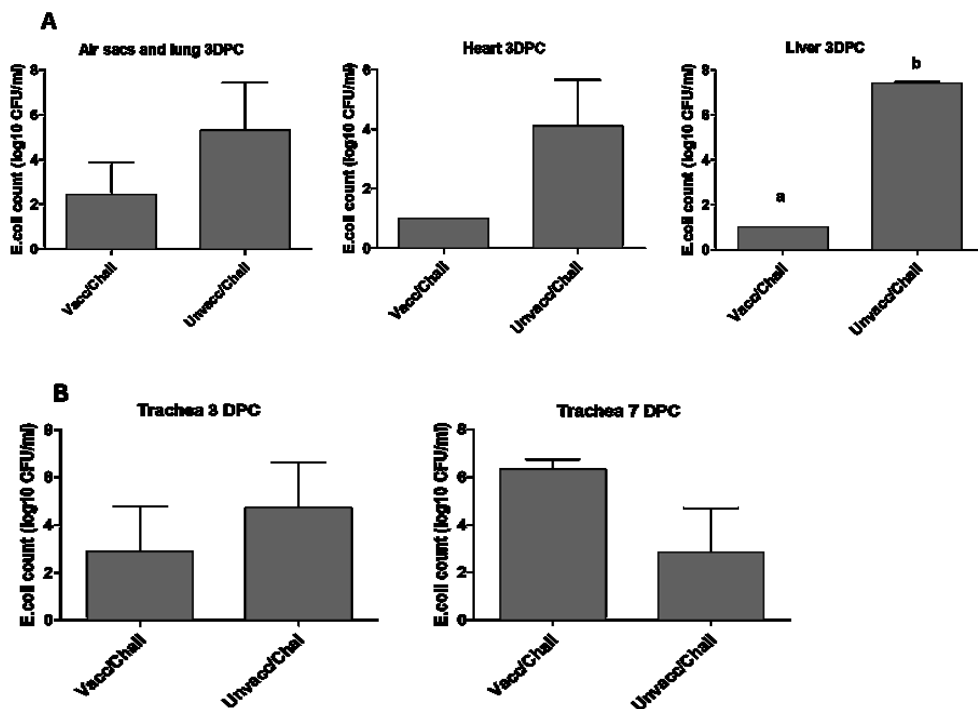


Fig. 5. Bacterial count 3 DPC from air sacs and lung, heart, liver (A), and 3 & 7 DPC Trachea (B). Different letters indicated a significant difference between groups. Transformed data were analyzed by nonparametric Kruskal-Wallis test and Dunns test (p value < 0.05).

References

1. Abozeid, H.H. Global Emergence of Infectious Bronchitis Virus Variants: Evolution, Immunity, and Vaccination Challenges. *Transbound Emerg Dis.*, **2023**, Article ID 1144924, 28 pages (2023). <https://doi.org/10.1155/2023/1144924>.
2. Ghetas, A.M. Infectious Bronchitis Virus Genotypes in the Middle East. *Avian Dis.*, **65**, 647–651 (2021). doi: 10.1637/aviandiseases-21-00097.
3. Cavanagh, D. and J. Gelb. *Infectious bronchitis*, in *Diseases of Poultry*, Y. M. Saif, et al., Editors. 2008, Blackwell Publishing: Ames, Iowa. p. 117-135.
4. Abdel-Moneim, A.S., Afifi, M.A. and El-Kady, M.F. Emergence of a novel genotype of avian infectious bronchitis virus in Egypt. *Arch of Virol.*, **157**(12), 2453-2457 (2012). doi: 10.1007/s00705-012-1445-1.
5. Abdel-Moneim, A.S., El-Kady M.F., Ladman, B.S. and Gelb J.J. S1 gene sequence analysis of a nephropathogenic strain of avian infectious bronchitis virus in Egypt. *Virol. J.*, **20** (3), 422X-3-78,78 (2006). doi: 10.1186/1743-422X-3-78.
6. Abdel-Moneim, A.S., Madbouly, H.M., Gelb, J.J. and Ladman, B.S., Isolation and identification of Egypt/Beni-Seuf/01 a novel genotype of infectious bronchitis virus. *Veterinary Medicine Journal, Giza*, **50**(4), 1065-1078 (2002).
7. Abdel-Sabour, M.A., Al-Ebshahy, E.M., Khaliel, S.A., Abdel-Wanis, N.A. and Yanai, T. Isolation and molecular characterization of novel infectious bronchitis virus variants from vaccinated broiler flocks in Egypt. *Avian Dis.*, **61**(3), 307-310 (2017). doi: 10.1637/11566-121516-RegR.
8. Al-Beltagi, S.E., Torkey, H.A. and Seddeek, M.E. Antigenic Variations of Infectious Bronchitis Virus from Broiler flocks in Al Behera Governorate. *Alex. J. Vet. Sci.*, **40**, 44-51 (2014). doi: 10.5455/ajvs.46652
9. Houta, M.H., Hassan, K.E., El-Sawah, A.A., Elkady, M.F., Kilany, W.H., Ali, A. and Abdel-Moneim, A.S. The emergence, evolution and spread of infectious bronchitis virus genotype GI-23. *Arch. of Virol.*, **166**, 9-26 (2021).
10. Abd El Rahman, S., Hoffmann, M., Lueschow, D., Eladl, A. and Hafez, H.M. Isolation and characterization of new variant strains of infectious bronchitis virus in Northern Egypt. *Adv. Anim. Vet. Sci.*, **3**, 362-371 (2015). DOI:10.14737/journal.aavs/2015/3.7.362.371
11. Ghetas, A.M., Kutkat, M.A., Amer, M.M. and Awaad, M.H.H. Isolation and molecular identification of IBV isolates in different governorates in Egypt. *J. Egypt. Soc. Parasitol.*, **46**, 341-346 (2016). DOI: 10.21608/JESP.2016.88682

12. Selim, K., Arafá, A.S., Hussein, H.A. and El-Sanousi, A.A. Molecular characterization of infectious bronchitis viruses isolated from broiler and layer chicken farms in Egypt during 2012. *Int. J. Vet. Sci. Med.*, **1**(2), 102-108 (2013). doi: 10.1016/j.ijvsm.2013.10.002.
13. Zanaty, A., Arafá, A.S., Hagag, N. and El-Kady, M. Genotyping and pathotyping of diversified strains of infectious bronchitis viruses circulating in Egypt. *World J. Virol.*, **5**, 125-134 (2016). doi: 10.5501/wjv.v5.i3.125.
14. Matthijs, M.G., Van Eck, J.H., Landman, W.J. and Stegeman, J.A. Ability of Massachusetts-type infectious bronchitis virus to increase colibacillosis susceptibility in commercial broilers: a comparison between vaccine and virulent field virus. *Avian Pathol.*, **32**, 473-481 (2003). doi: 10.1080/0307945031000154062.
15. Muchon, J.L., Garcia, R.G., Gandra, E.R.S., Assunção, A.S.A., Komiyama, C.M., Caldara, F.R., Nääs, I.A. and Santos, R.A. Origin of broiler carcass condemnations. *Revista Brasileira de Zootecnia*, **48**, e20180249 (2019). doi:10.1590/rbz482018024
16. Omer, M.M., Abusalab, S.M., Gumaa, M.M., Mulla, S.A., Omer, E.A., Jeddah, I.E., AL-Hassan, A.M., Hussein, M.A. and Ahmed, A.M. Outbreak of colibacillosis among broiler and layer flocks in intensive and semi intensive poultry farms in Kassala state, Eastern Sudan. *Asian J. Poult. Sci.*, **4** (4), 173-181 (2010). DOI: 10.3923/ajpsaj.2010.173.181
17. Wang, J., Tan, P., Tan, D., Wang, L., Zhang, S., Qiu, Y., Dong, R., Wanhua Liu, W., Huang, J., Chen, T., Ren, J., Li, C. and Liu, H. The pathogenicity of chicken pathogenic *Escherichia coli* is associated with the numbers and combination patterns of virulence-associated genes. *OJVM.*, **5**, 243-254 (2015). DOI: 10.4236/ojvm.2015.512033
18. Ghunaim, H., Abu-Madi, M.A. and S Kariyawasam. Advances in vaccination against avian pathogenic *Escherichia coli* respiratory disease: Potentials and limitations. *Vet. Microbiol.*, **172**, 13-22 (2014). doi: 10.1016/j.vetmic.2014.04.019.
19. Abd El-Mongy, M., Abd-El-Mon, G.M., Moawad, A.A. and Mohammed, A.A. Serotyping and Virulence Genes Detection in *Escherichia coli* Isolated from Broiler Chickens. *J. Biol. Sci.*, **18**(1), 46-50 (2017). DOI: 10.3923/jbs.2018.46.50
20. Ahmed, A.A., H.M. Salem, M.M. Hamoud, and M.M. Amer, Molecular Identification of Resistance and Pathogenicity Genes of *E. coli* Isolated From Broiler Chicken Farms. *Egypt. J. Vet. Sci.*, **55**, 1787-1800 (2024). DOI: 10.21608/EJVS.2024.252030.1691
21. Amer, M.M., Mekky, H.M. Amer, A.M. and Fedawy, H.S. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. *Vet. World*, **11**(8), 1082-1088 (2018). doi: 10.14202/vetworld.2018.1082-1088.
22. Younis, G., Awad, A. and Mohamed, N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. *Vet. World*, **10**(10), 1167-1172 (2017). doi: 10.14202/vetworld.2017.1167-1172.
23. Koutsianos, D., Athanasiou, L.V., Mossialos, D., Franzo, G., Cecchinato, M. and Koutoulis, K.C. Investigation of serotype prevalence of *Escherichia coli* strains isolated from layer poultry in Greece and interactions with other infectious agents. *Vet. Sci.*, **9**(4), 9040152 (2022). doi: 10.3390/vetsci9040152.
24. Kumar, A.D., Sarma, B.J., Rao, A.S. and Mishra, S.K. Serogroups of *Escherichia coli* isolates from chickens and their antibiogram. *Indian J. Poult. Sci.*, **38**(3), 270-273 (2003).
25. McPeake, S.J.W., Smyth, J.A. and Ball, H.J. Characterization of avian pathogenic *Escherichia coli* (APEC) associated with colisepticaemia compared to faecal isolates from healthy birds. *Vet. Microbiol.*, **110**, 245-253 (2005). <https://doi.org/10.1016/j.vetmic.2005.08.001>
26. Wen-Jie, J., Zhi-Ming, Z., Yong-Zhi, Z., Ai-Jian, Q., Hong-xia, S., Yue-long, L., Jiao, W. and Qian-qian, W. Distribution of virulence-associated genes of avian pathogenic *Escherichia coli* isolates in China. *Agric. Sci. China*, **7**(12), 1511-1515 (2008). [https://doi.org/10.1016/S1671-2927\(08\)60410-1](https://doi.org/10.1016/S1671-2927(08)60410-1)
27. Cook, J.K.A., Smith, H.W. and Huggins, M.B. Infectious bronchitis immunity: its study in chickens experimentally infected with mixtures of infectious bronchitis virus and *Escherichia coli*. *J. G. Virol.*, **67**, 1427-1434 (1986). doi: 10.1099/0022-1317-67-7-1427.
28. Zanaty, A., Naguib, M.M., El-Husseiny, M.H., Mady, W., Hagag, N. and Arafá, A. The sequence of the full spike S1 glycoprotein of infectious bronchitis virus circulating in Egypt reveals evidence of intra-genotypic recombination. *Arch Virol.*, **161**, 3583-3587 (2016). doi: 10.1007/s00705-016-3042-1
29. Ali, A., Kilany, W.H., Zain El-Abideen, M.A., Sayed, M.E. and Elkady, M. Safety and efficacy of attenuated classic and variant 2 infectious bronchitis virus candidate vaccines. *Poult. Sci.*, **97**, 4238-44 (2018). doi: 10.3382/ps/pey312.
30. Ben-David, A. and Davidson, C.E. Estimation Method for Serial Dilution Experiments. *J. Microbiol. Methods*, **107**, 214-221 (2014). <https://doi.org/10.1016/j.mimet.2014.08.023>
31. Suvarna, K., Layton, C. and Bancroft, J.D. *Bancroft's theory and practice of histological techniques*. Vol. 8th ed. 2018, London, UK: Elsevier.
32. Kathayat, D., Lokesh, D., Ranjit, S. and Rajashekara, G. Avian pathogenic *Escherichia coli* (APEC): An overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. *Pathogens*, **10**, 467 (2021). doi: 10.3390/pathogens10040467.
33. Matthijs, M.G.R., Ariaans, M.P., Dwars, R.M., van Eck, J.H.H., Bouma, A., Stegeman, A. and Vervelde,

- L. Course of infection and immune responses in the respiratory tract of IBV infected broilers after superinfection with E. coli. *Vet. Immunol. Immunopathol.*, **127**, 77-84 (2009). doi: 10.1016/j.vetimm.2008.09.016.
34. Matthijs, M.G., van Eck, J.H., de Wit, J.J., Bouma, A. and Stegeman, J.A. Effect of IBV-H120 vaccination in broilers on colibacillosis susceptibility after infection with a virulent Massachusetts-type IBV strain. *Avian Dis.*, **49**, 540-545 (2005). doi: 10.1637/7380-051305R.1.
35. Weerts, E.A.W.S., Matthijs, M.G.R., Bonhof, J., D van Haarlem, A., Marius, D. Wars, R., Gröne, A., Hélène Verheije, H. and Jansen, C.A. The contribution of the immune response to enhanced colibacillosis upon preceding viral respiratory infection in broiler chicken in a dual infection model. *Vet. Immunol. Immunopathol.*, **238**, 110276 (2021). doi: 10.1016/j.vetimm.2021.110276.
36. Ariaans, M.P., Matthijs, M.G., van Haarlem, D., van de Haar, P., van Eck, J.H.H., Hensen, E.J. and Vervelde, L. The role of phagocytic cells in enhanced susceptibility of broilers to colibacillosis after Infectious Bronchitis Virus infection. *Vet. Immunol. Immunopathol.*, **123**, 240-250 (2008). <https://doi.org/10.1016/j.vetimm.2008.02.003>

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

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المستخلص

جريت دراستنا لوصف القدرة المرضية لبكتريا 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 0H12 وال عترة IB var2 على التوالي، وتم اجراء عدوى بمعزولة بكتيريا 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 I و B var2) تليها العدوى بكتريا الأيشريشيا كولاي تسبب في زيادة الأعراض 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 سلبياً 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.