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Recent approaches for control of *E. coli* and respiratory complex in Middle EastHussein M. Galal <sup>a</sup>, Azza M. Tawfek <sup>b</sup>, Mohamed I. Abdrabou <sup>c</sup>, Ashgan Mohamed Hessain <sup>d,\*</sup>, Jwahrer Haji Alhaaji <sup>d</sup>, Saleh A. Kabli <sup>e</sup>, Ayman Elbehiry <sup>f</sup>, Waleed K. Alwarhi <sup>g</sup>, Ihab M. Moussa <sup>g,\*</sup><sup>a</sup> Department of Microbiology, Faculty of veterinary Medicine, Cairo University, Giza, Egypt<sup>b</sup> Department of Clinical Pathology, Faculty of veterinary Medicine, Cairo University, Giza, Egypt<sup>c</sup> Department of Cytology and Histology, Faculty of veterinary Medicine, Cairo University, Giza, Egypt<sup>d</sup> Department of Health Science, College of Applied Studies and Community Service, King Saud University, Saudi Arabia<sup>e</sup> Department of Biology, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah, Saudi Arabia<sup>f</sup> Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, University of Sadat City, Egypt<sup>g</sup> Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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## ABSTRACT

This study was conducted on 100 one-day-old broiler chicks to evaluate the effect of Poulvac *E. coli* vaccine in reduction of clinical signs and complications after concurrent infectious bronchitis virus (variant 02) and virulent *E. coli* O78 challenges. The birds were evaluated for clinical signs, mortality for 7 days post-infection, PM lesion score, average body weight and serological evaluation. Re-isolation and RT-PCR for the challenging infectious bronchitis virus (IBV) variant 02 were conducted thereafter. The results showed that the Poulvac *E. coli* at one-day old chicks in the presence of co-infection with virulent *E. coli* and IBV variant 02 provides better body weight gain at 35 days than the other groups. The challenge with IBV variant 02 alone in non-vaccinated birds doesn't give any mortality; this indicated that the severity of IBV variant 02 increased by the presence of co-infection with Avian Pathogenic *E. coli* (APEC). The mortality percentage associated with both *E. coli* and IBV variant 02 infections in the none vaccinated group by Poulvac *E. coli* was 25% while this percentage was 10% of the vaccinated group. The Poulvac *E. coli* is not negatively affecting the immune response against different concurrent viral vaccines like Infectious bursal disease (IBD), and moreover, it improves the immune response against some others like Newcastle disease virus (NDV), Avian Influenza (AI) H5 and IBV.

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## 1. Introduction

Broiler industry is facing several challenges from viral and bacterial infections; the most common in the Middle East (ME) is the Respiratory Complex Syndrome, where APEC plays an important role in the severity of this syndrome. Morbidity of IBV has been almost always 100%, but mortality can vary between 0% and 82%, depending on the age and the immune status of the birds, the

strain of the virus, and if secondary bacterial or viral pathogens are involved (Jackwood and De wit, 2013). Mixed infection of avian respiratory viruses, including IBV may induce similar clinical signs/lesions and thus complicate diagnostic decisions (Nguyen et al., 2013), as well as complicating its control.

Viral infections of the respiratory tract, such as IBV infection, facilitate the pathway of both colibacillosis of the respiratory tract and systemic colibacillosis (Nakamura et al., 1992; Peighambari et al., 2002; Matthijs et al., 2005) and thus resulting in increased severity and mortality associated with the disease. The mechanisms behind enhanced susceptibility to bacterial super-infection after viral infection have been studied extensively, but are still not well understood. A first set of hypotheses suggests increased susceptibility due to tissue damage in the respiratory tract resulting in functional impairment. Three possible causes have been described as mechanisms for functional damage. Viral replication in the upper respiratory tract causes loss of cilia and ciliated cells

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(Bakaletz, 1995), decreased ciliary activity impairs mucociliary clearance (Wilson et al., 1996) and finally, damage to epithelium may provide more attachment sites for bacteria (El Ahmer et al., 1999). A second set of hypotheses suggests altered innate immune responses. Impairment of innate effector functions, i.e. adhesion and entry, phagocytosis, killing, nitric oxide (NO) and superoxide production (Ariaans et al., 2008). So this study came as a response to evaluate the effect of the commercially available Poulvac *E. coli* vaccine in reduction of complications and clinical signs associated with IBV variant 02 infection and to evaluate the effect of the vaccine on the immune response of concurrent viral vaccines being used in broilers.

## 2. Materials and methods

### 2.1. Experimental chickens

One hundred of one-day old broiler chicks were floor reared under strict hygienic condition, in a previously cleaned and disinfected experimental unit. The chicks were provided with commercial broiler ration, water and feed were provided ad libitum. The chicks were vaccinated with the common vaccination program applied in the ME; all chicks were vaccinated at day old with inactivated H5N1 reassortant vaccine (Egyflu prepared from A/ch/Egypt/A-18-H-09 strain, Harbin weike biotechnology CO., China.), and inactivated oil emulsion NDV-vaccine (OL-VAC, prepared from La Sota strain, FATRO CO., Italy), Poulvac IB primer and Poulvac NDW (Zoetis) were applied by coarse spray. At 14 days old birds were vaccinated with Nobilis, Ma5 + Clone 30 (MSD), for control of IBD, Bursine<sup>®</sup>2 (Zoetis) was applied in drinking water at 8 and 16-day old. The chicks were divided into 5 groups (20 each) according to Poulvac *E. coli* vaccination scheme and APEC and IBV variant 02 challenges (Table 1).

### 2.2. Challenging virus and bacteria

The IBV variant 02 strain used in the challenge was obtained from (MEVAC company for vaccine production, Egypt), (EG/1212B, accession no. JQ839287.1). While the strain of Avian pathogenic *E. coli* O78 was isolated from outbreaks of colibacillosis, typed serologically and identified for virulence factors, by Microbiology Department, Faculty of Veterinary medicine, University of Sadat City.

### 2.3. Clinical examination and necropsy

All chicks in the different experimental groups were observed for clinical signs of IBV and colibacillosis. PM examination was done according to the established system outlined by Peighambari et al. (2002) with recordings of the characteristic lesions for IBV/colibacillosis infection, including mucosal thickening with serous or

catarrhal exudates in the nasal passage, sinuses and trachea. Presence of cloudy air sacs, which may contain caseous exudates, caseous material in the abdominal cavity, swollen and pale kidneys, with tubules and ureters distended with urates.

### 2.4. Antigens

The heterologous antigen of inactivated H5N2 Influenza vaccine (A/chicken/Mexico/232/94/CPA) was used in the Haemagglutination (HA) & Haemagglutination Inhibition (HI) test to evaluate the antibody titers for AI H5 while Live la Sota vaccine titrated and used as antigen for HA& HI to evaluate the antibody titers for NDV.

### 2.5. Blood samples

Chicken blood samples were collected from wing vein or by slaughtering and kept in slop position at 37 °C for one hour then at 4 °C overnight. Sera then separated by centrifugation at 3000 rpm/10 min and stored at –20 °C till tested, sera were used for detection of specific IBV, IBD antibodies using ELISA (Synbiotic) and specific H5N1 and NDV antibodies using HI test according to OIE (OIE terrestrial manual, 2008).

### 2.6. RT-PCR for IBV variant 02 S1 gene

The RT-PCR was carried out the 3 days post challenge on tracheas, 5 and 7 days post challenges on kidneys for detection of IBV (variant 02), according to the manufacturer's instructions using reagents provided in the kits; Thermo scientific. The amplified region of the RNA of the IBV (variant 02) was analyzed by gel electrophoresis according to Adzhar et al. (1997).

### 2.7. Sequencing of the amplified part of the IBV S1 gene

The RT-PCR product of positive samples were sent to (lab technology) for sequencing the amplified part (464 bp) of the S1 gene. Sequence chromatograms are edited using Mega 5 software. Edited sequences of IBV isolates were characterized using BLASTn for nucleotide or BLASTp for protein analysis (<http://www.ncbi.nlm.nih.gov/BLAST/>) (OIE Terrestrial Manual, 2013).

## 3. Results

### 3.1. Clinical signs

The clinical signs observed after IBV challenge were mild respiratory manifestations (ruffled feathers, rales, gasping, nasal discharge and diarrhea). The severity was increased in the groups challenged by Avian Pathogenic *E. coli* 3 days post challenge,

**Table 1**  
Concurrent infectious bronchitis virus (variant 02) and *E. coli* O78 challenges.

Group no.	Chick no.	Vaccination (Age/day)		Challenge (Age/day)	
		IBV <sup>a</sup>	Poulvac <i>E. coli</i> <sup>b</sup>	IBV <sup>c</sup>	<i>E. coli</i> <sup>d</sup>
1	20	1 + 14	1	25	28
2	20	1 + 14	–	25	28
3	20	–	–	25	–
4	20	1 + 14	–	–	–
5	20	–	–	–	–

<sup>a</sup> IBV vaccination dose at 1-day old by coarse spray (Poulvac IB primer) & at 14 days (Ma5) according to the company instructions.

<sup>b</sup> *E. coli* vaccination dose at 1-day old by coarse spray with Poulvac *E. coli* according to the company instructions.

<sup>c</sup> IBV challenge virus. Oculonasal challenge at 25 day of age with 100 µl/bird from 10<sup>6.2</sup> EID<sub>50</sub> per ml of IBV variant 2.

<sup>d</sup> *E. coli* challenge bacteria. Intratracheal challenge at 28 day of age with 1 ml/bird 10<sup>9</sup> CFU per ml *E. coli* O78.

especially the enteric signs while the non-challenged groups were apparently healthy.

### 3.2. Average body weight

The average body weight was evaluated at the 23, 30 and 35 days of age. In the 1st group was 1.3 kg, 2.04 kg and 2.26 kg, respectively, while in the 2nd group 1.3 kg, 1.83 kg and 2.14 kg, respectively, and in the 3rd group 1.11 kg, 1.7 kg and 2 kg, respectively, in the 4th group 1.33 kg, 1.88 kg and 2.1 kg, respectively, and the 5th group 1.1 kg, 1.92 kg and 2.2 kg, respectively.

### 3.3. Mortality and PM lesions

Mortalities were observed for one week after *E. coli* challenge. The mortality percentage were negative in non-challenged groups or group challenged with IBV only, but mortalities were 10% in the 1st group (vaccinated with IBV vaccines and Poulvac *E. coli* and challenged by IBV (variant O2) and Avian pathogenic *E. coli* O 78 and 25% in the 2nd group (vaccinated with IBV vaccines only and challenged with IBV (variant O2) and *E. coli* O 78 as shown in (Table 2). The P.M lesions score included cloudiness, turbidity or deposition of fibrin membrane on the liver, heart and air sacs for *E. coli* challenged groups were 3 and 3.2 in the 1st group (vaccinated by IBV and *E. coli* and challenged by IBV and *E. coli*) and the 2nd group (vaccinated by IBV and challenged by IBV and *E. coli*), respectively, as shown in Table 2.

### 3.4. Serological evaluation of antibody titers for AI H5 and NDV by HI

The antibody titers against avian influenza H5N1 virus was evaluated at 21, 27 and 37 days of age. The HI titer ( $\log_2$ ) for the 1st group was 3.2, 5.7 and 6.2, respectively, in the 2nd group was 4, 5.5 and 5.2, respectively, for the 3rd group, this titer was 4, 4.2 and 5.7, respectively, for the 4th group 4, 5.7 and 6, respectively, and the 5th group 4, 5 and 5.7, respectively. The antibody titers against NDV were evaluated at 21, 27 and 37 days of age. The HI titer ( $\log_2$ ) for the 1st group was 5.2, 5.7 and 6.2, respectively, for the 2nd group was 3.5, 5.5 and 5.2 respectively, for the 3rd group was 6, 5.7 and 6 respectively, the 4th group was 3.5,

### H5 HI titer (co-infection)

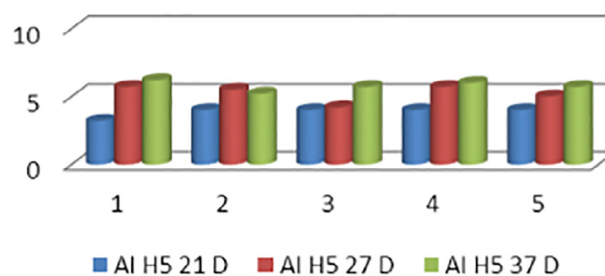


Fig. 1. HI Titers for AI H5 ( $\log_2$  GM) for different experimental groups at different ages.

5.5 and 5.7 respectively, the 5th group was 6, 5.7 and 5.2 respectively (Table 3 and Figs. 1 and 2).

### 3.5. Serological evaluation of antibody titers for IB and IBD by ELISA

The antibody titers against IBV evaluated by ELISA at 21, 27 and 37 days of age, and the result showed that the antibody titer for the 1st group was  $860 \pm 97$  and  $517 \pm 229$  at 27 and 37 days respectively, for 2nd was  $574 \pm 204$ ,  $417 \pm 117$  and  $1095 \pm 983$ , respectively, but, the 3rd group was  $433 \pm 73$  and  $2160 \pm 1733$  at 27 and 37 days of age, respectively, for the 4th group was  $574 \pm 204$ ,  $596 \pm 228$  and  $1520 \pm 1536$ , respectively. Antibody titers against IBDV were evaluated by ELISA at 21, 27 and 37 days of age, for the 1st group was  $4162 \pm 1172$  and  $2978 \pm 581$  at 27 and 37 days respectively, for the 2nd was  $4204 \pm 746$ ,  $3039 \pm 850$  and  $3486 \pm 427$ , respectively, for the 3rd group was  $2864 \pm 604$  and  $4145 \pm 561$  at 27 and 37 days, respectively, for the 4th group was  $4204 \pm 746$ ,  $4311 \pm 502$  and  $2464 \pm 785$  at 21, 27 and 37 days of age, respectively (Table 4).

### 3.6. Results for re-isolation of IBV var 2 and *E. coli* post challenge

Avian Pathogenic *E. coli* was re-isolated according to Murray et al. (19), 3 and 5 days post challenge from the heart, blood, liver

Table 2  
Post mortem lesion score and mortality rate in different experimental groups.

Group no.	Chick no.	Vaccination age/day		Challenge Age/day		BW ratio Age/day			Mortality %	PM lesion score
		Poulvac <i>E. coli</i>	IBV	IBV	<i>E. coli</i>	23	30	35		
1	20	1	1 + 14	25	28	1.334	2.04	2.26	10	3
2	20	-	1 + 14	25	28	1.334	1.83	2.14	25	3.2
3	20	-	-	25	-	1.105	1.7	2	0	ND
4	20	-	1 + 14	-	-	1.334	1.88	2.1	0	ND
5	20	-	-	-	-	1.105	1.92	2.2	0	ND

Table 3  
The results of HI for (AI H5 and NDV) for different experimental groups at different ages.

Group no.	Chick no.	HI Titer ( $\log_2$ ) GM age/day					
		AI H5			NDV		
		21	27	37	21	27	37
1	20	3.2	5.7	6.2	5.2	5.7	6.2
2	20	4	5.5	5.2	3.5	5.5	5.2
3	20	4	4.2	5.7	6	5.7	6
4	20	4	5.7	6	3.5	5.5	5.7
5	20	4	5	5.7	6	5.7	5.2

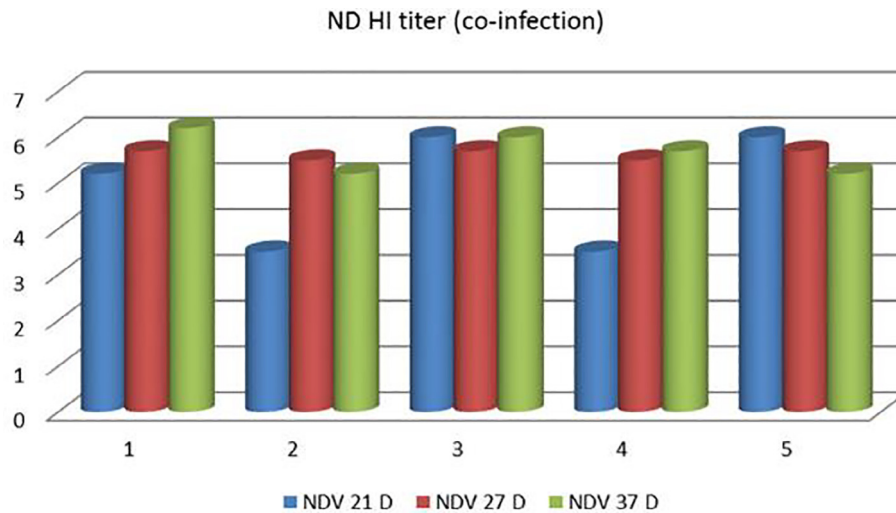


Fig. 2. HI Titers for NDV (log<sub>2</sub> GM) for different experimental groups at different ages.

Table 4

The results of ELISA titers for IBV and IBD for different experimental groups at different ages.

Group no.	Vaccination age/day		Challenge age/day		ELISA Titer mean ± SD					
	Poulvac <i>E. coli</i>	IBV	IBV	<i>E. coli</i>	IBV			IBD		
					21	27	37	21	27	37
1	1	1 + 14	25	28	ND	860 ± 97	517 ± 229	ND	4162 ± 1172	2978 ± 581
2	–	1 + 14	25	28	574 ± 204	417 ± 117	1095 ± 983	4204 ± 746	3039 ± 850	3486 ± 427
3	–	–	25	–	ND	433 ± 73	2160 ± 1733	ND	2864 ± 604	4145 ± 561
4	–	1 + 14	–	–	574 ± 204	596 ± 228	1520 ± 1536	4204 ± 746	4311 ± 502	2464 ± 785

Table 5

Results of RT-PCR for IBV Variant O2.

Group no.	Vaccination age/day		Challenge age/day		IBV RT-PCR			
	Poulvac <i>E. coli</i>	IBV	IBV	<i>E. coli</i>	Trachea 3 days post challenge	Kidney		
						5 days post challenge	5 days post challenge	
1	1	1 + 14	25	28	+	+	+	
2	–	1 + 14	25	28	+	+	+	
3	–	–	25	–	+	+	+	
4	–	1 + 14	–	–	ND <sup>a</sup>	–	–	
5	–	–	–	–	ND	–	–	

<sup>a</sup> Not detected.

parenchyma, air sacs and gall bladder in *E. coli* challenged groups and then the colonies tested for agglutination against O-78 specific antisera and all groups were positive for this strain. Re-isolation of IBV was performed from trachea 3-day post challenge and kidneys 5 and 7 days post challenge by intrallantoic inoculation in 9–11 days SPF eggs, the allantoic fluid was collected after 48 h from inoculation and examined by RT-PCR, all samples from all groups were RT-PCR positive (Table 5).

#### 4. Discussion

Many viral and bacterial diseases are affecting the broiler industry in Middle East causing severe economic losses by billions of dollars per year. Poultry meat represents more than 70% from protein eaten by humans, so controlling such diseases and minimizing

the mortality rates is very essential to save both the human and animal health (FAO, 2002). It is commonly believed that in broiler chickens maintained under commercial conditions the lesions caused by the IBV are sufficiently severe to permit *E. coli* to invade the affected tissues and give rise to generalized infection and often with high mortality. There is an evidence to support this view in that a condition resembling the natural disease can be produced by inoculating chickens with a mixture of IBV and *E. coli* (Smith et al., 1985).

In this study 100 one-day old broiler chicks were floor reared under strict hygienic condition, divided into 5 groups (20 chicks each) for studying the effect of virulent *E. coli* challenge in the severity of IBV infection and the role of Poulvac *E. coli* in reduction of the severity of IBV disease, especially that is caused by IBV variant O2 which is endemic in ME. The chicks in experimental group 1 were vaccinated with Poulvac *E. coli* vaccine and commercially



available IBV vaccines and challenged for both virulent *E. coli* O78 and IBV variant O2, while those in group 2 were vaccinated with commercially available IBV vaccines and challenged by both virulent *E. coli* O78 and IBV variant O2. Chicks in group 3 were not vaccinated while challenging by IBV variant O2 only. Chicks in group 4 were vaccinated with IBV only. Chicks in group 5 were neither vaccinated nor challenged. In the present study the effect of Poulvac *E. coli* in reduction of the severity of the co-infection of IBV and *E. coli* was evaluated on the basis of average body weight, mortality, PM lesion scoring, serology, clinical signs, histopathology and re-isolation of both *E. coli* and IBV.

The clinical signs observed after IBV variant O2 challenges were a mild respiratory manifestation (ruffled feathers, rales, gasping, nasal discharge and diarrhea) the severity were increased in the groups challenged with *E. coli* O78 3 days post challenge, especially the enteric signs, while non challenged groups were apparently healthy, these results are going in harmony with that of Smith et al. (1985). The previously mentioned results indicated that challenge with IBV variant O2 gave very mild disease reflected on the percentage of mortality which was clear in the group 3, non-challenged by *E. coli* O78 (0% Mortality). On the other hand, the mortality in groups co-challenged with *E. coli* O78 and IBV variant O2 varied according to the administration of Poulvac *E. coli* vaccine (10% in vaccinated group and 25% in non-vaccinated group) and these results were in agreement with the data mentioned by Jackwood (2012). The average body weight for the group 1, 2, 3, 4 and 5 were at 35 days old 2.26, 2.14, 2, 2.1 and 2.2 kg respectively. The results indicated that the vaccination against *E. coli* using Poulvac *E. coli* could reduce the effect of the challenge or natural infection occurred by Avian Pathogenic *E. coli* on average body weight as previously reported by La Ragione et al. (2013). The PM lesions score included cloudiness, turbidity or deposition of fibrin membrane on the liver, heart and air sacs for Avian Pathogenic *E. coli* challenged groups were 3 and 3.2 in groups 1 and 2 respectively. The reduction of the lesion score might be due to the protective effect of Poulvac *E. coli* vaccination at day old, these findings are supported by that reported by La Ragione et al. (2013).

The effect of Poulvac *E. coli* on the immune response to concurrent vaccination against AI H5, NDV, IBV and IBD have been evaluated through detection of the antibody titers at different ages against each particular agent by the standard serological tests. The serological evaluation for both AI H5 and NDV revealed that the HI titer (log 2) for the groups 1, 2, 3, 4 and 5 were at 37-day old for AI H5 6.2, 5.2, 5.7, 6 and 5.7 respectively and for NDV were 6.2, 5.2, 6, 5.7 and 5.2, respectively. Our reading of these results indicates that the application of Poulvac *E. coli* at day old concurrently with oily AI H5 + NDV vaccine and live NDV vaccine is not affecting the immune response to these vaccines, moreover, it showed improvement in the immune responses as it's clear in group 1 (vaccinated and challenged), these results are reported as well by Tobias et al. (2013). The ELISA antibody titer for IBV at 37 days were  $517 \pm 229$ ,  $1095 \pm 983$ ,  $2160 \pm 1733$  and  $1520 \pm 1536$  in groups 1, 2, 3 and 4 respectively, the significant differences in the antibody titers in the different groups are due to the effect of vaccinations/challenges differences. The high antibody titer in group 3 is resulted from challenging with IBV variant O2 in non IBV vaccinated birds. The application of Poulvac *E. coli* at day old improves the immune response against IBV challenge and this was clear in group 1 where we did not observe an increase in the antibody titers like that observed clearly in group 3, these results are closely similar to that reported in De wit et al. (1998, 2010). The ELISA antibody titers for IBD at 37 days were  $2978 \pm 581$ ,  $3486 \pm 427$ ,  $4145 \pm 561$  and  $2464 \pm 785$  in groups 1, 2, 3, and 4 respectively. Poulvac *E. coli* vaccine improves the early immune response at 27 days old for IBDV 4162+ than the non-vaccinated group 2 while no significant differences observed between the all

different groups at 37 days. Our readings indicate that the vaccine is not negatively affecting the immune response to IBD vaccination; these results were in agreement also with that mentioned in De wit et al. (1998, 2010) and Tobias et al. (2013).

## 5. Conclusions

It's concluded that Poulvac *E. coli* at day old aid in reduction of mortality from 25% to 10% in groups challenged by virulent *E. coli* O78 and IB variant O2 strain, Poulvac *E. coli* at one-day old together with IB primer at day old in the presence of co-infection with virulent *E. coli* and IBV variant O2 (group 1) provide better BW gain at 35 days than the all other non-vaccinated groups. The challenge with IB variant O2 alone in non-vaccinated birds didn't give any mortality (group 3); this indicated that the severity of IBV variant O2 is being increased by the presence of co-infection with APEC O78. Poulvac *E. coli* at day old is not negatively impacting the immune response to concurrently applied vaccines for AI H5, NDV, IBV and IBD and moreover improves the immune response even better than the non-vaccinated non challenged groups. Difference of epidemiological data between countries in poultry production industry requires efforts to position any veterinary drug or vaccine in a way to get the best effective results in terms of high body weight and low mortality rate, which will reflect on the animal health and consequently the human health.

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