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COMPARATIVE STUDY ON COMMERCIAL VACCINES AGAINST *E.COLI* IN BROILER CHICKENS

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Received: 5 March 2019; **Accepted:** 12 March 2019

ABSTRACT

E. coli infections in avian species have become an economic threat to the poultry industry worldwide. The objective of this study is to determine whether the immunization using commercially available living *Escherichia coli* vaccines as Nisseiken Avian Colibacillosis Vaccine (Nisseiken Co., Ltd., Ome, Tokyo, Japan) and O78 aroA deleted vaccine (Poulvac ® *E.coli*, Zoetis) are protective against APEC challenges or not. Ninety eight chicks (Arbor, Acres) of both sexes were divided into seven groups (14 birds/each); two groups were vaccinated at day 1 of age by spray route using Nisseiken Avian colibacillosis Vaccine, then one of them challenged intratracheally with homologous *E.coli* O78 and the other with heterologus O1 at day 14, the other two groups were vaccinated at day 1 of age by eye drop route using Poulvac ® *E.coli*, Zoetis vaccine then one of them challenged intratracheally with homologus *E.coli* O78 and the other with heterologus O1 at day 14. The other two groups were positive control (challenged, unvaccinated); one challenged with O78 and the other with O1 at day 14 using intratracheal route. The last group served as environmental control (non vaccinated, non challenged). At day 28, birds were necropised and examined to evaluate the efficacy of both of the two different vaccines. The best obtained results were recorded to the vaccinated challenged groups with the homologous and heterologous strains and vaccinated by spraying and eye drop methods which showed a decrease in organ lesion scores in comparison to the other groups (non-vaccinated, challenged broilers). These results suggest that the two different vaccines used in our study are efficient in reducing lesion scores against homologous and heterologous challenge using spray and eye drop methods that could lead to minimizing the time for treatment and cases of condemnation in processing plants.

Key words: APEC, broiler chickens, Vaccine, challenge, air sacculitis, pericarditis, perihepatitis.

INTRODUCTION

Colibacillosis is considered as one of the most important diseases affecting poultry industry worldwide. It leads to great economic losses every year. Economic losses are caused by increasing morbidity and mortality rates in poultry flocks, antibiotic treatment costs, reduced weight gains and numerous carcass condemnation at the abattoir (Barnes *et al.*, 2008; Mombarg *et al.*, 2014). Colibacillosis are responsible for many visible gross lesions as pericarditis, perihepatitis, air sacculitis, salpingitis, peritonitis, omphalitis, cellulitis, coligranuloma and osteomyelitis/arthritis (Barnes *et al.*, 1997).

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Most studies on colibacillosis refer to broilers, even though laying hens can also be severly affected (Zanella *et al.*, 2000). Serotypes O78, O1 and O2 are the most frequent isolates of APEC in the field (Dho-Moulin and Fairbrother, 1999; Lutful Kabir, 2010; Persoons *et al.*, 2011). Hence, for controlling the majority of avian colibacillosis infections; the vaccine must be able to induce protection against these three serotypes. Many trials have been made to develop an effective vaccine against colibacillosis in poultry. The poultry industry requires cheap vaccine, that can be applied easily and has adequate efficacy against virulent *E. coli* strains as well as wide narrow of safety (La Ragione *et al.*, 2013). Vaccines depend on the defined genetic deletion m.os. may be preferable candidates for live vaccines. Nisseiken Avian colibacillosis vaccine (Nisseiken Co., Ltd., Tokyo, Japan) is made up of 10^7–10^9 colony-forming-units (CFU)/dose of AESN1331 O78 APEC strain which has a delated crp gene and has been freeze-dried with skim-milk (Nagano *et al.*, 2012). Poulvac® *E. coli* is a defined aroA deletion mutant deficient for the biosynthesis of aromatic amino

acids constructed in an APEC O78:K80 strain (Mombarg *et al.,* 2014). Referred to information published about this vaccine (Poulvac® *E.coli*, Zoetis), it can develop sufficient protection against O78 as well as O1, O2 and O18. The aim of our study was conducted to evaluate the safety and the efficacy of both of the two different used vaccines against *E.coli* O78 (homologous strain) and O1 (heterologous strain) in broilers.

MATERIALS AND METHODS

Chickens

Ninety eight of 1 day old broiler chicks (Arbor, Acres) of both sexes and with an average weight of 40-45 gm were obtained from a commercial hatchery and grown over a 28 day experimental period. Food and water were provided ad libitum with no antibacterial or anticoccidial components. The same conditions of temperature, humidity and ventilation were adjusted. No antibiotics were administered during this experiment. Vaccination against diseases other than *E. coli* was applied. Birds were vaccinated against Newcastle disease at day 5 and 17 of age by eye drop route (Newcastle Cloned N-79 and lasota vaccines respectively). Chicks were also vaccinated at day 8 with Volvac® B.E.S.T (AI+ND) by injecting 0.5 ml per bird, subcutaneously in the middle third part back of the neck and they were vaccinated with Bursal Disease Vaccine (BURSA-VAC) at day 11 of age by eye drop route. Birds were divided in to 7 groups; group 1 and 2 were vaccinated with avian colibacillosis vaccine at day 1 by spray while the other two groups (3 and 4) were vaccinated with Poulvac® *E.coli*, Zoetis. By eye drop route. The remaining groups (5, 6 and 7) in contrast were not vaccinated with any type of the used *E.coli* vaccines (control groups). The experiment was done at Poultry Diseases Department, Faculty of Veterinary Medicine, Assiut University.

Nisseiken Avian Colibacillosis Vaccine

(Nisseiken Co., Ltd. 9-2221-1 Shin-machi, Ome, Tokyo 198-0024, Japan)

The vaccine is made up of 10^7–10^9 colony-forming-units (CFU)/dose of AESN1331 O78 APEC strain which has a delated crp gene and has been freeze-dried with skim-milk (Nagano *et al.,* 2012). The vaccine is dissolved in 100 to 300 ml physiological saline per 1000 doses, and administrated by a fine sprayer. All instructions were followed before and during vaccination according to manufacturer's instructions.

Poulvac® *E. coli*, Zoetis vaccine:

Poulvac® *E. coli* live vaccine containing as active substance aroA gene-deleted Escherichia coli, serotype O78, strain EC34195 was used. The freeze-dried vaccine was reconstituted and then diluted with distilled water according to the manufacturer's instructions and was administered by eye drop route. The correct diluent and size bottle was ensured (i.e. 1000ds / 35 diluent for 1000d vaccine vial) before usage. The diluents was sterile and contains a dye (usually blue) which is used to monitor the vaccine application. The vaccine was refrigerated and kept cold at 3 °c before use and while transferring to the place of experiment.

Challenge

Groups 1, 3 and 5 were challenged intratracheally with *E.coli* O1, and groups 2, 4 and 6 were challenged intratracheally with E.coli O78 on day 14 with 1 ml (containing $6x10^8$/bird). This dose of challenge has been determined according to (La Ragione *et al.,* 2013). Group 7 was the environmental control group (non-vaccinated, non-challenged). Birds in each group were monitored until the end of the experiment. In the event of mortality, necropsy of dead birds was carried out and macroscopic lesions were recorded. At the end of the experiment, (14 days after challenge), necropsy of all birds was done after euthanasia (table 1).

Table 1: Grouping chicks for efficacy of two different live attenuated *E.coli* O78 vaccines

Groups	Vaccination	Vaccination method	Vaccination date	Applied vaccine	Challenge with O1	Challenge with O78
Group 1	+	Spray	Day 1	Nisseiken	+	-
Group 2	+	Spray	Day 1	Nisseiken	-	+
Group 3	+	Eye drop	Day 1	Poulvac	+	-
Group 4	+	Eye drop	Day 1	Poulvac	-	+
Group 5	-	-	-	-	+	-
Group 6	-	-	-	-	-	+
Group 7	-	-	-	-	-	-

Mortality rate, clinical signs and necropsy findings were evaluated before and after challenge. Individual body weights were calculated at day 28 of age and feed conversion rate also were estimated which was defined as the total amount of feed consumed by each group between days 1–28 and

dividing it by the increase in mass of the chickens over the same time period (Rawiwet and Chansiripornchai 2009). Scores for gross pathologic findings were assigned as follows: air sacs (normal = 0, mild cloudiness and thickness = 1, moderate cloudiness and thickness accompanied by serous exudate or fibrin spots = 2, extensive cloudiness and thickness accompanied by muco- or fibrinopurulent exudate = 3), heart and pericardium (normal = 0, turbid with excessive or cloudy fluid in the pericardial cavity = 1, marked pericarditis = 2), and liver (normal = 0, slight amount of fibrinous exudate = 1, marked perihepatitis = 2) according to (Peighambari *et al.*, 2002).

Statistical analysis of results

The obtained data of body weight and lesion scores was analyzed using SAS system for estimating the significant differences between the groups.

RESULTS

Clinical signs

No obvious signs were noticed after vaccination with live attenuated E.coli O78 vaccines (Nisseiken avian colibacillosis or poulvac® *E.coli*, Zoetis vaccine). This means that both of the vaccines are safe to be used and have no unfavourable reactions on the bird. The clinical signs observed after challenge were more pronounced in group 5 and group 6 (ruffled feathers, gasping, nasal discharge, respiratory rales and diarrhea). In vaccinated groups of 1 and 3 (challenged with O1), signs were milder in comparison to group 5 (O1 challenged). In groups 2 and 4 (Vaccinated and challenged with O78), signs were milder than group 6 (O78 challenged). In group 7 (non-vaccinated, non-challenged), no obvious signs were noticed.

Weight gain:

The average body weight was evaluated at the day 28 and the results are showed in table (2) and figure (1). Weighing data analysis with SAS showed no significant difference between vaccinated groups, but there was a great difference between them and non-vaccinated ones (group 5 and 6). Group 5 (O1challenged) had significantly lower weight gain in comparison to vaccinated groups of 1 and 3 (O1 challenged). Group 6 (O78 challenged) had significantly lower weight gain than vaccinated ones of group 2 and 4 (O78 challenged). There was also a significant difference between group 7 and the others (best results of weight gain were obtained). Feed conversion rate results are summarized in table (3).

Table (2): Weighing data analysis with SAS for body weight (LSD 5%)

Group	Mean	t Grouping
Group 1	1542.29	BC
Group 2	1542.14	BC
Group 3	1546.43	BC
Group 4	1598.86	BA
Group 5	1410	C
Group 6	1419.29	C
Group 7	1721.86	A

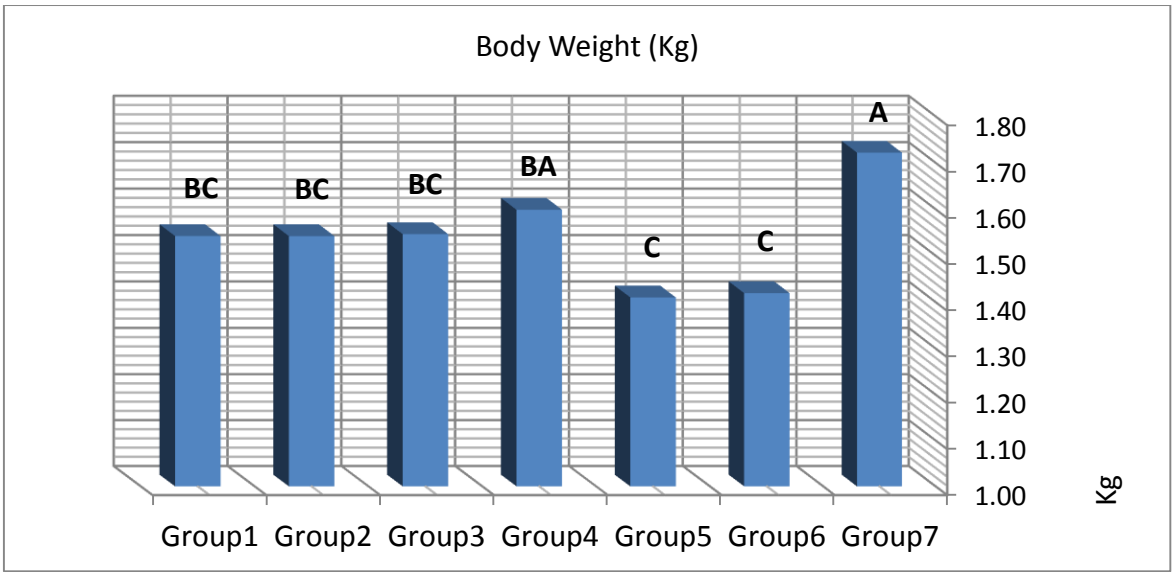

Fig. (1): Average body weights of groups

Table (3): Showing feed conversion rate of different groups

Groups	Total feed consumption	Total body weight	Feed conversion rate
Group 1	25.800 kg	21.592 kg	1.19
Group 2	27.260 kg	21.590 kg	1.26
Group 3	25.870 kg	21.650 kg	1.19
Group 4	27.240 kg	22.384 kg	1.216
Group 5	23.630 kg	19.740 kg	1.197
Group 6	22.460 kg	19.870 kg	1.13
Group 7	28.830 kg	24.106 kg	1.195

Necropsy examination results

Necropsy signs (air-sacculitis, pericarditis and perihepatitis) were evaluated and graded according to (Peighambari *et al.*, 2002). Necropsy data are described in table (4) and figures (2,3,4,5,6,7).

Table (4): Data analysis with SAS for differences between air sacs, heart and liver lesion scores of different groups (LSD 5%)

Means with the same letter are not significantly different

Groups	Air sacs			Heart and pericardium			Liver		
	N	Mean	t Grouping	N	Mean	t Grouping	N	Mean	t Grouping
Group 1	14	0.2857	B	14	0.2143	B	14	0.2143	B
Group 2	14	0.2143	B	14	0.1429	B	14	0.1429	B
Group 3	14	0.3571	B	14	0.2143	B	14	0.1429	B
Group 4	14	0.2857	B	14	0.2143	B	14	0.1429	B
Group 5	14	1.1429	A	14	0.8571	A	14	0.7857	A
Group 6	14	1.4286	A	14	1	A	14	0.9286	A

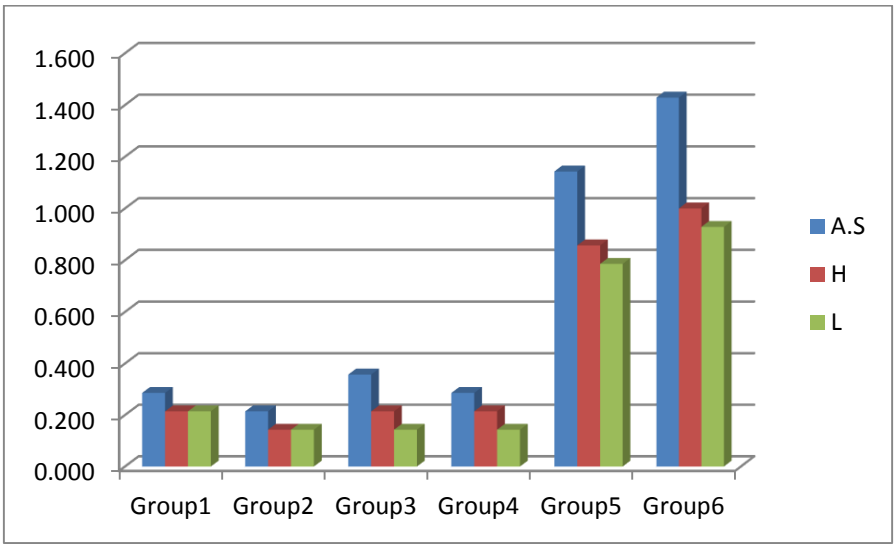

Fig. (2): Lesion scores of groups

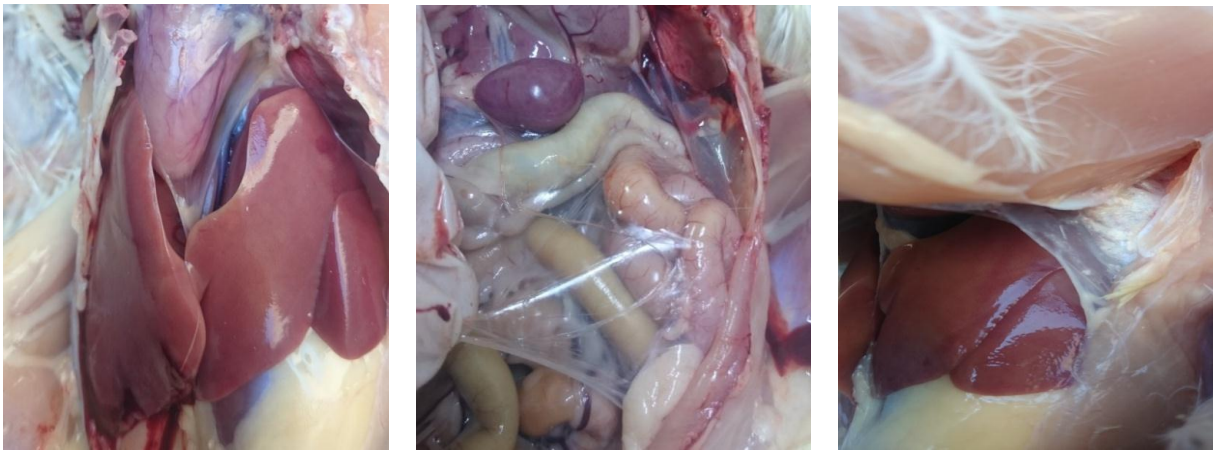

Fig. (3): 28 days old broiler chicken showing normal liver, heart and air sacs (vaccinated with Nisseiken avian colibacillosis vaccine and challenged with O78).

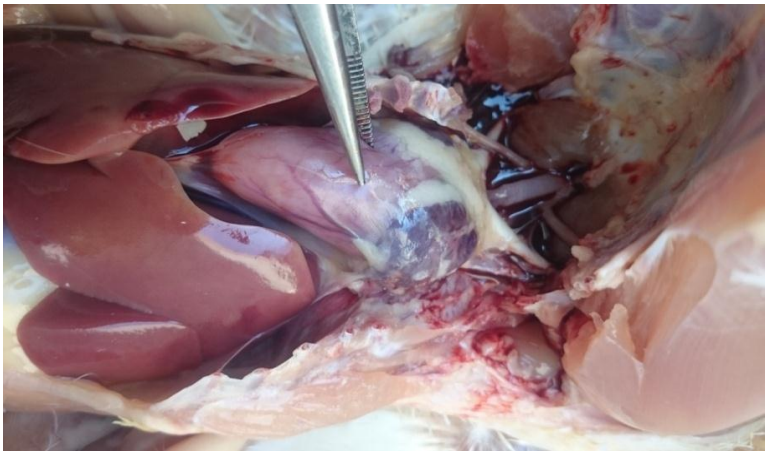

Fig. (4): 28 days old broiler chicken showing normal liver and heart (vaccinated with Poulvac ® *E.coli* and challenged with O78).

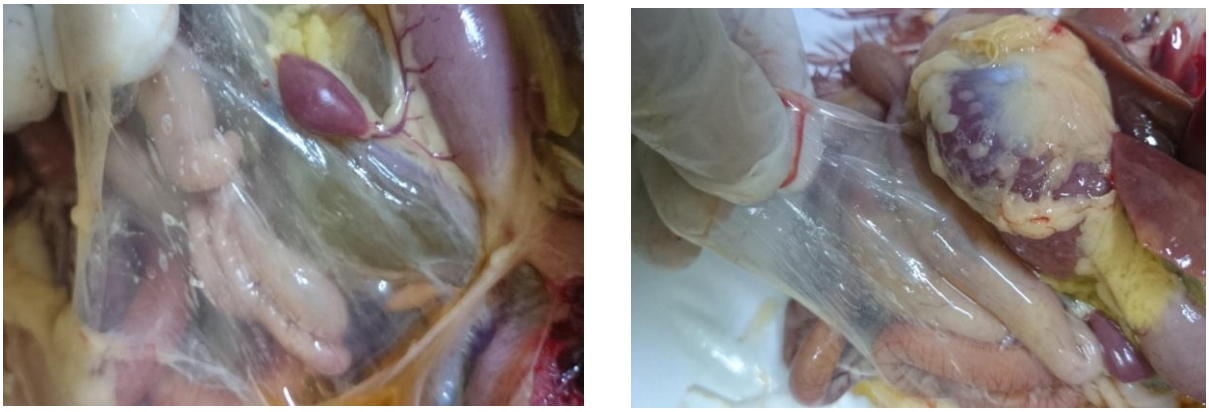

Fig. (5): 28 days old broiler chicken showing moderate thickening and cloudiness of air sacs (score 2); poulvac ® *E.coli* vaccinated and challenged with O1 and O78.

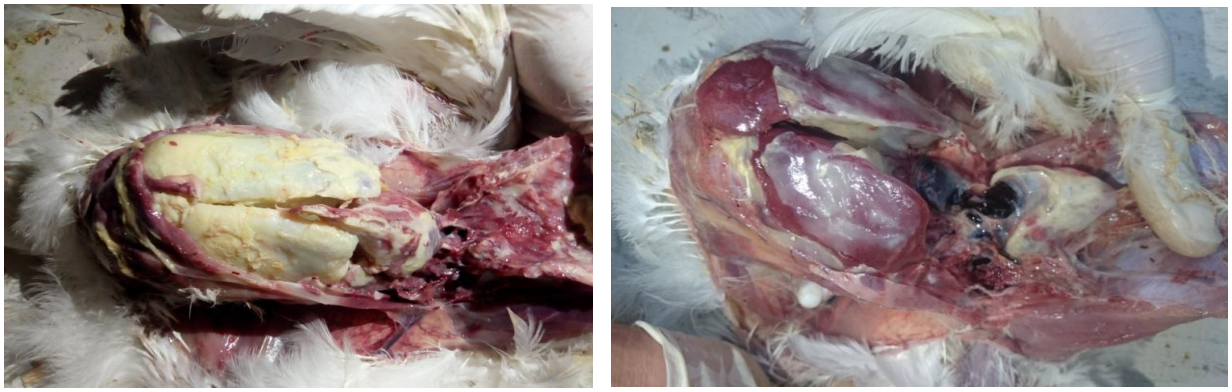

Fig. (6): 28 days old broiler chicken showing marked pericarditis and perihepatitis (score 2); positive control group (O1 and O78 challenged).

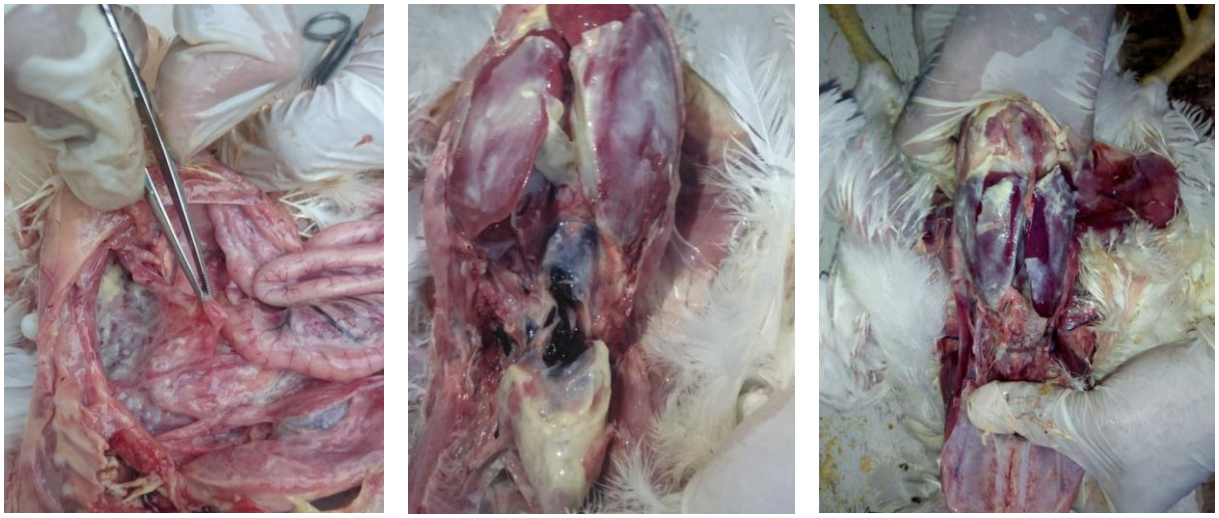

Fig. (7): 28 days old broiler chicken showing extensive air sacculitis (score 3) and marked pericarditis and perihepatitis (score 2); non-vaccinated and challenged birds.

DISCUSSION

No one can deny the great effect of colibacillosis on poultry industry in Egypt and world wide. Colibacillosis causes huge economic losses due to mortality, decreased feed conversion rate, carcass condemnations and costs spent in its control and prevention (Barnes *et al.,* 2008). In this study, we used two different commercial vaccines against E.coli (Live attenuated O78 *E.coli* vaccines) to measure their efficacy and differentiate between each one of them. One of them was given by spray route (nisseiken Avian Colibacillosis Vaccine), the other was given by eye drop route (poulvac ® *E.coli*, Zoetis vaccine) at 1 day of age. There were no obvious clinical signs attributed to the process of vaccination or unfavourable reactions recorded after giving the vaccine. Thus both of the two different vaccines used in the experiment are safe to be used in broiler chickens. These results were also recorded by others (La Ragione *et al.,* 2013; Mombarg *et al.,* 2014 and Mohamed *et al.,* 2016). Following challenge, the clinical signs (general and respiratory signs) were more obvious and severe in unvaccinated groups in comparison to vaccinated ones (milder signs) which means that the vaccine can decrease clinical signs. The same findings were also obtained by (Sadeghi *et al.,* 2018).

Based on mortality rate results, no meaningful difference was observed regarding mortality between vaccinated and non vaccinated broilers. Only one bird died in groups 2 and 5. Similar studies were made on current vaccine in Egypt, Thailand and Iran resulted in non significant difference between vaccinated and non vaccinated birds (Rawiwet and Chansiripornchai 2009; Mombarg *et al.,* 2014; Mohamed *et al.,* 2016), while (Fan *et al.,* 2008) indicated significantly lower mortality rate in vaccinated birds.

Regarding to weight gain, best results obtained in negative control group and there was no significant

difference between it and the vaccinated ones (1, 2, 3 and 4), also there was no difference between the vaccinated groups each other. There was significant difference between vaccinated and unvaccinated ones. This means that the vaccine gives best results in maintaining proper weight gain in comparison to non vaccinated groups.

Fernandes Filho *et al.* (2013) reported more weight gain in control group in the second and third week post vaccination. In contrast, (Salehi *et al.,* 2012; Mombarg *et al.,* 2014; Mohamed *et al.,* 2016 and sadeghi *et al.,* 2018) reported that there was no significant difference between vaccinated and unvaccinated groups.

For the necropsy findings of air sacs, pericardium and liver, there were no significant differences between vaccinated groups (group 1, 2, 3 and 4). By comparing groups 1 and 3 (vaccinated with two different vaccines and heterologously challenged with O1) with its equivalent group 5 (unvaccinated, challenged with O1), there was a major significant difference. This means that there was heterologous protection provided by both of the two different vaccines. The same result obtained when comparing groups 2 and 4 (vaccinated with two different vaccines and homologously challenged with O78) with its positive control group 6. This means that there was homologous protection achieved.

Both of the four vaccinated groups play an important role in minimizing lesions score of air sacs, heart (pericardium) and liver. This efficacy of spray method in reducing the lesion scores may be due to the living bacteria that delivered by spray, allowing stimulation of eye, conjunctiva, and bronchus-associated lymphoid tissues (Peighambari and Gyles, 1998; kariyawasam *et al.,* 2004; Chansiripornchai, 2009).

Some researchers were agreeable to our results as (La Ragione *et al.,* 2013; Mombarg *et al.,* 2014; Sadeghi *et al.,* 2018). They reported a decrease in gross visible lesions of colibacillosis in vaccinated birds.

It was showed that aroA deleted vaccine have a successful effect in controlling colibacillosis in chickens challenged with homologous APEC O78 and also against heterologous untypeable APEC strain by La Ragione *et al.* (2013).

Many research articles were disagreed to the results of our study, they said that vaccination against E.coli infection is not fully successful in chicken protection (Chaffer *et al.,* 1997; Peighambari *et al.,* 2002; Amoako *et al.,* 2004; Salehi *et al.,* 2012). It was reported by Mohamed *et al.* (2016) a reduction in gross lesions in homologous challenge only but not in heterologous challenge.

CONCLUSION

Nisseiken Avian Colibacillosis vaccine (Nisseiken Co., Ltd. Ome, Tokyo, Japan) and Poulvac® *E.coli*, Zoetis vaccine are safe to be used in poultry industry and had no undesired effect. Based on the results of this study, they showed an successful role in minimizing the severity of the lesions and clinical signs in vaccinated birds than those unvaccinated ones and consequently they may lead to decrease the economic losses spent every year in the farm.

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دراسة مقارنة على لقاحات الميكروب القولونى التجارية فى بدارى دجاج التسمين

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أصبحت عدوى الميكروب القولونى مهددا أقتصاديا لصناعة الدواجن على مستوى العالم. الهدف من هذه الدراسة هو تحديد ما اذا كان اللقاحات التجارية (لقاح بولفاك ولقاح نيسيكن)

Poulvac® E.coli vaccine, Zoetis and Nisseiken avian colibacillosis vaccine

لديها قدرة على صد العدوى أم لا. تم تقسيم عدد ٩٨ كتكوت (أربو أيكرز) من كلا الجنسين الى ٧ مجموعات (١٤ طائر/المجموعة)، تم تحصين مجموعتين منهم (١،٢) بواسطة لقاح نيسكين عند عمر يوم بواسطة الرش، ثم تم اصابة أحدهم عند عمر ١٤ يوم بواسطة عترة مماثلة للتحصين والاخرى بعترة مغايرة عن التحصين من خلال الحقن داخل القصبة الهوائية. المجموعتين التاليتين (٣،٤) تم تحصينهم بواسطة لقاح بولفاك عند عمر يوم بواسطة التقطير فى العين وتم أصابة أحدهم بواسطة عترة مماثلة للتحصين والمجموعة الأخرى بعترة مغايرة من خلال الحقن داخل القصبة الهوائية عند عمر ١٤ يوم. المجموعتين التاليتين (٥،٦) كانتا غير محصنين وتم اصابتهم بعترتين مختلفتين مثل التى استخدمتا فى التحصين عند عمر ١٤ يوم من خلال الحقن داخل القصبة الهوائية. المجموعة الأخيرة (٧) لم يتم اعطائها التحصين أو أصابتها. عند عمر ٢٨ يوم تم تشريح الطيور وفحصها جيدا لتقييم مدى كفاءة كلا من التحصينتين. تم تسجيل أفضل النتائج فى المجموعات المحصنة بواسطة الرش والتقطير فى العين والتى تمت أصابتها بعترة مماثلة ومغايرة على عكس المجموعات الغير محصنة. هذه النتائج أكدت مدى أهمية كلا من اللقاحين المستخدمين فى تقليل الافات الباثولوجية ضد الاصابة المماثلة والمختلفة لعترة التحصين مما يترتب عليه تقليل الوقت اللازم للعلاج وكذلك تقليل حالات الطيور الغير مقبولة فى المجزر.