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Cross Immunity of a Sonicated Trivalent Avian Colibacillosis Vaccine to Pathogenic Escherichia coli O26 and O78 Strains in Broiler Chickens

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ABSTRACT

Colibacillosis outbreaks are a global issue affecting the poultry industry. There is no cross-immunity among the strains of APEC. If a vaccine induces cross-immunity, it will play a key role in preventing colibacillosis. Herein, a sonicated trivalent colibacillosis vaccine containing O78:K80, O2:K1, and O1:K1 serotypes, with Alum as an adjuvant, was used to assess cross-immunity against E. coli O26. Ninety-six broiler chickens were randomly assigned to four groups: Group A was vaccinated and exposed to O78; Group B was unvaccinated but exposed to O78; Group C was vaccinated and exposed to O26; Group D was unvaccinated but exposed to O26. At 14 days old, chickens in groups A and C received a single dose of the vaccine, while groups B and D received normal saline subcutaneously. At 35 days old, all groups were challenged with O78 and O26 as described above. Clinical signs and lesions, isolation of the bacterium, weight gain, food intake, FCR, and antibody titers against the O antigens of the vaccine strains and O26 were recorded. The results indicated that 2 weeks post-vaccination, titers to the O antigens of the vaccine strains were significantly higher in the vaccinated groups than in the unvaccinated groups ($p \leq 0.05$). Following the challenge, no significant difference was observed in food intake and FCR between the groups ($p > 0.05$); however, the growth rate in group A was significantly higher than in group B ($p \leq 0.05$). At 42-49 days old, the vaccinated groups had the highest growth rate, which was statistically significant compared to the unvaccinated groups; and FCR in group A was significantly better than in group B ($p \leq 0.05$). In conclusion, it appears that in addition to homologous immunity, the vaccine also induces cross-immunity against O26.

Keywords

Colicepticemia, Heterologous, Homologous, Prevention, Poultry

Abbreviations

APEC: Avian Pathogenic Escherichia coli
FCR: Feed Conversion Ratio

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Introduction

Colibacillosis, especially colisepticemia as the acute form, is one of the most common bacterial diseases in the poultry industry with economic losses worldwide [1-3]. Colibacillosis which is caused by APEC, often occurs simultaneously with other diseases and due to predisposing factors, such as avian vaccination, particularly infectious bronchitis vaccines, and stress [2, 4]. Several methods have been applied to prevent and control colibacillosis, such as better management of the litter, improving drinking water, and better ventilation in farms, and also using antibiotics, bacteriophages, and nutrient synergy [1, 2, 5]. Despite these efforts, incidences and economic losses due to colibacillosis continue in poultry houses all over the world. Several studies indicated that colibacillosis can be prevented in poultry by vaccination and different vaccines, including inactivated, live, recombinant, mutant, and molecular vaccines, which have been prepared and tested experimentally [1, 6, 7]. The great diversity among APEC serogroups and the different mechanisms and stages of infection by the serogroups [1] are the main reasons vaccines have not been able to induce cross-immunity. Melamed et al. reported that by using the ultrasonic inactivation method for preparing an inactivated vaccine against APEC infection a certain degree of heterologous protection is possible because of the expression of some of the important internal immunogenic determinants [7].

Although APEC has diverse serogroups, the ones isolated from diseased birds in most countries are O78, O2, and O1 [1, 2, 7]. Moreover, we know that antigen particulates can increase the activation of antigen-presenting cells [8]. Alum [9], if used as an adjuvant, may enhance the efficacy of a vaccine because of its particulate form. It may also enable the use of a potential inactivated colibacillosis vaccine in broiler chickens due to its safety [8]. *E. coli* O26 strain is one of the APECs that has been isolated from chickens in Iran and other countries [10-12].

In the present study, we used a sonicated trivalent avian colibacillosis vaccine, including the O2:K1, O78:K80, and O1:K1 serotypes of APEC to evaluate the cross-immunity of this vaccine against O26 strain in broiler chickens.

Result

Clinical signs, gross lesions, mortality rate, and isolation of E. coli

Subsequent to the challenge, both the vaccinated and unvaccinated cohorts displayed a period of lethargy and listlessness, spanning approximately one day. Notably, avian subjects within the unvaccinated clusters, especially group B which was challenged with the O78 strain, exhibited more pronounced clinical signs. Dullness, lethargy, lack of movement, decreased appetite, hanging of stool on the anus, diarrheal stool, green stool in the bedding, and decreased reaction to movements were observed.

A chicken, that died in the unvaccinated group 8 days after challenge with the O78 strain and also three other euthanized chickens from different groups had gross lesions, including septicemia, pericarditis, perihepatitis, peritonitis, breast blister, emaciation, and airsacculitis (Table 1). *E. coli* bacteria were isolated from the liver and heart blood of two dead chickens that belonged to the unvaccinated group challenged with the O78 strain.

Growth rate

The analysis indicated no significant difference in weight gain between the examined groups during the periods of 14, 14-21, 21-28, and 28-35 days. However, a significant difference was observed among the groups in the age period of 35-42 days. The vaccinated group challenged with O78 exhibited the highest weight gain, while the unvaccinated group challenged with O78 demonstrated the lowest weight gain ($p \leq 0.05$). Furthermore, in the age period of 42-49 days, there was a significant difference between vaccinated and unvaccinated groups challenged with O26 and also between vaccinated and unvaccinated groups challenged with O78 ($p \leq 0.05$) (Table 2).

Feed consumption

Before the challenge, across some age periods, various groups exhibited equivalent consumption patterns, and from a statistical standpoint, no noteworthy distinctions were identified between the analyzed cohorts. After the challenge, at 35-42 days old, food consumption in group B decreased, but there was no significant difference with other groups ($p > 0.05$) (Table 3).

Feed conversion ratio

During the age intervals of 14-21, 21-28, 28-35, and 35-42 days, no statistically significant differences were observed between the examined cohorts. However, a notable disparity in FCR emerged during the 42-49-day period. The unvaccinated group challenged with O78 exhibited the highest FCR, whereas the vaccinated group exposed to O26 demonstrated the most favorable FCR outcome (Table 4).

Microagglutination test to O antigen

Before vaccination, antibodies to the O antigens

Table. 1
Post-mortem lesions and bacteria titers in the vaccinated broiler chicks challenged with O26 and O78

No	group	Autopsy symptoms	Isolation of *E.coli*
1	Carcass belonged to the vaccinated group challenged with O78	Perihepatitis	-
2	Carcass belonged to the unvaccinated group challenged with O78	Breast blister, emaciation, pericarditis, perihepatitis	+
3	Carcass belonged to the vaccinated group challenged with O26	Breast blister, pericarditis	-
4	Deceased carcass belonged to the unvaccinated group challenged with O78	Septicemia, pericarditis, airsacculitis	+

- Isolation of *E. coli* was negative
+ Isolation of *E. coli* was positive

Table. 2
Mean growth rate (g) of the chickens of different ages vaccinated and unvaccinated and challenged with O26 and O78

groups[1]	Age (day-old)					
	14	14-21	21-28	28-35	35-42	*42-49*
A	288.25 ± 4.77	328.41 ± 9.82	436.71 ± 13.30	498.16 ± 21.45	431.12 ± 41.00b	515.70 ± 41.44bc
B	307.21 ± 7.52	344.25 ± 10.62	440.00 ± 14.20	505.66 ± 24.00	284.25 ± 23.06a	*398.25 ± 46.65a*
C	286.37 ± 8.76	309.10 ± 9.48	426.16 ± 14.00	531.54 ± 13.08	389.00 ± 20.49ab	586.60 ± 33.30c
D	289.54 ± 6.12	328.54 ± 9.07	448.21 ± 11.00	507.08 ± 10.60	382.37± 15.32a	464.41 ± 34.31ab
P-value	**0.13**	**0.09**	**0.70**	**0.60**	**0.002**	**0.008**

1 The chickens of groups A and C received vaccine, while the chickens of groups B and D received sterile normal saline. Groups A and B were challenged with the O78:K80 strain, and groups C and D with the O26 strain.
*The data shown with different letters are significantly different.

Table 3.
Mean Feed consumption ± standard error of the chickens of different ages vaccinated and unvaccinated and challenged with O26 and O78

groups[1]	Age (day-old)				
	14-21	21-28	28-35	35-42	42-49
A	502.53 ± 2.00	758.33 ± 4.16	937.50 ± 0.00	887.50 ± 0.00	1225.00 ± 0.00
B	502.60 ± 1.80	760.43 ± 2.06	937.50 ± 0.00	800.00 ± 25.00	1314.26 ± 57.13
C	500.03 ± 9.53	762.53 ± 3.60	937.50 ± 0.00	862.50 ± 19.09	1257.13 ± 57.13
D	509.60 ± 1.80	760.43 ± 2.06	937.50 ± 0.00	865.83 ± 28.33	1257.13 ± 57.13
P-value	**0.46**	**0.82**	**1**	**0.08**	**0.65**

[1]A and C are the vaccinated groups challenged 16 and 26 days post-vaccination, respectively. B and D are the unvaccinated groups challenged 16 and 26 days post-vaccination, respectively.
*The data shown with the same letter are not significantly different.

of the vaccine strains were negative. Statistical analysis revealed significant differences between the examined groups on days 28 and 42 ($p \leq 0.05$). On 28 days, the vaccinated group challenged with O78 exhibited the highest titer, while the unvaccinated group challenged with O78 demonstrated the lowest titer. Similarly, at the age of 42 days, the vaccinated group treated with O26 displayed the highest microagglutination rate, whereas the unvaccinated group treated with O26 exhibited the lowest microagglutination rate (Table 5).

Table. 4
Mean FCR ± standard error of the broiler chickens of different ages vaccinated and unvaccinated and challenged with O78 and O26

groups[1]	Age (day-old)				
	14-21	21-28	28-35	35-42	42-49
A	1.54 ± 0.08	1.70 ± 0.02	1.86 ± 0.11	2.06 ± 0.09	2.64 ± 0.11ab
B	1.48 ± 0.02	1.73 ± 0.03	1.90 ± 0.06	2.87 ± 0.32	3.33 ± 0.31c
C	1.61 ± 0.02	1.80 ± 0.07	1.76 ± 0.03	2.25 ± 0.18	2.15 ± 0.13a
D	1.56 ± 0.08	1.70 ± 0.01	1.86 ± 0.11	2.27 ± 0.02	2.73 ± 0.20ab
P-value	**0.50**	**0.38**	**0.11**	**0.15**	**0.02**

[1] The chickens of groups A and C received vaccine, while the chickens of groups B and D received sterile normal saline. Groups A and B were challenged with the O78:K80 strain, and groups C and D with the O26 strain.
*The data shown with the same letter are not significantly different.

Table. 5
Geometric mean antibody titers to the O antigen of the O2, O78, O1, and O26 strains of APEC in the vaccinated broiler chickens

groups[1]	Age (day-old)		
	21	28	42
A	0.85 ± 0.21	4.25 ± 0.27b	8.2 ± 0.36b
B	0.65 ± 0.15	2.41 ± 0.25a	7.78 ± 0.82b
C	0.45 ± 0.15	3.5 ± 029b	9.59 ± 0.33c
D	0.54 ± 0.15	2.5 ± 0.25a	5.5 ± 0.89a
P-value	**0.43**	**0.000**	**0.000**

[1] The chickens of groups A and C received vaccine, and the chickens of groups B and D received sterile normal saline. Groups A and B were challenged with the O78:K80 strain, and groups C and D with the O26 strain.
*The data shown with the same letter are not significantly different.
*The data shown with the same letter are not significantly different.

Discussion

There is a need for an ideal APEC vaccine that can provide cross-protection against multiple APEC serotypes [13]. The results of the present study indicated that the alum adjuvanted-sonicated trivalent avian colibacillosis vaccine can induce homologous and heterologous immunity 21 days post-vaccination in a single dose. After the challenge, the incidence of the clinical signs and gross lesions decreased in the vaccinated challenged groups.

Common gross lesions of colibacillosis in broiler chickens are pericarditis, perihepatitis, and airsacculitis [1]. According to a study in Egypt (2021), chickens infected with *E. coli* strains O78 and O26 exhibited pronounced clinical manifestations compared to those infected with other strains. Consequently, these two strains are noteworthy for their potential to induce colibacillosis in poultry [14]. In this study, following challenges, in contrast to the challenged vaccinated group, the dead chickens of the unvaccinated group challenged with O78 typically developed pericarditis, perihepatitis, and airsacculitis, with *E. coli* bacteria isolated from the carcasses. Colibacillosis is characterized by variable polyserositis, but none of them are pathognomonic signs, and isolation of APEC is needed for diagnosis [2, 3, 15]. The challenge of chickens with O26 in both unvaccinated and vaccinated groups, despite the incidence of clinical signs, did not lead to mortality, which may be due to the low pathogenicity of the O26 strain. APEC has numerous strains with widespread pathogenic bacteria in poul-

try [16].

The weight loss of affected chickens is one of the reasons for the economic importance of colibacillosis. Colibacillosis causes the affected chickens to lose about 84 g/bird of average body weight [3]. The present study showed that the weekly mean body weight gain of challenged unvaccinated chickens in both groups which were challenged with O78 and O26 significantly decreased in comparison with the challenged vaccinated groups ($p \leq 0.05$). Colibacillosis reduces feed intake in affected chickens [17]. In this study food consumption insignificantly dropped immediately after a challenge on 35-42 days old. However, the chickens in all groups were compensated throughout the study.

Avian colibacillosis usually increases FCR [18]. The results of FCR at 42-49 days old indicated that the increase in food consumption in the vaccinated groups, especially in the vaccinated group challenged with O78, significantly raised the body weight of the chickens. The significant difference in FCR between the vaccinated group challenged with O78 and the unvaccinated group challenged with O78 means that although food intake increased one week after the challenge, it failed to improve the body weight of chickens in the unvaccinated challenged groups, especially in the unvaccinated group challenged with O78. The results related to FCR are in line with the findings of Amen et al. (2023) [19].

Regarding the two groups challenged with the O26 strain, although the FCR difference at the age of 42-49 days was not significant between the two groups, the FCR of the vaccinated group was better than the unvaccinated group and compared to all groups, this group had the highest growth rate at 42-49 days old. It seems that the pathogenicity of E. coli strains affects the FCR. As described above, APEC has numerous strains with widespread pathogenic bacteria in poultry [16].

The results of the microagglutination test showed that the O antigens of the three strains of the vaccine-induced immune responses in the vaccinated chickens 14 days post-vaccination. The O antigens in E. coli bacteria are among the highly immunogenic antigens [20]. The results also confirmed the challenge because antibody titers against O78 and O26 antigens rose in the challenged chickens.

Future vaccine development requires a multi-dimensional approach, focusing on identifying conserved antigens that confer broad protection across different APEC serotypes or incorporating such antigens. Multivalent vaccines targeting multiple serogroups or incorporating diverse antigens may offer enhanced efficacy and broader coverage [21].

Conclusion

The results demonstrated that it is possible to produce heterologous immunity with the alum-adjuvanted sonicated trivalent avian colibacillosis vaccine. These results support the data reported by Melamed et al. (1991) that demonstrated that chickens vaccinated with the sonicated O2:K1 strain were protected from challenges with the heterologous O78:K80 strain. Sonication increases the presentation of internal and common antigens to the immune system [22].

Materials and Methods

E. coli strains

Two strains of APEC, including O78:K80 and O26, were used in this study. These strains had been isolated from affected broiler chickens in Iran and their pathogenicity had been confirmed by experimental studies (Microbiology Laboratory, Faculty of Veterinary Medicine, University of Tehran).

Sonicated trivalent avian colibacillosis vaccine

The vaccine contains three inactivated serotypes of *E. coli,* including O78:K80, O2:K1, and O1:K1, and alum as an adjuvant.

Experimental design

A total of 96 broiler chickens (Ross 308®) were randomly assigned to four groups of 24 chicks each (female and male chicks were equal in each group). Each group had three subgroups of eight chicks per cage. Groups A and C were vaccinated, while groups B and D were not vaccinated (received normal saline instead of the vaccine). All the four groups were challenged 21 days post-vaccination. Groups A and B were challenged with the O78:K80 strain, while groups C and D were challenged with the O26 strain.

On day 14 of life, just before vaccination, and 7, 14, 21, 27, and 36 days after vaccination, the growth rate of chickens in the different groups was recorded. The mortality rate, feed consumption, and FCR were also assessed. After vaccination and challenge, the clinical signs and gross lesions of euthanized, moribund birds and dead chickens were recorded and also, using colony morphology and biochemical features attempts were made to isolate *E. coli* bacteria from the heart blood of fresh dead chickens [23]. Please replace the highlighted phrase with " using colony morphology and biochemical features.

Vaccination

Chickens in groups A and C received sonicated trivalent avian colibacillosis vaccine in the dorsal cervical region via the subcutaneous route on day 14 (0.5 ml/chick). Groups B and D received sterile normal saline at the same site and via the same route (0.5 ml/chick) (Table 6).

Challenge

Chickens of groups A and B were subcutaneously [10, 24] challenged 21 days post-vaccination with 0.5 ml of a suspension containing 1.5×109 CFU/ml of O78:K80, while chickens of groups C and D were challenged with 0.5 ml of a suspension containing 1.5×109 CFU/ml of O26 (Table 6).

Microagglutination test to O antigen

Blood samples were taken randomly from the chickens on days 14, 21, 28, and 42 for serology study. A volume of 50 µL of normal saline was placed in each well of a rounded-bottom microtiter plate. In the first well, 50 µL of serum sample was added and the mixture was serially diluted. Next, 50 µL of E. coli O antigens, including O2, O78, and O1 antigens or O26 antigens, was added to all nine wells. After overnight incubation at 4°C, the results were recorded.

Statistical analysis

Table. 6
Experimental design

Chikens[a] Groups	Vaccination Age (day) 14	Challenge Age (day) 35
A	Vb	+d
B	NVc	+
C	V	+
D	NV	+

[a]The chickens of groups A and C received sonicated trivalent avian colibacillosis vaccine via subcutaneous injection in the dorsal cervical region (0.5 ml/chick); the chickens of groups B and D received sterile normal saline (0.5 ml/chick).
Groups A and B were challenged with strain O78:K80 and groups C and D with strain O26.
[b]V: vaccinated, NV: unvaccinated, +: chickens were challenged.

Authors' Contributions

Sima Alempour Rajabi and Zolfaghar Rajabi conceived and planned the experiments. Sima Alempour Rajabi carried out the experiments. Sima Alempour Rajabi and Zolfaghar Rajabi planned and carried out the simulations. Sima Alempour Rajabi contributed to sample preparation. Sima Alempour Rajabi and Zolfaghar Rajabi contributed to the interpretation of the results. Sima Alempour Rajabi and Zolfaghar Rajabi took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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