Journal of Advanced Veterinary Research

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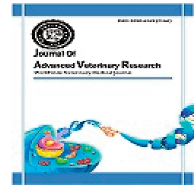

Evaluation of The Living *Escherichia coli-O78* Deleted *aroA* Vaccine Against Homologous and Heterologous *E. coli* Challenge in Broiler Chickens

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ARTICLE INFO

Original Research

Received:
15 June 2016

Accepted:
28 June 2016

Keywords:

APEC
Living vaccine
Genetically modified
Chickens

ABSTRACT

To determine whether the immunization using commercially available living *Escherichia coli-O78 aroA* deleted vaccine (Poulvac® *E. coli*) is protective against APEC challenges or not. Ninety chicks were divided into six groups (15 birds/each); two groups were vaccinated at day 1 by spray and drinking routes then challenged intratracheally with homologous *E. coli* O78 at day 21, the other two groups were similar to the previously mentioned groups but challenged with heterologous *E. coli* O1 in parallel with the four challenged-vaccinated groups there were two positive control (challenged-not vaccinated) groups; one challenged with O78 and the other one with O1 at day 21 using intratacheal route. The best obtained results were recorded to the vaccinated-challenged group with the homologous strain and vaccinated by spraying method which exhibited decreases in organ lesion scores in comparison to the other groups (non-vaccinated challenged chickens and groups of chickens either homologous challenged-vaccinated through drinking water or heterologous challenged-vaccinated groups). These findings suggest that vaccine is a suitable for minimizing lesion scores against homologous challenge using spraying method that could lead to minimizing the time for treatment and cases of condemnation in processing plants.

J. Adv. Vet. Res. (2016), 6 (3), 89-92

Introduction

Avian pathogenic *Escherichia coli* (APEC) is a major reason for a number of extraintestinal diseases in broiler chickens, including colisepticemia, air sacculitis, pericarditis and perihepatitis (Howell, 1996; kariyawasam *et al*., 2004). These case conditions can lead to huge economic losses to the poultry industry each year as a result of increased percentages of low weights, carcass condemnation in processing plants, and increased number of mortalities among affected birds (Dziva and Stevens, 2008). There are many serogroups of *E. coli* are considered pathogenic commonly isolated O1, O2, O78, O8, and O35, that can cause the previous conditions (Bélanger *et al*., 2011; La Ragione and Woodward, 2002).

E. coli infections are controlled mainly by shielding birds against major respiratory pathogens, as Newcastle Disease (ND), Infectious Bronchitis (IB), Mycoplasmosis and immunosuppressive viral infections, Infectious Bursal Disease (IBD) and Chicken Anemia (CA). In addition to the methods that are essential for minimizing environmental stress (temperature imbalance, improper ventilation, mycotoxins, and contaminated drinking water), which all help to the incidence of *E. coli* infection (Shane, 2009).

Among the methods for controlling *E. coli*–associated diseases, vaccines have received extra focusing because antimicrobials have faced partial success due to the generated resistance to the existing antimicrobial agents and the lack of new effective agents.

The control of *E. coli* infections has been mostly depended on vaccination with killed autologous bacterins (Trampel and Griffith, 1997), but these deliberate short-lived serotype-specific protection and their usefulness is hindered by the different serovars of *E. coli* could infect poultry. So, the use of live vaccines was emerged, which require a low-cost in their preparation and their easiness in administration as well as their cross protection efficacy. Vaccines depend on the defined genetic deletion m.os. may be preferable candidates for live vaccines, especially if it cannot revert to wild type. A number of researchers have described attempts to develop live attenuated vaccines for avian *E. coli* targeting several genes to develop genetically defined mutants and none have emerged as suc-

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cessful commercial vaccines for colibacillosis in chickens or turkeys (Peighambari and Gyles, 1998; Roland *et al*., 1999; Peighambari *et al*., 2002; Roland *et al*., 2004).

The *aroA* gene is responsible for the biosynthesis of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) in the virulent *E. coli* parent strain. Aromatic biosynthetic (*aroA*) mutants (Hoiseth and Stocker, 1981) have unique advantage that the surface appendages, such as fimbriae and flagella that have showed their importance in the pathogenesis of avian colibacillosis are still expressed. When the *aroA* gene is deleted, the candidate *E. coli* becomes avirulent and unable to continue in growth due to losing the capability to synthesize the amino acids necessary for its existence.

The manufacturer of *E. coli aroA*-live vaccine has proved that the produced vaccine protects chickens against the homologous and heterologous serogroups of *E. coli*. In contrary, this vaccine has never been confirmed to have the efficacy in protection against wild *E. coli* serotype O78 isolated from Thailand (Chansiripornchai, 2009).

Due to the previously mentioned debates among the efficacy of this vaccine and the wide use of antimicrobial agents in the Egyptian poultry farms and the rapid increase of microbial resistance to these agents. Our study designed to evaluate this commercial living *E. coli* vaccine (Poulvac®*E. coli*) to show its efficacy in minimizing the percentages of morbidity, mortality and lesion scores among the challenged chickens that may lead to decrease the economic losses facing owners that is coming from purchasing antimicrobial agents and shortening the period of treatment, in addition to decreasing the mortality rates.

Materials and methods

Chickens

Ninety, unvaccinated broiler chickens (Ross) of mixed sex were obtained on the day of hatching from a commercial hatchery. The chickens were fed *ad libitum* with commercial diets suitable for their age has no antibacterial or anticoccidial components, before and during the experiments. Vaccination against diseases other than *E. coli* was applied using vaccines of different manufacturers and included Newcastle Disease vaccines (Hitchner B1 and LaSota vaccines were applied by eye drop route at 7 and 17 days of age, respectively), and Infectious Bursal Disease vaccine (live intermediate vaccine was applied by eye drop route at 13 days of age).

Vaccine

E. coli aroA vaccine (Poulvac®*E. coli*, Pfizer Animal Health, Exton, PA 19341, USA). The vaccine dosages were calculated according to a titer of 7.0 x10^7CFU per dose in 0.5 ml for spray application, the size of the droplets was adjusted to 100 ± 10 µm by means of cold fogger (ULV Fogger SFYM-YM02B, Saintfine®), and in 5 ml for drinking water application.

Challenge

Challenged strains were APEC O78 and O1 that had been originally isolated from heart blood of chickens with a field case of colisepticemia from farms at different localities in Egypt. For preparation of bacterial suspensions, the bacterial strains were inoculated into brain heart infusion broth and incubated at 37°C for 18 hours. Then, bacterial cells were harvested by centrifugation at 5000 rpm for 30 minutes and washed thrice with PBS and resuspended in PBS to yield an approximate concentration of 6 x 10^8 CFU/ml. Each chicken was challenged with 1 ml of the inocula by intratracheal route. After challenge, all birds were observed daily for a week; any bird that died during the observation period was necropsied. The surviving chickens at the end of the experiment were all euthanatized, necropsied, and examined for colibacillosis lesions.

Assessment of protection against homologous challenge

Forty five chickens were divided into 3 groups of 15 each. Chickens in groups 1 and 2 were vaccinated at one day of age with Poulvac®*E. coli* vaccine by coarse spray and drinking water routes, respectively. Chickens in group 3 were not vaccinated and kept as positive control. Since the onset of immunity for Poulvac®*E. coli* is demonstrated to be 14 days post vaccination (CVMP, 2012), chickens in all groups were challenged at 21 days of age with APEC O78.

Assessment of protection against heterologous challenge

Forty five chickens were divided into 3 groups of 15 each. Chickens in groups 4 and 5 were vaccinated at one day of age with Poulvac®*E. coli* vaccine by coarse spray and drinking water routes, respectively. Chickens in group 6 were not vaccinated and kept as positive control. Chickens in all groups were challenged at 21 days of age with APEC O1.

Evaluation criteria

Individual body weights in grams were recorded at 10, 20, 24 and 28 days of age using sensitive scale (Five grams difference sensitivity). Mortalities were recorded as the number of chickens that had died before the end of the experiment. Scores for gross pathologic findings were assigned according to data from (Peighambari *et al*., 2002) 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).

Statistical analysis

Analysis of mortalities in different groups was performed by Chi-square test. ANOVA and Duncan multiple range tests were used for the statistical comparison of the body weights and the lesion scores with $P \leq 0.05$. All tests were performed using SAS v9.2.

Results

Safety variables

No adverse events (death or clinical signs) potentially attributable to the vaccine were observed. The weight gain of the chickens during the first three weeks of life showed that there was no negative effect because of vaccination.

Protection against homologous challenge

Regarding mortalities and average body weights, there were no significant differences between the studied groups. For the gross pathological findings, the average lesion scores of air sacs and the pericardium in the group vaccinated with

Table 1. Mortalities, average body weights and lesion scores of groups in the protection study against homologous challenge

Group*	Mortality	Average body weights (g)				Lesion scores		
		Day 10	Day 20	Day 24	Day 28	Heart	Liver	Air sacs
Group 1	1/15^{a}	230.33^{a}	693.67^{a}	788.93^{a}	879.28^{a}	0.867^{b}	0.333^{a}	1.400^{b}
Group 2	3/15^{a}	230.67^{a}	718.00^{a}	770.00^{a}	856.25^{a}	1.533^{a}	0.667^{a}	1.933^{ab}
Group 3	2/15^{a}	216.00^{a}	688.33^{a}	774.21^{a}	966.92^{a}	1.467^{a}	0.533^{a}	2.267^{a}

$^{a, b}$The superscripts that differed in each column have significantly different at confidential 95% ($p \leq 0.05$)
* Group 1: Poulvac®*E. coli*/Coarse spray/Challenged O78; Group 2: Poulvac®*E. coli*/Drinking water/Challenged O78; Group 3: Not vaccinated/Challenged O78

Table 2. Mortalities, average body weights and lesion scores of groups in the protection study against heterologous challenge

Group*	Mortality	Average body weights (g)				Lesion scores		
		Day 10	Day 20	Day 24	Day 28	Heart	Liver	Air sacs
Group 4	1/15^{a}	209.67^{a}	702.33^{a}	867.00^{a}	975.00^{a}	0.933^{a}	0.200^{ab}	1.867^{a}
Group 5	1/15^{a}	219.33^{a}	695.67^{a}	815.67^{a}	967.50^{a}	1.067^{a}	0.600^{a}	1.800^{a}
Group 6	1/15^{a}	213.33^{a}	699.33^{a}	885.36^{a}	1059.28^{a}	1.067^{a}	0.133^{b}	1.667^{a}

$^{a, b}$The superscripts that differed in each column have significantly different at confidential 95% ($p \leq 0.05$)
* Group 4: Poulvac®*E. coli*/Coarse spray/Challenged O1; Group 5: Poulvac®*E. coli*/Drinking water/Challenged O1; Group 6: Not vaccinated/Challenged O1

Poulvac®*E. coli* by coarse spray but not by drinking water route were statistically significantly less than those of the positive control group (Table 1).

Protection against heterologous challenge

Mortalities, average body weights, and average lesion scores did not differ significantly between the studied groups (Table 2).

Discussion

A vaccine containing a Δ*aroA* mutant of an O78:K80 *E. coli* is currently marketed by Zoetis (Poulvac®*E. coli*, Zoetis). In this study, we evaluated the protective ability of this vaccine by spray and drinking methods against homologous (*E. coli* O78) and heterologous infections (*E. coli* O1) in broiler chickens.

The obtained results showed, there were no unfavorable reactions (death or other clinical signs) attributable to the administration of the vaccine, an indication of its safety for mass administration.

The weight gain of the chickens (Table1 and 2) during the first three weeks of life, before the birds being challenged with APEC, showed that there was no negative effect because of vaccination, as there were no significant differences between vaccinated and control groups, similar results were obtained by Salehi *et al*. 2012 and Mombarg *et al*. 2014, unlike Filho *et al*. (2013), who reported that, there was a tendency of increase on the weight of the control group during the second week and with significant difference during the third week post vaccination in comparison to the vaccinated groups.

Also, from the gained results, there were statistical significant differences in minimizing lesions score of heart (pericardium) and air sacs between the vaccinated groups with homologous challenge using spray method (Table 1). In contrast, no statistical significant results were obvious in the other groups either by homologous using drinking water method and heterologous challenges vaccinated using either routes (Table 1 and 2). 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).

Several research articles were comparable to the results of our study, which mentioned 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) may be for the mentioned reasons; firstly, this could be due to the existence of a maternally derived antibodies, even if they are present in little levels (Elazab *et al*., 2009). Secondly, Lynne *et al*. (2006) observed that the vaccination with Iss protein encouraged protection against *E. coli* infection in chickens, while in our research, it is probable that the used vaccine did not live lengthy enough in the organs of chickens to express a suitable amount of Iss protein. Thirdly, may be due to administration of single dose of the vaccine when birds are still immunologically immature at one day old so may be a need for another shot to induce the immunity (Heller *et al*., 1990; Sadeyen *et al*., 2015). Fourthly, may be the size of sprayer droplet, in the present study the size of sprayer droplets that used with tested vaccine were about 100 µm, which were larger in their size than those used in the studies of Kariyawasam *et al*. (2004) and La ragione *et al*. (2013). These large droplets can't reach deeply in the respiratory tract to induce a strong immune response as happened when used a small droplet size that can move to the lower parts of the respiratory tract.

In the present study, the vaccination in drinking water proved its failure to protect chickens against challenging with *E. coli* that may be due to the chickens need high number of *E. coli* (10^{10}CFU/ml) as proposed by DuPont *et al*. 1970 and Amoako *et al*. 2004).

In concern to the failure of vaccine to protect against heterologous challenge was in parallel with previous studies (Deb and Harry, 1976; Peighambari, *et al*., 2002; Kariyawasam *et al*., 2004) that traced this to role of LPS in relationship with protection during vaccination with live *E. coli* vaccine.

On reverse to our results, La Ragione *et al*. (2013) stated that the *aroA* construct was shown to be successful as a vaccine against colibacillosis in chickens and turkeys caused by a homologous APEC O78 and also against an untypeable APEC strain in chickens. Also, Sadeyen *et al*. (2015) verified that the two vaccines (Poulvac®*E. coli* and a formalin-inactivated vac-

cine) were protective against homologous intra-air sac challenge with *E. coli* in turkeys.

Conclusion

The living *Escherichia coli-O78* deleted *aroA* vaccine effective in reducing the lesion scores against homologous infection by using the spray method that may lead to decrease the downgrading carcasses and condemnation rate, but could not protect against homologous infection when the vaccine was used in drinking water as well as heterologous infected-vaccinated groups. Based on the results of the current study, further investigations may be required to determine the factors that may play a role in the efficacy of this vaccine.

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