Short Communication

The Efficacy of *Escherichia coli* AroA-Live Vaccine in Broilers against Avian *E. coli* Serotype O78 Infection

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Abstract

The efficacy of an *Escherichia coli (E. coli)* aroA-live vaccine in the prevention of colibacillosis in chickens following intratracheal challenge with a virulent strain of *E. coli* O78 was investigated. Thirty-six, one day old broiler chickens were divided into 3 groups of 12 each. Chickens in each group were randomly divided into 2 replicates. The chickens in group 1 that were not vaccinated and challenged served as a negative control. The chickens in group 2 that were not vaccinated but received *E. coli* serotype O78 served as a positive control. The chickens in group 3 were vaccinated by the oral route at 5 days of age with *E. coli* aroA-vaccine and challenged with *E. coli* serotype O78. All the chickens in groups 2 and 3 were challenged intratracheally at 4 weeks of age with 0.5 ml ($1.2x10^9$ cfu/ml) per dose of *E. coli* O78. The chickens were monitored for 7 days after infection for feed conversion ratio (FCR), and the post-mortem pathology was assessed. The results revealed that the vaccine tends to prevent *E. coli* infection. The chickens in group 3 tended to show lower pathological findings including airsacculitis, pericarditis, perihepatitis, peritonitis and arthritis than the chickens in group 2 but the FCR was not different in each group ($p>0.05$).

Keywords : broilers, colibacillosis, *Escherichia coli* aroA-live vaccine, *E. coli* O78,

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บทคัดย่อ

ประสิทธิภาพของวัคซีนเชื้อเป็น *เอสเชอริเชีย โคไล* อะโรเอ ในไก่เนื้อ ต่อการติดเชื้อ *เอสเชอริเชีย โคไล* ซีโรไทป์ O78 ในสัตว์ปีก

วิสุทธิ์ ระวิเวช นิวัตร จันทร์ศิริพรชัย*

ศึกษาประสิทธิภาพของวัคซีนป้องกันโรค *เอสเชอริเชีย โคไล (อี. โคไล)* เชื้อเป็นอะโรเอ ในการป้องกันการเกิดโรคโคไลบาซิลโลซิสในไก่ เมื่อได้รับเชื้อพิษ *อี. โคไล* สายพันธุ์รุนแรง O78 ทางหลอดลม ไก่อายุ 1 วัน จำนวน 36 ตัว แบ่งเป็น 3 กลุ่ม กลุ่มละ 12 ตัว โดยสุ่มแบ่งไก่แต่ละกลุ่มออกเป็น 2 กลุ่มย่อย ไก่กลุ่ม 1 เป็นกลุ่มควบคุมลบ ไม่ได้รับวัคซีนและเชื้อพิษ ไก่ในกลุ่ม 2 เป็นกลุ่มควบคุมบวก ไม่ได้รับวัคซีน แต่ได้รับ *อี. โคไล* ซีโรไทป์ O78 ไก่ในกลุ่ม 3 ได้รับวัคซีนโดยการป้อนปากที่อายุ 5 วัน ด้วยวัคซีน *อี. โคไล* เชื้อเป็นอะโรเอ และได้รับ *อี. โคไล* ซีโรไทป์ O78 ไก่ทุกตัวในกลุ่ม 2 และ 3 ได้รับเชื้อพิษทางหลอดลมที่อายุ 4 สัปดาห์ ขนาด 0.5 มล. (1.2×10^9 cfu/มล.) ต่อโดสของ *อี. โคไล* O78 ไก่ทุกตัวได้รับการตรวจประเมินเป็นเวลา 7 วันหลังจากติดเชื้อ ในด้านอัตราการแลกเนื้อ และพยาธิสภาพ ผลการทดลองพบวัคซีนมีแนวโน้มป้องกันการติดเชื้อ *อี. โคไล* ไก่ในกลุ่ม 3 มีแนวโน้มพบการแสดงออกของรอยโรค ถุงลมอักเสบ ถุงหุ้มหัวใจอักเสบ เยื่อหุ้มตับอักเสบ เยื่อบุช่องท้องอักเสบ และ ข้ออักเสบ ต่ำกว่าไก่ในกลุ่ม 2 แต่ไม่พบความแตกต่างของอัตราแลกเนื้อในแต่ละกลุ่ม ($p>0.05$)

คำสำคัญ : ไก่เนื้อ โคไลบาซิลโลซิส วัคซีนเชื้อเป็น *อี. โคไล* อะโรเอ *อี. โคไล* O78

หน่วยปฏิบัติการวิจัยสุขภาพสัตว์ปีก ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330
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Introduction

Colibacillosis, caused by *Escherichia coli (E. coli)*, is a common systemic disease and has economic importance in poultry industry. *E. coli* infection occurs as an acute fatal septicemia or subacute pericarditis and airsacculitis, as well as perihepatitis, arthritis, and also cellulitis. Among bacterial infections, colibacillosis is very often the first cause of morbidity and mortality in poultry. A large number of *E. coli* are maintained in the poultry house environment through fecal contamination. Systemic infection occurs when a large number of pathogenic *E. coli* gain access to the blood stream via the respiratory tract or intestines. Bacteremia progresses to septicemia and death, or the infection extends to the serosal surfaces, the pericardium, the joints and the other organs. Surveys have been conducted in many parts of the world to determine the serotypes that are most frequently associated with diseases caused by *E. coli* variations according to geographic region but in most studies the common serotypes have been O1, O2, O35, and O78 (Sojika et al., 1965; Heller et al., 1977; Chansiripornchai and Sasipreeyajan, 2002). Treatment strategies include the control of predisposing infections or environmental factors and the early use of antibiotics. Unfortunately, a high frequency of resistance to tetracycline, oxytetracycline, chlortetracycline and doxycycline has occurred and more than 93% and 100% of *E. coli* isolates are resistant to erythromycin (Chansiripornchai et al., 1995). Furthermore, using of antibiotic drugs in the future will tend to be reduced and restricted in commercial farms so *E. coli* vaccines are an alternative way to prevent and control of *E. coli* infection.

E. coli vaccines include an inactivated vaccine, a live attenuated vaccine and a recombinant vaccine. Effective inactivated vaccines against various serotypes, including O2:K1 and O78:K80 have been produced (Deb and Harry, 1976; Deb and Harry, 1978; Cessi, 1979).

The inactivated vaccines provide protection against the homologous serogroups but little or no protection against the heterologous serogroups. Recently, a commercial live vaccine for chickens has been developed. The inventor has proclaimed that the *E. coli* aroA-live vaccine can protect against the homologous and heterologous serogroups. However, the *E. coli* aroA-live vaccine has never been proved to have the efficacy of vaccine protection for the virulent *E. coli* serotype O78 isolated from Thailand.

The Poulvac® *E. coli* vaccine contains an *E. coli* strain that has been genetically-modified by the deletion of the aroA gene responsible for the biosynthesis of amino acids in the virulent *E. coli* parent strain (The GMO is named aroA- PTA-5094). The aroA gene-deleted vaccine is capable of triggering a protective immunity in poultry against infection and disease from wild, virulent *E. coli* bacteria found in the environment. However, because the aroA gene is deleted, the live vaccine bacterium is avirulent and unable to form a self-sustaining population since the vaccine strain has lost the capability to synthesize the amino acids necessary for its survival. The objective of the experiment was to prove the efficacy of the *E. coli* aroA-live vaccine against *E. coli* serotype O78 isolated from Thailand.

Materials and methods

Chickens: Thirty-six, unvaccinated broiler chickens (Arbor Acres) of mixed sex were obtained on the day of hatching from a commercial hatchery (Krungthai Farm, Thailand). The chickens were fed ad libitum before and during the experiments. At the onset of the experiments, no significant difference in average body weight between the experimental groups was found. The guidelines and legislative regulations on the use of animals for scientific purposes of Chulalongkorn University, Bangkok, Thailand were followed as is certified in permission No. 0831071.

Bacterial strains: The chickens were challenged with an *E. coli* serotype O78 that had been originally isolated from the diseased air sacs of a chicken with a field case of colisepticemia (Chansiripornchai and Sasipreeyajan, 2002). In the experiment, 0.5 ml of the *E. coli* suspension, containing $1.2x10^9$ cfu/ml was used for intratracheal challenge. At the end of experiment, the livers of all the birds were collected for *E. coli* identification.

Vaccination and Experimental designs: *E. coli* aroA-vaccine (Poulvac *E. coli*, Fort Dodge Animal Health, Iowa, USA) was orally administered to the chickens according to the manufacture's recommendation. The *E. coli* vaccine dosages were calculated according to a titer of $5.0x10^6$ cfu per dose. Thirty-six, unvaccinated broiler chickens of mixed sex were divided into 3 groups of 12 each. Chickens in each group were randomly divided into 2 replicates. Group 1 that was not vaccinated and challenged served as a negative control group. Group 2 that was not vaccinated but was challenged served as a positive control group. Group 3 was orally vaccinated at 5 days of age with *E. coli* aroA-vaccine and received *E. coli* serotype O78. All the chickens in groups 2 and 3 were intratracheally challenged at 28 days of age with 0.5 ml ($1.2x10^9$ cfu/ml) per dose of *E. coli* O78. At 7 days post challenge, the number of dead birds was noted and all the surviving birds were necropsied and examined for the presence of grossly visible lesions of colibacillosis.

Efficacy criteria and definitions: Mortality was defined as the number of chickens that were killed or that had died before the end of the trial. Morbidity was defined as the number of birds with lesions in either airsac, pericardium or perihepatic.

The airsac, pericardial and perihepatic lesions of colisepticemia in each bird were scored. The airsac lesions of colisepticemia were scored according to Kleven et al. (1972) as follows: 0: no lesions, 1: cloudiness of air sacs, 2: air sac membranes are thickened, 3: "meaty" appearance of membranes, with large accumulations of a cheesy exudate confined to one air sac, 4: lesions with the same score as score 3 but with lesions in two or more air sacs. The pericardial lesions of colisepticemia were scored according to

Charleston et al. (1997) as follows: 0: no lesions, 1: excessive clear or cloudy fluid in the pericardium, 2: extensive fibrination in the pericardial cavity. The perihepatic lesions of colisepticemia were scored according to Charleston et al. (1997) as follows: 0: no visible lesions, 1: definite fibrination on the surface of the liver, 2: extensive fibrination, adhesions, liver swelling and necrosis.

Chickens with severe lesions were characterized as having an air sac lesion score of 4 and pericarditis and perihepatitis scores of either 1 or 2. The average body weight of the birds in each group was measured at 1 day, 28 days and 35 days of age. A feed conversion ratio (FCR) was calculated for each group by taking the total amount of feed consumed by each group between days 1-35 and dividing it by the increase in mass of the chickens over the same time period.

Statistical analysis: ANOVA and Duncan multiple range tests were used for the statistical comparison of the body weight. The mortality, morbidity and the lesion scores were analysed by Chi-square and Mann-Whitney U test, respectively. SPSS for Windows was used for statistical analysis.

Results and Discussion

Pathological findings and production parameters: One chicken in each group was culling before vaccination due to drowning. The pathological findings and production parameters measured for vaccinated or control groups are summarized in Table 1. Two birds died in the positive control and the vaccinated groups at one and two days post challenge with *E. coli* O78, respectively. The birds were found to have acute, severe septicemia and *E. coli* could be isolated from the livers at postmortem examination.

The mortality and morbidity rate of the birds vaccinated with *E. coli* aroA-live vaccine was no different from the positive control group ($p>0.05$). For the pathological findings, the average lesion scores of air sacs and the number of birds with arthritis in the group vaccinated with *E. coli* aroA-live vaccine were statistically significantly less than those of the positive control group ($p<0.05$). There was no difference in the FCR among the 3 groups ($p>0.05$).

Bacteriology: All the isolates of birds in groups 2 and 3 were *E. coli* serotype O78 which was the same serotype as the *E. coli* challenge strain. The birds in group 2 with no vaccination had a significantly higher number of the *E. coli* isolates than those birds in group 3 vaccinated with one dose at 5 days old ($p<0.05$) (table 1).

In the positive control group, broilers that were challenged with *E. coli* serotype O78 revealed 9% mortality and 91% morbidity. Normally, the mortality rate in field cases caused by *E. coli* infection is 0.25% in the primary and increases to 1% after being infected for 5 days and can increase up to 10% (Shane, 1981; Wrey et al., 1996). Fan et al. (2004) showed a mortality of 28.1% for chickens that were intratracheally challenged with 1.0 x 109 cfu *E. coli* O78 at 6 weeks of age. In contradiction of Rosenberger et al. (1985), 0% mortality was found in the one day old chickens after being intratracheally challenged with the concentration of less than 10^4 cfu of *E. coli*, but the mortality increased to 60% when the challenge dose was increased to 10^7 cfu. At 15 days of infection, the chickens apparently developed an age-associated resistance to *E. coli* introduced intratracheally. In our model, mortality could be found after the chickens had been challenged with 0.6 x 10^9 cfu/ml *E. coli* O78 at 28 days old. Thus, the birds vaccinated with *E. coli* aroA-live vaccine could have reduced morbidity but not a reduced mortality rate. These results do not all agree with Fan et al. (2004), showing that birds vaccinated with *E. coli* aroA-live vaccine can have a reduce morbidity and mortality rate. In this experiment, there was no difference in mortality rate between the vaccinated and the positive control group but the chickens in the vaccinated group died later than the chickens in the positive control group. In the pathological findings, lesion scores of airsacs, pericardium,

Table 1. Pathological findings and feed conversion ratio of broilers

Group	Morbidity	Mortality	No. of birds with gross lesions		Mean gross lesion score ± SD and No. of birds with gross lesions			% birds with severe lesions*	No. of bird positive for *E. coli*	FCR day 1-35
			Peritonitis	Arthritis	Airsacculitis[x]	Pericarditis[y]	Perihepatitis[z]			
1 (n=11)	0/11[a]	0/11[a]	0/11[a]	0/11[a]	0.00[a] (0/11[a])	0.00[a] (0/11[a])	0.00[a] (0/11[a])	0.00 (0/11[a])	0/11[a]	1.55±0.18[a]
2 (n=11)	10/11[b]	1/11[a]	2/11[a]	6/11[b]	2.82±1.33[b] (10/11[c])	0.91±0.70[b] (8/11[b])	0.18±0.60[a] (1/11[a])	36.36 (4/11[b])	5/11[b]	1.54±0.22[a]
3 (n=11)	7/11[b]	1/11[a]	0/11[a]	1/11[a]	0.82±1.40[a] (4/11[b])	0.55±0.52[b] (6/11[b])	0.00[a] (0/11[a])	0.00 (0/11[a])	1/11[a]	1.58±0.06[a]

*Birds with severe lesions were characterized as having an air sac lesion score of 4 and pericaditis and perihepatitis scores of either 1 or 2

[x]Air sac lesions were scored on a scale of from 0 to 4.

[y]Pericardium lesions were scored on a scale of from 0 to 2.

[z]Perihepatic lesions were scored on a scale of from 0 to 2.

[a,b]The superscripts that differed in each column have significantly different at confidential 95% ($p<0.5$)

Group 1: negative control; Group 2: positive control; Group 3: vaccination and challenge

perihepatic and the number of birds with peritonitis and arthritis were investigated. The birds in the positive control group had a significantly higher airsac lesion score and also a higher number of birds with arthritis than the birds in the vaccinated group ($p<0.05$). Compared to the vaccination and the positive control group, chickens in the vaccinated group tended to show lower morbidity and fewer pericarditis- and perihepatitis lesion scores than the chickens in the positive control group. In conclusion, the *E. coli* aroA-live vaccine tends to reduce the pathological lesions of the chickens challenged with *E. coli* serotype O78 isolated in Thailand.

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