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A STUDY ON THE EFFICACY OF INACTIVATED AVIAN *E.COLI* VACCINE IN LAYER CHICKS

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ABSTRACT

The efficacy of avian *Escherichia coli* vaccine was studied in the prevention of colibacillosis in layer chicks by using intramuscular and subcutaneous routes. Sixty, one day old Layer chicks were divided into 3 groups (A, B and C) of 20 each. Chicks in each group were randomly divided into 2 replicates. On 15th day of age, chicks in group C (control-non-vaccinated) were injected with 0.9% saline through intramuscular (ten chicks) and subcutaneous routes (ten chicks). Whereas, chicks in group A and B were injected with Avian *E.coli* vaccine through intramuscular and subcutaneous routes, respectively. Ten birds in each group (A and B) were injected with 0.3ml and ten with 0.5ml of vaccine. Chicks were monitored for 21 days post vaccination and antibody titer through ELISA and AGP were noted. The results obtained through AGP and ELISA shows a marked difference between control and treated birds. Moreover, a high antibody titer was observed in the group which had received 0.5 ml of vaccine through subcutaneous route. Thus it was concluded that a dose of 0.5 ml per bird through subcutaneous elicits a high antibody titer that may provide protection against avian *E. coli.*

Key-words: *E. coli* vaccine, layer chicks, colibacilosis

INTRODUCTION

Colibacillosis is the most common infectious bacterial disease in poultry that causes high morbidity and mortality which is associated with significant economic losses (Barnes and Gross, 1997). Avian pathogenic *E. coli* (APEC) infections or Collibacilosis are characterized by multiple body lesions including pericarditis, airsacculitis, salpingitis perihepatitis, arthritis, synovitis and cellulitis with acute fatal septicemia (Barnes *et al.,* 2008, Ewers *et al.,* 2003, Chen *et al.,* 2011). Systemic infection occurs when avian pathogenic *E. coli* (APEC) gain access to the blood stream through 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. Flocks with airsacculitis at processing had lower average body weights, more processing errors, fecal contamination and Campylobacter contamination (Russell, S. M., 2003). E. coli was isolated from 88.2% of chickens with airsacculitis from flocks in Jordan (El-Sukhon *et al.,* 2002). In Greece colibacillosis was the most frequent respiratory disease of broilers over a 10year period (1992-2001) and caused considerable economic losses (Georgopoulou, J., *et al.,* 2005). APEC infections were identified as a major factor in poultry disease in Pakistan also. Most APEC isolated from poultry is specific clonal types that are pathogenic only for birds and represent a low risk of disease for people or other animals (Caya, F., *et al.,* 1999 & Ron, E. Z., 2006). This difference may be due to the lack of toxin production or that toxins produced by avian strains are not detectable with tests for toxins produced by mammalian strains.

The most common serotypes that have been reported to be associated with *E. coli* are O1, O2, O35 and O78 (Chansiripornchai and Sasipreeyajan, 2002), but this varies according to geographic regions. Treatment strategies that have been employed include controlling predisposing infections or environmental factors in addition to the early use of antibiotics. A high resistance to antibiotics such as tetracycline, oxytetracycline, chlortetracycline and doxycycline has been reported and more than 93% of *E. coli* isolates are resistant while 100% are resistant to erythromycin (Chansiripornchai and Sasipreeyajan, 2002). Furthermore, the use of antibiotics against *E. coli* will tend to reduce giving room for the *E. coli* vaccines to be used in commercial farms.

Vaccines against *E.coli* are available in the form of inactivated, live attenuated and recombinant vaccine. While inactivated vaccines are effective against various serotypes (Cessi, 1979). These provide protection against the homologous serogroups while partial protection is achieved against the heterologous serogroups. However, the pathogenesis and the role of virulence factors in colibacillosis have not yet been completely elucidated, which makes its control more difficult (Lynne *et al*., 2006).

The main objective of the present study was to prepare an effective *E.coli* vaccine from the strain isolated from broiler chicks, to check its efficacy in broiler and to recommend an effective route and dose for the same.

MATERIALS AND METHODS

Chicks

Sixty, day old chicks were obtained from local hatchery and reared in the poultry shed of Poultry Research Laboratory, Department of Physiology, University of Karachi and fed with feed and water ad libitum. They were not vaccinated from hatchery as requested.

Avian *E.coli* Vaccine

The formalized inactivated Avian *E.coli* vaccine was prepared using indigenous *E.coli* culture isolated from commercial layer poultry farm. The inactivated formalized vaccine was prepared as per our lab protocol and injected in chickens as outlined in Table 1.

Table 1. Dosage and routes of immunization.

Groups	**A**	**B**	**C**
Dose (ml)	**Avian *E.coli* Vaccine**		**Saline**
	0.3	**0.5**	**0.5**
Intramuscular route	√	√	√
Subcutaneous route	√	√	√

Study Trial

Sixty, one day old Layers chicks were divided into 3 groups (A, B and C) of 20 each. Chicks in each group were randomly divided into 2 replicates and reared up to the 15 days. Pre-vaccination titres were determined on 15 day of age. On 15th day chicks in group C (non-vaccinated-control) were injected with saline (0.9%) through intramuscular (ten chicks) and subcutaneous routes (ten chicks), while chicks in group A and B were injected as outlined in Table 1.

Serum Collection

At twenty one (21) days post vaccination the chicks were bled through wing vein. Blood was allowed to stand overnight and the serum separated, stored at -20°C till further use.

Serologic Identification

The blood samples were collected from chicks prior to vaccination at day 15 of their age to determine the maternal antibodies level. To get good quality of sera, the tubes were kept in incubator for one hour at 37ºC and then collected into small vials. Samples were inactivated in water bath at 56ºC for 30min. After inactivation, they were stored at -40ºC till further use.

Agar Gel Immunodiffusion Test (AGID)

An overnight culture of *E.coli* was inactivated using formalin at a final concentration of 2% for 24 hrs. Later the culture was centrifuged and supernatant discarded. The cells were resuspended and density comparable to Tube No. 3 of a McFarland Nephelometer was achieved with formalized saline. For the AGID 1% (w/v) of purified agar in 8% (w/v) NaCl in 0.1 M phosphate buffer, pH 7.2 was prepared and poured to a thickness of 2–3 mm in a petri dish and left to solidify. With the help of a cutter wells approximately 5 mm in diameter were in cut in agar. Approximately 50µl of antigen (center) and antisera (periphery) were added to the wells. The plates were left in a moist chamber for the antigen antibody reaction and precipitin lines to be produced.

Whole Cell Elisa

Whole cell ELISA for the *E.coli* was carried out as descried by Hassan *et al.*, (2011), with certain modification. In brief, the cells from overnight culture were harvested through centrifugation at 2,000g and fixed with 4% (w/v) paraformaldehyde for 20 min at RT. This was followed by three washing with 1X PBS. Later the cells were incubated with blocking solution [3% (w/v) BSA in 1x PBS] for 30 min at RT. After 3 times washing with 1x PBS, the cells were incubated with 500 µl of primary antibody solution (1 µl of vaccinated bird serum in 200 µl of 1% BSA (w/v) in 1x PBS), followed by 60 min incubation at RT. The cells were then harvested and washed with 1x PBS (x 3) for 10 min. After washing, the cells were incubated with 500µl of horseradish peroxidase conjugated anti-chicken antibody (at a ratio of 1:200 in 1% BSA in 1x PBS) for 60 min at RT, followed by 3 times washing for 10 min. After that, the cells were centrifuged at 2,000 g for 10 min and the pellet was then resuspended in 200 µl of 1x PBS. In the wells of ELISA plate, 10 µl of the bacterial suspension and 50 µl of substrate (BM Blue, Roche) was

mixed and then incubated at RT for 20 min. The reaction was then stopped by adding 50 µl of stop solution (1M H_2SO_4). The absorbance was measured at 490 nm by using ELISA reader.

RESULT AND DISCUSSION

Avian colibacillosis, an infectious disease of broilers is caused by *Escherichia coli*. The infection is one of the major causes of mortality and morbidity in commercial poultry and is associated with heavy economic losses. Vaccination against *E.coli* is constrained by the degree of variability in APEC. A variety of killed and attenuated vaccines have been developed and tested which provide considerable protection against certain strains but are less effective against heterologous strains (Dho- Moulin and Fairbrother, 1999). It has been reported by Melamed *et al.,* 1991 that a considerable degree of protection may be obtained with heterologous inactivated vaccine. Passive immunization of young birds via the breeder hens is efficient for two weeks (Heller *et al.,* 1990). Various strains of *E. coli* inactivated either by formalin, or attenuation have been used for immunization of chickens to prevent colibacillus infections. However due to repeated outbreaks a need for an effective vaccines has always existed (Vaez zadeh *et al.,* 2004). Present study was carried out keeping in view the current morbid situation of poultry industry. A formalized inactivated vaccine from a local isolate of *E.coli* from broilers was prepared and tested for its efficacy through either sub-cutaneous or intramuscular route and with a dose of 0.3 ml and 0.5 ml per bird.

The maternal antibody titers against avian *E.coli* were evaluated which showed negligible amount of antibodies (Table 2) being present at day one of age, suggestive that no passive immunization is being carried out at the breeder level which may result in the transmission of antibodies from mother to progeny, making chicks susceptible to the infection.

Table 2. Maternal Antibody levels at one day old Chicks.

Groups	**A**	**B**	**C**
ELISA titres	0.020	0.019	0.022
AGP	Negative	Negative	Negative

The chicks were later reared up to the age of 15 days to minimize the influence of any other antibody which might be circulating in the blood. The results of pre-vaccination analysis of through AGP and ELISA at 15 days of age are summarized in table 3.

Table 3. Pre-vaccination titers of 15 days old Chicks.

Groups	**A**	**B**	**C**
ELISA titres	0.025	0.022	0.029
AGP	Negative	Negative	Negative

Table 4. 21 Days post-vaccination titers.

Group	**Avian *E.coli* Vaccine Dose (ml)**	**Route of Vaccination**	**AGP**	**ELISA**
A	0.3	Intramuscular	+	1.108
		Subcutaneous	++	1.452
B	0.5	Intramuscular	++	1.352
		Subcutaneous	++++	1.982
C	Control (non-vaccinated)	Intramuscular	-	0.030
		Subcutaneous	-	0.034

The birds were immunized through SC and IM routes (Table 1). The birds who had received 0.3 ml of vaccine developed a significant amount of antibody when compared with control group. It was also observed that the control

chicks remained fully susceptible against avian *E. coli* and did not develop any antibodies till the end of experimentation which was evaluated through AGP and ELISA (Table 4).

A non-significant difference was observed between the routes of IM and SC at a dose of 0.3 ml. The birds who had received 0.5 ml either through IM or SC route developed a significant amount of antibody when compared with control group. A significant difference was also noted among the birds who had received 0.3 ml and 0.5 ml with higher antibody levels in birds that were immunized with 0.5 ml through SC route. The study concluded that avian *E.coli* vaccine if administered through SC route with a dose of 0.5 ml per bird can elicit a good immune response, which may prevent birds from colibacillosis.

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