International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Susceptibility and Interleukins-6 Serum Level of Broilers Chickens Vaccinated with Baculovirus Expressed H5N1-ND after Experimental Infection with Virulent *E. coli*

Ismael Radwan[1], Ahmed Orabi[2]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Beni-suief University, Egypt

[2] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract: ***Avian pathogenic Escherichia coli cause severe respiratory and systemic disease in poultry yet the nature and consequences of host immune responses to infection are poorly understood. Here, an experimental study was conductedon broiler chickens for evaluating the effect of BEST; Baculovirus Expressed H5N1-ND "inactivated vaccine on controlling non-specifically of virulent E.coli infection with special reference to serum IL6 as indicator for potent immune response.Two groups from broilers chickens each one consisted of 250 birds, 1st group was vaccinated with "BEST®; at 10 day of age, while 2nd group was vaccinated with inactivated ND vaccine at 6 day of age" and also inactivated H5N3 vaccine at 10 day of age".E.coli suspension 1.5x10^8 cfu/ml from selected virulent and multidrug resistantE.coli isolate was used in experimental infection. Results revealed that 1st group showed slight respiratory signsand slight diarrhea with 8% mortalities begin at 3rd day post experimental infection and ended in 6th day with slight air sacculitis and slight pericarditis, in the other hand 2nd group recorded severe respiratory signs with nasal discharge , eye lid inflammation and diarrhea accompanied by 12 % mortalities with perihepatitis , pericarditis and air sacculitis .IL6 serum level also was recorded for each experimentally infected groupsas follow , 1st group IL6 conc. was "55 , 62 , 35 , 40 and 55pg/ml " while for 2nd group IL6 conc.was "32 ,40,30,35and 40 pg/ml".Such data inform the rational design of strategies to control of endemic Collibacillosis disease of poultry.***

Keywords: *E.coli*, BEST®, IL6

1. Introduction

Escherichia coli is a normalinhabitant chicken's microflora. Some avian *E. coli*serotypes are pathogenic and induce significant economic problems in broiler chickens (**Russell, 2003**).Avian pathogenic *E.coli* "APEC", which causes of avian collibacillosis .APEC strains are commensal classified as extra intestinal pathogenic *E.coli* "EXPEC" (**Ron ,2006**; **Johnson and Russo , 2002**) .Several factors have been shown to contribute the virulence of avian *E.coli* as Congo red binding , serum resistance and toxins production (**Gjessing and Berkhoof, 1989 and Fecteau*et al* ., 2001**).The role of *E. coli* in chronic respiratory diseases in meat-type chickens is well documented and its pathogenicity has been correlated with numerous extrinsic and intrinsic bird related factors and conditions. The extrinsic factors include environment, exposure to other infectious agents, virulence and level of duration of exposure, active and passive immune status and breed of chicken (**Gross, 1990**). Since intensive breeding has been widely applied in the poultry industry, outbreaks of acute mortality in flocks due to avian colibacillosis have frequently been observed, and are responsible for the significant economic losses (**Vandekerchove *et al* ., 2004**). Multiple antimicrobial resistance maybe acquired through mobile genetic elements which help in transfer of genes responsible for creation of "MDR" pathogens .**Koga *et al* ,(2015)**;detected the following virulence genes " *hly* F , *iss* , *omp*T , *iron* and *iut*A" in Extended Spectrum β-Lactamase producing *E.coli* isolated from chicken carcasses .Currently, the control of APEC has become not only an urgent issue but also a great challenge. Vaccination is an effective method for controlling infectious diseases (**Yang, 2003**).To date, a number of experimental vaccines have been developed to prevent colibacillosis (**Nagano *et al*2012; Lynne *et al*., 2012**).Cytokines as interleukins-6 (IL6) are secreted proteins involved in cell recruitment and regulation of both innate and adaptive responses as they are essential for effective host immune response to pathogens. Chicken IL6 has been confirmed to have a role in pro-inflammatory response (**Kaiser *et al* ., 2000**).So the present investigation was recording the susceptibility of broiler chickens group vaccinated with "BEST vaccine" to virulent "APEC" when compared with broiler chickens groups vaccinated with other programs.

2. Materials and Methods

1) Strain

E.coli strain was recovered from broiler chickens suffering from pericarditis, perihepatitis and airsaculitis then completely identified according to (**Quin *et al*., 2002**) as well as API 20E (BioMerieux) .

2) *Isolation and identification of E.coli.*(Murray*et al.* 2003).

Swabs were inoculated into Trypticase soya broth medium and incubated at 37°C for 72 hours.A loop-full from the inoculated medium was then sub cultured onto the surface of MacConkey's agar. All of the inoculated plates were incubated aerobically at 37°C for 24 hours. Smears from the suspected lactose fermenting colonies were stained with Gram stain and examined microscopically. Straight non sporulated Gram negative rods of medium size were selected

for further identification. The suspected colonies were picked up and examined for their colonial morphology followed by convential biochemical tests and confirmed by API 20 then preserved in semisolid agar for experimental infection.

3) *E.coli*virulence study

a) Congo red binding assay (Berkhoff and Vinal ., 1989)
Congo red (CR) binding test Congo red binding is one of the indicators of virulence among *E. coli* isolates. All of the *E. coli* isolates were tested for their growth status on Congo red medium. Incubation at 37°C then left at room temperature for an additional 2 days (not to exceed 4 days). The Congo red positive (CR+) isolates were indicated by the development of bright or orange red colonies. Different intensities in the dye uptake were (+) and (++). Congo red negative (CR-) isolates did not bind the dye and appeared as white colonies.

b) Hemolysis assay (Marilda *et al.*,1990).
E. coli isolates were propagated on blood agar base supplemented with 5% washed sheep erythrocytes. Blood agar plates were then incubated at 37°C for 24 hr. and colonies producing clear zones of hemolysis were then recorded as hemolysis positive.

4) Antimicrobial resistance (CLSI, 2016)
All purified isolates were tested by the standard disc diffusion method and were subjected to a susceptibility panel of antibiotics (Oxoid) belonging to different drug classes. Isolates were cultured in trypticase soy broth (TSB) supplemented with 0.6% yeast extract, and transferred to Mueller– Hinton agar (Oxoid). The plates were incubated at 37^{o}C for 48 hours.

a) Serum resistance assay (Fecteau *et al* ., 2001)
0.05 ml from cell suspension equal to 2.5x10^{8}cfu/ml in HBSS add to the same amount serum and incubated at 37^{o}C then 10 µl were plated on MHA 0 min and 180 of incubation , the plates were further incubated at 37^{o}C . Susceptibility of bacteria to serum bactericidal activity expressed as percentage of bacteria surviving after 180 min and overnight incubation in relation to the original growth of bacteria at 0 min in the controls.

5) Experimental design

a) Broiler chickens
Two groups each one consisted of 500 birds in separate partition

b) Vaccination program
- The two groups were vaccinated against (infectious bronchitis, infectious bursal disease and Lasota strains was given in drinking water at 16 day of age.
- 1st group was vaccinated with "BEST; Baculovirus Expressed H5N1-ND "inactivated vaccine at 10 day of age " 0.5 ml for each bird S/C".
- 2nd group was vaccinated with inactivated ND vaccine "0.3 ml for each bird S/C at 6 day of age" and also inactivated H5N3 vaccine "0.3 ml for each bird S/C at 10 day of age"

c) Bacterial strain used in experiment
1.5x10^{8} cfu/ml cell suspension in HBSS from the selected *E.coli* isolate which was serum resistance , multidrug resistance and Congo red binder was prepared according to (**Colle *et al* ., 1996**).

d) Experimental infection with *E.coli*(**Gjessing and Berkhoof ., 1989**)
100 bird from each group were eye dropped with 0.2 ml for each bird using the previously prepared bacterial suspension for 3 successive days and kept under natural conditions of the original flock with daily observation for any clinical signs and mortality till the end of rearing period of the flock "35 days of age ".

e) Serum samples
5 randomly serum samples were collected from each group in the 2 nd post last dose of *E.coli* for detection of IL6 level according to (**Kaiser *et al*., 2000**)

3. Result and Discussion

Colibacillosis is one of the main causes ofeconomic losses in the poultry industry worldwide (**Ewers et al., 2003**). Majority of economic losses result from mortality, decreased production, condemnations, costs of chemotherapy and eradication programmes. *E. coli*strains are often resistant to antimicrobials such as cephradine, tetracyclines, chloramphenicol (**Rahman et al., 2004; Hooda, 2009**), sulfonamides, b-lactam antibiotics (**Li et al., 2007; Renu, 2010**) and amino-glycosides (**Hooda, 2009; Renu, 2010**). Resistance to fluoroquinolones was reported withinseveral years of the approval of this class of drugs for use in poultry (**Li et al., 2007**). The selected *E.coli* isolate was Congo red binder which indicated by appearance of red colonies within 24 hr. at 37^{o}c, also it resist serum for 12 hr. and show hemolytic activity on sheep blood agar.Antibiogram revealed that it was highly resistance for Cefotaxime , Gentamycin , Colistin sulphate , Doxycycline , Ciprofloxacin and Sulfamethoxazole-trimethoprime and that is indication for multidrug resistant strain "MDR" .**BerKhoff and Vinal ., (1986),**found a direct correlation between the ability of clinical *E.coli* isolates to bind Congo red dye and their ability to cause septicemic infection in chickens , while (**Gaylen *et al* ., 2014**) recorded that there was a relationship between Congo red binding of *E.coli* and its biofilm formation with resistance to antimicrobial agents .

Serum resistance and hemolytic activity are important virulence associated properties in avian pathogenic *E.coli* (**da Rocha ., *et al* 2002**),and (**Guabirba and Schouler ., 2015**) who concluded that APEC strains constitute a heterogeneous group and different isolates may harbor associations of virulence factors each one is able to induce colibacillosis .In the present study , clinical signs , mortalities and PM lesions for each group were recorded in table (1) as 1st group showed slight respiratory manifestation , slight diarrhea and eye lid inflammation and lacrimation , with 8% mortalities begin at 3rd day post experimental infection and ended in 6th day with slight air sacculitis ,slight pericarditis, and slight liver enlargement, while the incontact broilers showed slight respiratory signs with 1.5% mortalities, in the other hand 2nd group recorded severe respiratory signs with nasal discharge , eye lid inflammation and diarrhea accompanied by 12 % mortalities with perihepatitis, pericarditis and air sacculitis as PM lesions while the incontact broilers recorded 2% mortalities .

Interleukin-6 (IL-6) is a highly pleotropic molecule, with demonstrated roles in diverse biological functions, is an endogenous chemical which is active in inflammation, and in B cell maturation. IL-6 participates in the short-term defense against infection or injury, and warns the immune system against the source of inflammation. However, defective regulation of this molecule results in disease. On the other hand, interleukin-6 overexpression has equally important effects. IL-6 has been demonstrated to be important for primary resistance to several pathogens, including *L. monocytogenes* (**Dalrymple *et al.*, 1995**), *Chlamydia trachomatis* (**Williams *et al.*, 1998**), *Escherichia coli* (**Dalrymple *et al.*, 1996**), and *Yersinia enterocolitica* (**Dube *et al.*, 2004**). The IL-6 receptor is present on normal T-lymphocytes in the resting phase, normal activated B-cells, and cells in the myeloid and hepatic cell lines. The present workIL6 serum level also was recorded for each experimentally infected groups in(fig 1) as follow , 1st group IL6 conc. was "55 , 62 , 35 , 40 and 55pg/ml " while for 2nd group IL6 conc.was "32 ,40,30,35and 40 pg/ml"

References

[1] BerKhoff, H.A. and Vinal, A.C. (1986): Congo red medium to distinguish between invasive and non-invasive *Escherichia coli* pathogenic for poultry. Avian Dis., 30(1):117-121.
[2] CLSI, 2016: Antimicrobial susceptibility testing standards.
[3] Collee, J.G; Fraser, A.G;Marmion, B.P and Simmons, A. (1996): Practical Medical Microbiology. 14th Ed.
[4] da Rocha A.c, Silva A.B, de Birto A.B , Moraes H.L , Potens A.B, (2002):Virulence factors of avian pathogenic *E.coli* isolated from broilers from south of brazil .Avian .Dis.46:749-753.
[5] Dalrymple, S.A., Lucian, L.A., Slattery, R, McNeil, T, Aud, D.M., Fuchino, S., Lee, F., and Murray, R., 1995. Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia. Infect. Immun. Vol. 63, p: 2262–2268.
[6] Dalrymple, S.A., Slattery, R., Aud, D.M., Krishna, M., Lucian, L.A., and Murray, R., 1996. Interleukin-6 is required for a protective immune response to systemic Escherichia coli infection. Infect. Immun. Vol. 64, p: 3231–3235.
[7] Dube, P.H., Handley, S.A., Lewis, J., and Miller, V.L., 2004. Protective role of interleukin-6 during Yersinia enterocolitica infection is mediated through the modulation of inflammatory cytokines. Infect. Immun. Vol.72, p: 3561–3570.
[8] Ewers, C., Janseen, T. and Wieler, L.H. (2003). Avain pathogenic E. coli (APEC). Berl. Munch. Tierarztl. Wochenschr. 116:381-395.
[9] Fecteau,G ; Fairbrother,J.M;Higgins,R;Van Metre , D.C. ;Smith , B.P; and Jang,S.(2001):Virulence factors in *Escherichia coli* isolated from blood of bacterimemic neonatal calves. Veterinary Microbiology, 78(3):241-249.
[10] 10-Gaylen A.ulich, Chin-yi chen, Bryan J.cottrell and Ly-Huong Nguyen,(2014): Growth media and temperature effects on biofilm formation by serotype O_{157}-$H_7$and non O_{157} shiga toxin producing *E.coli* .FEMS Microbial Lett. 354 (133-138).
[11] Gjessing,K.M and Berkhoof .H.A. (1989):Experimental production of air saccaulitis and septicemia by aerosol exposure of 1 day old chicks using Congo redpositive Escherichia coli .Avian Dis.,33(3):473-478.
[12] Gross W B. 1990. Factors affecting the development of respiratory disease complex in chickens. Avian Dis. 34: 607- 610.
[13] Guabirabay, R and Schouler,C .(2015):Avian colibacillosis still black holes .FEMS.Microbiol ,2015.
[14] Hooda, A. (2009). Etio-pathological studies on poultry mortality with special reference to gastrointestinal tract disorders. M.V.Sc. thesis submitted to CCS Haryana Agricultural University, Hisar.
[15] KaiserJ.T,Clausen,T.,Bourenkow,G.P,Bartunik.H.D,Steinbacher,S.andHuber,R.(2000):Crystal structure of NIFS-like protein from thermotoga maritime :implication for iron sulphur cluster assembly .J.Mol.Biol.297,451-464.
[16] Li, X.S., Wang, G.Q., Du, X.D., Cui, B.A., Zhang, S.M. and Shen, J.Z. (2007). Antimicrobial susceptibility and molecular detection of chloramphenicol and florfenicol resistance among Escherichia coli isolates from diseased chickens. J. Vet. Sci.8: 243-247.
[17] Lynne AM, Kariyawasam S, Wannemuehler Y, Johnson TJ, Johnson SJ, Sinha AS, Lynne DK, Moon HW, Jordan DM, Logue CM, Foley SL, Nolan LK. 2012. Recombinant Iss as a potential vaccine for avian colibacillosis. Avian Dis. 56(1):192–9.
[18] Marlida,C ;Ernest,E;Julio,C;Amauri,A;Lvens,G;and Diognes,S.(1990):Virulence factors of avian *Escherichia coli* .Avian Dis. 34:531-538 .
[19] Murray.P.R, Baron.E.J, Jorgensen.J.H, Pfaller.M.A, Yolken.R.H, 8th Ed. American Society for Microbiology; Washington, DC (2003): Manual of clinical Microbiology; PP.110-122.
[20] Nagano T, Kitahara R, Nagai S.2012. An attenuated mutant of avian pathogenic Escherichia coli serovar O78: a possible live vaccine strain for prevention of avian colibacillosis. Microbiol Immunol. 56(9): 605–12.
[21] Quinn, P.J; Markey, B.K; Carter, M.E; Donnelly, W.J.C; Leonard.F.C. and Maguire, D.(2002):Veterinary Microbiology and Microbial Disease .Published by Blackwell .PP.113-116.
[22] Rahman, M.A., Samad, M.A., Rahman, M.B. and Kabir, S.M.L. (2004). In vitro antibiotic sensitivity and therapeutic efficacy of experimental salmonellosis, colibacillosis and pasteurellosis in broiler chickens. Bangladesh J. Vet. Med. 2: 99-102.
[23] Renu (2010). Pathological investigation of the diseases affecting gastrointestinal tract of poultry. M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar.
[24] Ron, E.Z. (2006): Host Specificity of septicemic *E.coli*: human and avian pathogens .Curropin Microbiol, 9:28-32.
[25] Russell, S. M. (2003): The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of Campylobacter spp. and Escherichia coli. PoultSci., 82: 1326-1331.
[26] Vandekerchove D, de Herdt P, Laevens H, Pasmans F.2004. Colibacillosis in caged layer hens:

characteristics of the disease and the aetiological agent. Avian Pathol. 33(2):117–125

[27] Williams, D.M., Grubbs, B.G., Darville, T., Kelly, K., and Rank, R.G., 1998. A role for interleukin-6 in host defense against murine Chlamydia trachomatis infection. Infect. Immun. Vol. 66, p: 4564–4567.

[28] Yang H.2003. Animal immunology. 2nd ed. Beijing: China Agriculture University Press.

Table 1: Susceptibility and IL-6 serum level of broilers chickens after experimental infection with virulent *E. coli*

Items	1st group	2nd group
Live vaccines	• IB at 1 day old • IBD at 14 day old • Lasota at 16 day old	
Inactivated vaccine	BEST at 10 day old	ND 0.3 ml + H5N3 0.3 ml at 10 day old
Experimental infection with field virulent *E.coli* strain	100 birds from each group eye instilled with $1.5X10^8$ cfu/ml three successive days	
Clinical signs	• Slight respiratory manifestation • Slight diarrhea • Slight lacrimation and eye lid inflammation	• Sever nasal discharge • Severe diarrhea • Sever lacrimation • Sever eye lid inflammation
Mortality rate	8%	12%
PM lesion	Slight inflammation in serous membranes and liver enlargement	Pericarditis , peri hepatitis , air saculitis and enteritis
Mean average of IL6 conc.	50 pg/ml	35 pg/ml

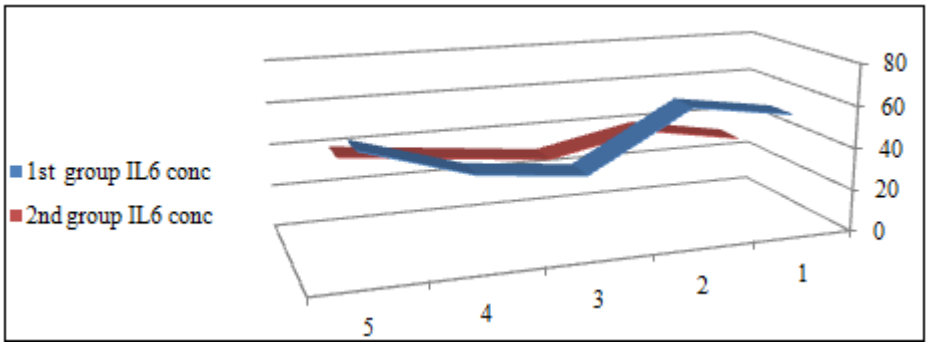

Figure 1: IL-6 Serum level of broilers chickens after experimental infection with virulent *E. coli*