Studies on the effects of cephradine and colibacillosis on immunological status of broiler chicken vaccinated with Newcastle virus vaccine

Ashraf Elkomy1, Mohamed Aboubakr1*, Elsayed Emam2, Mohammed Kassem3

1Pharmacology Department, Faculty of Veterinary Medicine, Benha University, 13736, Moshtohor, Toukh, Qalyubeya, Egypt
2Animal Health Research Institute (Zagazig branch), Egypt. 3Elanco Animal Health, New Cairo, Egypt
*Corresponding author E-mail: mohamed.aboubakr@fvtm.bu.edu.eg

Abstract

The present study was carried out using 100, one-day old broiler chicks to evaluate the immunological status of broiler chicks vaccinated with Newcastle virus vaccine and infected with E.coli and treated by cephradine. At day 15th of age, broilers chicks were divided into 4 equal groups (25 chicks in each). 1st group, healthy non infected non treated broilers (control group). 2nd, 3rd and 4th groups experimentally infected with E. coli was done at 15th day of age. 2nd group infected, non treated broilers, 3rd group infected broilers and vaccinated with Newcastle disease virus vaccine, 4th group infected broilers vaccinated with Newcastle vaccine and received 20 mg/kg b.wt cephradine in drinking water daily for 5 consecutive days. At 1st, 10th and 20th day post administration, blood samples were collected for determination cellular and humeral immune response. Infected broilers with E.coli only or infected broilers and vaccinated display significant increase in leukocyte, heterophils, phagocytic activity, phagocytic index, killing percentage, nitric oxide, lysozyme activity and gamma globulin. Beside significant decrease in lymphocyte, serum total protein, albumin, total globulin, A/G ratio and HI titer coupled with insignificant decrease in esinophils, basophils and monocyte, beta globulin associated with insignificant increase in alpha globulin allover experimental period post vaccination when compared with control broilers. Vaccinated-Infected broilers that received 20 mg/kg b.wt cephradine for five consecutive days revealed significant increase in leukocyte, heterophils, phagocytic activity, phagocytic index, killing % and gamma globulin at 1st day post treatment coupled with insignificant increase at 10th and 20th day post treatment. In addition to significant decrease in serum total protein, albumin, total globulin A/G ratio and HI associated with non significant decrease in esinophils, basophils, monocyte, beta globulin and non significant increase in nitric oxide, lysozyme activity and alpha globulin allover the experiment when compared with control broilers. It could be concluded that, colibacillosis in broiler chickens and cephradine induced some adverse effects on immunological status of broiler chickens. Therefore, it’s important not vaccinated broiler during colibacelosis or using cephradine in treatment.

Keywords: Broiler chickens; Cephradine; Colibacillosis; Immunity; Vaccine.

1. Introduction

Cephalosporins are a group of antibiotics derived from mould of cephalosporium spp. and are based on 7-aminocephalosporic acid which corresponds to 6-penicilanic acid in penicillins (El-Hewaily et al., 2014). Cephradine is a beta-lactam, first-generation cephalosporin antibiotic with bactericidal activity and available in both oral and parenteral dosage forms (Wilson and Gisvold, 1982). Cephradine have a good activity against Gram positive bacteria and moderate activity against some enterobacteria as strains of E.coli, Proteus mirabilis, Salmonella and Shigella (James, 1993). Antibacterial activity due to ability of beta-lactamase ring to bind bacterial enzyme transpeptid-ase, which important for proper cell wall synthesis (Thomson et al., 1984; El Sayed et al., 2016; Aboubakr and Elbadawy, 2017). Escherichia coli, usually abbreviated to E.coli, is one of the main species of bacteria normally inhabiting lower intestines of worm-blooded animals (birds and mammals) (Rosario et al., 2004). Colibacillosis affects poultry industry causing serious economic losses achieved by high mortality and lossbody weight (El-Nemr, 2011). Colibacillosis was associated with various disease conditions (Otaki, 1995). Acute form in poultry leads to septicemia and death (Calnek et al., 1997). E.coli infection in poultry associated with percarditis, perirepaticitis, airsacculitis, peritonitis, panopthalmitis and omphalitis (La Ragione and Woodward, 2002). Newcastle disease is an economically essential listed and highly frequent isolated worldwide virus due to its importance to the commercial poultry producers. Controlling of Newcastle disease virus by vaccination is and routinely applied by the majority of poultry production companies to supply immunological response against the disease (Darrell et al., 2013). The presentwork was conducted to throw light on the effect of E- coli infection and cephradine on immunological status of broilers during vaccination with Newcastle virus vaccine.
2. Materials and methods

2.1. Drugs

Cephradine (Atocef Forte®) water-soluble powder Each 100 gm of powder contain 20 gm cephradinee base. It is available as package containing 500 gm. It is produced by ATCO Pharma Company, Egypt.

2.2. Experimental broiler chickens

One hundred apparently healthy one day old Hubbard broiler chicks obtained from Cairo Poultry Company (CPC) were used in the present study. Chicks were floor reared under hygienic measures. Chicks were fed on balanced commercial ration free from any medications from Cairo Poultry Comp and water provided ad-libitum.

2.3. Newcastle vaccine

Avipro®Polybanco (Live Vaccine against Newcastle-Bronchitis B1 Type, B1 Strain, Mass. & Conn) Manufactured by Elanco animal health Co. was used as eye drops for vaccination of chicks on the 7th day of age against Newcastle disease.

2.4. Microorganisms and e. coli inoculum

E.coli strain (O.157) used in this study obtained from animal healthy research doki. Broth culture was standardized to give bacterial suspens-ion containing 3X 10⁹ viable organism/ml of E.coli O157 using Mac-Ferland tube. Each bird was given 0.3 ml via nasal route (Nakamura et al., 1992).

2.5. Experimental design

100 one-day-old Hubbard broiler chicks were divided in to four equal groups (25 chicks in each). 1st group; healthy broilers non-vaccinated non medicated served as control. 2nd, 3rd and 4th groups experimentally infected with E.coli was done at the 15th day of age. 2nd group infected, non-treated broiler chicks (+ve control) 3rd group infected broilers vaccinated with Newcastle disease vaccine only, 4thgroup infected broilers vaccinated with Newcastle disease virus vaccine and received 20 mg/kg b.wtcephradine daily for five consecutive days.

At 1st, 10th and 20th day post treatment 2 blood samples was collected. 1st sample was collected in test tub contain EDTA as anticoagulant for estimation total and differential leucocytic count according to Jain (1986). Phagocytic activity, Phagocytic index and killing % were determined according to Rouse et al., (1980) and Woldehiwet and Rowan (1990). 2nd sample was collected in test tub for obtain clear serum for estimation serum total protein according to Doumas et al., (1981), protein fractions were performed using cellulose acetate electrophoresis test according Henry et al., (1974). Serum Nitric oxide was measured according to Rajarman et al., (1998) and Ramadan and Atta (2003) and lysozyme activity (Schlitz, 1987).

2.6. Statistical analysis

Obtained data was analyzed (Petrie and Watson 1999).

3. Results

Infected broilers with E coli only or infected broilers and vaccinated display significant increase in leukocyte, heterophils, phagocytic activity, index, killing percentage, nitric oxide, lysozyme and γ globulin. In addition to significant decrease in lymphocyte, total protein, albumin, globulin and HI titer coupled with insignificant decrease in eosinophils, basophils, monocyte and β globulin associated with insignificant increase in α globulin allover experimental period post vaccination.

Vaccinated-Infected broilers, received 20 mg/kg b.wt cephradine daily for five days showed significant increase in leukocyte, heterophils, phagocytic activity, index, killing % and γ globulin at 1st day post treatment coupled with insignificant increase at 10th and 20th day post treatment. More-over significant decrease in total protein, albumin, total globulin and HI titers associated with insignificant decrease in eosinophils, basophils, monocyte, β globulin and insignificant increase in nitric oxide, lysozyme and α globulin allover experiment.

4. Discussion

Broiler chickens infected with E coli only or infected and vaccinated with Newcastle vaccine evoked significant leukocytosis, heterophilia and significant lymphocytopenia coupled with insignificant decrease in eosinophil, basophil and monocyte allover the experimental period. Leukocytosis in E coli infected bird may be due to inflammatory response in gastrointestinal tract due to bacterial infection (Doxey, 1983). Our results agreed with El-Nemr (2011) and Allam et al., (2014) they noticed that chickens infected with E. coli showed leukocytosis, heterophilia and lymphocytopenia associated with non significant decrease in eosinophil, basophil and monocyte count. Same change in leukogram was reported by Haq et al., (2015) in pigeons infected with E coli. Our result was parallel with result reported by Mohamed and Younis (2018) stated that colibacilosis induce leucocytosis due to heterophilia infected broilers with E coli, cephradine treated and vaccinated displayed significant leucocytosis and heterophilia beside significant lymphocytopenia at 1st day post treatment and insignificant effect in leukocyte, heterophil and lymphocyte at 10th and 20th day post treatment associated with insignificant decrease in esinophils, basophils and monocyte allover the experiment (Abd El-Aziz, 2006). These results are reinforced by Hassan (1996) who stated that rabbits infected with E coli treated with cefoperzone showed no harmful effects in leukogram. Our data clearly reinforced by those obtained by Mwafl (2000) who stated that another cephalosporin (Ceftiofur) im-
proved adverse effects of *E. coli* infection on leukogram. Same changes in leukogram were reported by Shawky (2007) who stated that infected broilers with *E. coli* cefoperazone treated improved leukogram.

Broilers infected with *E. coli* only or infected with *E. coli* and vaccinated displayed significant increase in phagocytic activity and index compared with control broilers. Same change in phagocytosis % and index was recorded in broiler infected with *E. coli* (Coles, 1986). These results were in agreement with those of El-Boushy et al., (2006) and EL-Sissi and Salman (2011) who stated that phagocytic activity and index were increased in *E. coli* infection in broilers. Our results go hand in hand with those obtained by Abd El-Ghany and Ismail (2014) and Allam et al., (2014) who stated that, *E. coli* infection induced elevation in phagocytic activity in broilers. Colibacelosis in broilers revealed significant elevation in phagocytic activity and killing % (Awad et al., 2015; Mohamed and Younis, 2018).

Chickens infected with *E. coli*, cephradine treated and vaccinated with Newcastle vaccine displayed significant increase in phagocytic activity, index and killing % at 1st day coupled with insignificant increase at 10th and 20th day post vaccination. Another cephalosporin (Cefodizime) capable of interfering with some bacteria as *E. coli* virulence parameters, thus facilitating host neutrophilic defenses such as phagocytosis, killing and oxidative bursts (Braga et al., 1999). Same changes in phagocytic activity, index and killing % were reported by Abd El hafez et al., (2006) in rabbits infected with *E. coli* and treated with cefotaxime.

Broilers infected with *E. coli* or vaccinated with Newcastle vaccine and infected with *E. coli* displayed significant increase in nitric oxide and lysozyme activity allowed the experimental period post infection. Nitric oxide and lysozyme increased in some bacterial infection and it acts as a free radical (Bredt and Snyder, 1994). This result comes in accordance with those of Tizard (1996) who reported that endotoxin in excreted by *E. coli* induced increase in nitric oxide and lysozyme. Increased Nitric oxide and lysozyme in bacterial infection is involved in innate immunity (Coleman, 2001). Chicken infected with *E. coli* infection and vaccinated with Newcastle vaccine increased in Nitric oxide in infected broilers and plays an important role in destroying invading microorganisms (Foley and Farrell, 2003). Same results were supported by Nakamura et al., (1994) who reported that chicken vaccinated with live Newcastle vaccine and infected with *E. coli* showed increased nitric oxide and lysozyme.

Chickens infected with *E. coli*, cephradine treated and vaccinated with Newcastle vaccine displayed insignificant increase in nitric oxide and lysozyme allowed the experimental period post infection. Same observation was reported by Abd El hafez et al., (2006) who reported that infected rabbits with *E. coli* treated with cefotaxime and vaccinated with another vaccine (hemorrhagic septicemia vaccine) induced insignificant increase in nitric oxide and lysozyme. Same changes in nitric oxide and lysozyme were reported by Huang and Matsumoto (2000) in chickens infected with *E. coli* and vaccinated with Newcastle disease virus vaccine.

Infected broilers with *E. coli* only or with vaccination displayed significant decrease on serum total protein, albumin, total globulin, A/G ratio and significant increase in γ globulin allowed experimental period post infection coupled with insignificant increase in α beside insignificant decrease in β globulin. Our findings were reinforced by El-Nemr (2011) which indicated that broiler chickens infected with *E. coli* showed significant decrease in serum total proteins, albumin and significant increase in globulins. This finding is in accordance with the finding of Yassin (2012) and Zaki et al., (2012) they found that serum total protein, albumin, and A/G ratio were significantly decreased associated with increase in globulin in broilers infected with *E. coli*. Our data coincides with El Sayed et al., (2014) and Mohamed (2015) who found that colibacelosis induced significant reduction in serum total protein and albumin. This result was supported by Kumari (2016) and Godbole (2017) who reported that broiler chicks experimentally infected *E. coli* revealed decrease in total protein and albumin concentration.

Infected chickens treated with cephradine or infected treated with cephradine and vaccinated showed significant decrease in serum total protein, albumin, total globulin and A/G ratio. Beside significant increase in serum γ globulin at 1st day post vaccination associated with non significant effect at 10th and 20th day post vaccination coupled with insignificant increase in α and insignificant decrease in β globulin allowed experimental period post vaccination when compared with control broiler chickens. Same changes were reported by Borowski et al., (1984) who stated that cephalosporins induced significant decrease in serum total protein, albumin, total globulin and A/G ratio. Our results were supported by results reported by Ahmad (2009) who mentioned that broiler vaccinated by Newcastle vaccine and received cetifluir sodium showed significant decrease in serum total protein, albumin, total globulin and A/G ratio beside significant increase in serum γ globulin. Same results were reported by Hegazy et al., (2006) who reported that broiler infected with *E. coli* and Newcastle disease vaccination revealed insignificant increase in α and insignificant decrease in β globulin.

Our results revealed that *E. coli* infection alone or *E. coli* infection and vaccination in broilers produced significant reduction in Ht titters allowed experimental period post infection. Meanwhile, infected with *E. coli*, Cephradine treated and vaccinated with Newcastle vaccine elicited significant decrease in Ht titters at 1st day post vaccination coupled with insignificant increase at 10th and 20th day post treatment when compared with control broilers. Reduction in Ht titters against Newcastle disease might be attributed to the immunosuppressive effect of *E. coli*. Colibacelosis in chickens induced vaccination failure immune response of chickens to Newcastle virus vaccine and induces significant reduction in Ht titters against Newcastle disease (Hassan and Hassanein, 1999). The obtained results were in agreement with El-Sissi and Mohamed (2011) who reported that broilers infected with *E. coli* showed significant reduction in Ht titters for Newcastle disease virus. Our observed data are in accordance with those reported by Abd El-Ghany and Ismail (2014) who stated that, *E. coli* infection induced reduction in Ht titters for Newcastle disease virus in broilers. Our data coordinates with those recorded by Awad et al., (2015) who recorded that *E. coli* infection decreased Ht titters humoral immune responses to Newcastle disease viruses.

**Table 1:** Effect of Vaccination and Cephradine (20 mg/Kg B. Wt) on Total and Differential Leucocytic Count of Broilers (N=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>10th day</th>
<th>20th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>T. Leukocyte</td>
<td>11.21±</td>
<td>12.18±</td>
<td>11.76±</td>
</tr>
<tr>
<td>10^9/µl</td>
<td>0.15</td>
<td>0.31*</td>
<td>0.13*</td>
</tr>
<tr>
<td>Hetero.</td>
<td>2.40±</td>
<td>3.69±</td>
<td>3.84±</td>
</tr>
<tr>
<td>Eosino.</td>
<td>0.11</td>
<td>0.0*</td>
<td>0.11*</td>
</tr>
<tr>
<td>Baso.</td>
<td>1.36±</td>
<td>1.30±</td>
<td>1.35±</td>
</tr>
<tr>
<td>Mono.</td>
<td>0.12</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>10^9/µl</td>
<td>1.65±</td>
<td>1.58±</td>
<td>1.44±</td>
</tr>
<tr>
<td>G1</td>
<td>0.24</td>
<td>0.26</td>
<td>0.27</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.
Table 2: Effect of Vaccination and Cephradine (20 mg/Kg B.Wt) on Phagocytic Activity Percentage, Phagocytic Index, Killing Percentage of Broilers (N=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>10th day</th>
<th>20th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>phagocytic activity%</td>
<td>40.13±</td>
<td>42.03±</td>
<td>41.95±</td>
<td>41.38±</td>
<td>40.15±</td>
<td>42.87±</td>
<td>41.67±</td>
</tr>
<tr>
<td>phagocytic index</td>
<td>4.86±</td>
<td>5.96±</td>
<td>5.86±</td>
<td>5.68±</td>
<td>4.86±</td>
<td>5.99±</td>
<td>5.80±</td>
</tr>
<tr>
<td>killing%</td>
<td>0.46±</td>
<td>0.23±</td>
<td>0.15±</td>
<td>0.14±</td>
<td>0.39±</td>
<td>0.24±</td>
<td>0.14±</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.

Table 3: Effect of Vaccination and Cephradine (20 mg/Kg B.Wt) on Serum Nitric Oxide and Serum Lysozyme Activity of Broilers (N=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>10th day</th>
<th>20th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide</td>
<td>26.16±</td>
<td>29.18±</td>
<td>29.21±</td>
<td>27.37±</td>
<td>27.11±</td>
<td>29.18±</td>
<td>29.05±</td>
</tr>
<tr>
<td>Lysozyme activity</td>
<td>94.13±</td>
<td>97.48±</td>
<td>97.44±</td>
<td>94.52±</td>
<td>93.89±</td>
<td>97.43±</td>
<td>97.21±</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.

Table 4: Effect of Vaccination and Cephradine (20 mg/Kg B.Wt) on Serum Total Protein and Protein Fractions of Broiler Chickens (N=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>10th day</th>
<th>20th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. protein (g/ml)</td>
<td>5.57±</td>
<td>4.99±</td>
<td>5.06±</td>
<td>5.12±</td>
<td>5.54±</td>
<td>5.09±</td>
<td>5.43±</td>
</tr>
<tr>
<td>Albumin (g/ml)</td>
<td>0.33±</td>
<td>0.41±</td>
<td>0.46±</td>
<td>0.15±</td>
<td>0.11±</td>
<td>0.45±</td>
<td>0.31±</td>
</tr>
<tr>
<td>a</td>
<td>0.21±</td>
<td>0.22±</td>
<td>0.18±</td>
<td>0.18±</td>
<td>0.02±</td>
<td>0.05±</td>
<td>0.02±</td>
</tr>
<tr>
<td>Globulin (g/ml)</td>
<td>0.63±</td>
<td>0.64±</td>
<td>0.63±</td>
<td>0.64±</td>
<td>0.62±</td>
<td>0.69±</td>
<td>0.66±</td>
</tr>
<tr>
<td>Total</td>
<td>6.83±</td>
<td>6.95±</td>
<td>7.02±</td>
<td>7.23±</td>
<td>6.99±</td>
<td>7.05±</td>
<td>6.97±</td>
</tr>
<tr>
<td>A/G</td>
<td>0.19±</td>
<td>0.20±</td>
<td>0.22±</td>
<td>0.19±</td>
<td>0.21±</td>
<td>0.23±</td>
<td>0.22±</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.18±</td>
<td>0.19±</td>
<td>0.19±</td>
<td>0.19±</td>
<td>0.19±</td>
<td>0.19±</td>
<td>0.19±</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.

References


