

Comparison of the Efficacy of Enrofloxacin Against *Escherichia coli* or *Pasteurella multocida* Infection in Chickens

Visut Rawiwet¹ Piyarat Chansiripornchai² Niwat Chansiripornchai^{1*}

Abstract

This study compared the efficacy of enrofloxacin, given immediately or 6 hrs after infection against *Escherichia coli* or *Pasteurella multocida*. In the first experiment, thirty, 12-week-old male layer chickens were divided into 3 groups of 10 each. Chickens in group 1, 2 and 3 were infected with *E. coli* serotype O78 by left thoracic air sac injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately receiving *E. coli*. Group 2 was given enrofloxacin 6 hrs after receiving *E. coli*. Group 3 served as a positive control. In the second experiment, twenty, 16-week-old male layer chickens were divided into 3 groups. Groups 1 and 2 have 7 chickens each and group 3 has 6 chickens. Chickens in group 1, 2 and 3 were infected with *Pasteurella multocida* by thigh muscle injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately after receiving *P. multocida*. Group 2 was given enrofloxacin 6 hrs after receiving *P. multocida*. Group 3 served as a positive control. The result revealed that enrofloxacin has efficacy to treat colibacillosis and fowl cholera. Chickens immediately treated with enrofloxacin showed lower mortality both of colibacillosis and fowl cholera infection. The lesion scores of airsacculitis, pericarditis and perihepatitis in the chickens immediately treated with enrofloxacin showed lower than the chickens treated 6 hrs later.

Keywords: Colibacillosis, enrofloxacin, fowl cholera, treatment

¹Avian Health Research Unit, ²Department of Veterinary Pharmacology, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri Dunant Rd., Bangkok 10330, Thailand.

Corresponding author E-mail: cniwat@chula.ac.th

บทคัดย่อ

เปรียบเทียบประสิทธิภาพของเอนโรฟลอกซาซินต่อการติดเชื้อ เอสเชอริเชีย โคไล และ พาสจูเรลลา มัลโตซิดา ในไก่

วิสุทธิ ะวิเวช¹ ปิยะรัตน์ จันทรศิริพรชัย² นิวัตร จันทรศิริพรชัย^{1*}

การศึกษานี้มีวัตถุประสงค์ในการเปรียบเทียบประสิทธิภาพของเอนโรฟลอกซาซิน โดยการให้ยาทันที หรือให้ยา 6 ชั่วโมงภายหลังการติดเชื้อ เอสเชอริเชีย โคไล และ พาสจูเรลลา มัลโตซิดา ในการทดลองแรก ไก่ไข่เพศผู้อายุ 12 สัปดาห์ จำนวน 30 ตัว แบ่งออกเป็น 3 กลุ่มๆ ละ 10 ตัว ไก่ในกลุ่ม 1 2 และ 3 ได้รับเชื้อ อี. โคไล ซีโรไทป์ O78 โดยฉีดเข้าถุงลมช่องท้องด้านซ้าย โดยกลุ่ม 1 ได้รับเอนโรฟลอกซาซิน (10 มก./กก.) โดยละลายยาในน้ำทันทีหลังจากได้รับเชื้อ อี. โคไล กลุ่ม 2 ได้รับเอนโรฟลอกซาซิน 6 ชั่วโมง หลังจากได้รับเชื้อ อี. โคไล กลุ่ม 3 เป็นกลุ่มควบคุมบวก ในการทดลองที่ 2 ไก่ไข่เพศผู้อายุ 16 สัปดาห์ จำนวน 20 ตัว แบ่งออกเป็น 3 กลุ่ม กลุ่ม 1 และ 2 ไก่กลุ่มละ 7 ตัว กลุ่ม 3 ไก่ 6 ตัว ไก่กลุ่ม 1 2 และ 3 ได้รับเชื้อ พี. มัลโตซิดา โดยการฉีดเข้ากล้ามเนื้อหน้าแข้ง โดยกลุ่ม 1 ได้รับเอนโรฟลอกซาซิน (10 มก./กก.) โดยละลายยาในน้ำทันทีหลังจากได้รับเชื้อ พี. มัลโตซิดา กลุ่ม 2 ได้รับเอนโรฟลอกซาซิน 6 ชั่วโมง หลังจากได้รับเชื้อ พี. มัลโตซิดา กลุ่ม 3 เป็นกลุ่มควบคุมบวก ผลการทดลองพบว่าเอนโรฟลอกซาซินมีประสิทธิภาพในการรักษาโรคโคไลบาซิลโลซิส และโรคอหิวาต์สัตว์ปีก ไก่ที่ได้รับยาเอนโรฟลอกซาซินทันทีมีอัตราการตายที่ต่ำกว่าทั้งการป่วยด้วยโรคโคไลบาซิลโลซิส และอหิวาต์สัตว์ปีก ค่าคะแนนรอยโรคของถุงลมอักเสบ ถุงหุ้มหัวใจอักเสบ เยื่อหุ้มตับอักเสบ ในไก่ที่ได้รับเอนโรฟลอกซาซินทันที ต่ำกว่าไก่ที่ได้รับการรักษาหลัง 6 ชั่วโมง

คำสำคัญ: โคไลบาซิลโลซิส เอนโรฟลอกซาซิน อหิวาต์สัตว์ปีก การรักษา

¹ หน่วยปฏิบัติการวิจัยสุขภาพสัตว์ปีก² ภาควิชาเภสัชวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 39 ถ.อรัญญิก ปทุมวัน กรุงเทพฯ 10330 ประเทศไทย

*ผู้รับผิดชอบบทความ E-mail: cniwat @chula.ac.th

Introduction

Escherichia coli is a normal organism of the gut microflora of the chickens, and most serotypes are not pathogenic. However, a number of serotypes of *E. coli* are pathogenic, they are inhaled into the respiratory tract and are probably the most frequent and economically significant cause of bacterial disease known as colibacillosis in chickens. The most common form of colisepticemia is characterized by an infection of the air sacs. The air sacs of poultry lie outside of the lungs in the single body cavity. These expandable air sacs function principally as airways; when they are forced to expand and contract air is moved through the relatively inexpandable lungs. Airsacculitis is often associated with bacteremia, septicemia, pericarditis and perihepatitis, usually resulting in approximately 5% mortality in commercial situations (Wray et al., 1996).

Fowl cholera caused by the Gram-negative, non-motile, coccobacillary bacterium *Pasteurella multocida*, has been known to occur in a variety of wild and domestic birds and to cause major economic losses in the poultry industry. Death results primarily from peracute and acute septicemia and less frequently from the chronic and localized form of the disease.

Infections of chickens with *E. coli* or *P. multocida* can be prevented and controlled by antibiotics. However, a serious drawback of the prophylactic use of such chemotherapy is the development of resistance in avian pathogenic *E. coli* (APEC) to most antibiotic (Chansirpornchai et al., 1995). Genes that are located on plasmids often encode resistance to antibiotics. These plasmids easily spread through bacterial populations, which lead to the spread of resistance, so rendering the drugs ineffective. One group of antibiotics, the fluoroquinolones, is less likely to undergo this fate, because they inhibit the synthesis of DNA gyrase A, which is essential for duplication of plasmid (Glisson, 1994; Chansirpornchai et al., 1995)

Fluoroquinolones are broad-spectrum drugs with a bactericidal activity and their uses for the prevention and control of avian infections with *E. coli* and *P. multocida*. Enrofloxacin is antibiotics belonging to the fluoroquinolone class of compounds. This drug is more potent than any earlier analogues, has a broad spectrum of activity and drug resistant bacteria are induced less frequently (Wolfson and Hooper, 1988).

The objectives of the current experiment were to compare the efficacy of enrofloxacin against *E. coli* and *P. multocida* infection in the different times of drug application.

Materials and Methods

Chickens: Unvaccinated male Babcock layer chickens were obtained on the day of hatching from a commercial hatchery. The chickens were fed *ad libitum* before and during the experiments. At the onset of the experiments there was no statistically significant difference in average weight between the experimental groups. Chickens in experiments 1 and 2 were brought from different batches of the same hatchery so that there might be differences among chickens in each experiment. 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.0831068.

Bacterial strains: The chickens were challenged with an *E. coli* strain of serotype O78 that was originally isolated from the diseased air sacs of a chick with a field case of colisepticemia. Sekizaki et al. (1989) showed that this *E. coli* strain produced high mortality in a very short time. In the first experiments, we injected 1.0 ml of the *E. coli* suspension, containing 10^6 cfu/ml. *E. coli* suspension was injected into the left caudal thoracic air sac of infected chickens. In the 2nd experiment, the chickens were challenged with *P. multocida* strain 8A that was also originally isolated from the liver of the fowl cholera infection chicken. One ml of *P. multocida* suspension, containing 10^5 cfu/ml was injected into each bird. *P. multocida* suspension was injected into the left thigh of infected chickens.

Medication: Enrofloxacin (Quinoxine-10, F.E. Pharma Co. Ltd., Thailand) was administered to the chickens in their drinking water at the concentrations specified in the experimental designs. The water intake for birds had been measured before the experiments were performed. So, we knew approximately the water intake for our chickens and the quantity of drugs dissolved in the water could be calculated so that we knew the amount of drug intake for each group per average chicken weight.

Experimental designs: The age of chickens of 1st and 2nd experiments was differed. Consequently, the positive control groups in both experiments were included in order to compare the data within each experiment. The bacteria were challenged immediately after the experiments started. In the experimental groups, enrofloxacin was given immediately or 6 hrs later in the drinking water of chickens for twice a day. The enrofloxacin was given to the chickens within 2 hrs for 5 consecutive days. The morbidity and mortality were recorded for the seven and three days following infection of the chickens with *E. coli* and *P. multocida*, respectively. The pathological lesions in the dead chickens were investigated at necropsy.

Experiment 1: To study the efficacy of enrofloxacin given immediately or 6 hrs later in the drinking water of chickens that were challenged with *E. coli*. Thirty, 12-week-old male layer chickens were divided into three groups of 10 each. Chickens in group 1, 2 and 3 were infected with *E. coli* serotype O78 by left thoracic air sac injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately after receiving *E. coli*. Group 2 was given enrofloxacin 6 hrs after receiving *E. coli*. Group 3 served as a positive control.

Experiment 2: To study the efficacy of enrofloxacin given immediately or 6 hrs later in the drinking water of chickens that were challenged with *P. multocida*. Twenty, 16-week-old male layer chickens were divided into three groups of 7, 7 and 6 chickens in each group, respectively. Chickens in groups 1, 2 and 3 were infected with *P. multocida* serotype 8A by left thigh muscles injection. Group 1 was given enrofloxacin (10 mg/kg), dissolved in drinking water immediately receiving *P. multocida*. Group 2 was given enrofloxacin 6 hrs after receiving *P. multocida*. Group 3 served as a positive control.

Efficacy criteria and definitions: Mortality was defined as the number of birds that were killed or that died before the end of the trial. Morbidity was defined as the number of birds with either air sac, pericardium or perihepatic lesions. The airsac, pericardial and perihepatic lesions of colisepticemia in each bird were scored. The air sac 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. (1998) 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. (1998) as follows: 0: no visible lesions, 1: definite fibrination on the surface of the liver, 2: extensive fibrination, adhesions, liver swelling and necrosis.

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

Efficacy of enrofloxacin treatments against *E. coli* infection: The chickens in groups 1 and 2 which received enrofloxacin immediately and 6 hrs after challenged with *E. coli* revealed less morbidity and mortality comparing to the positive control group. The mortality of chickens in group 1 was statistically significant less than ($p < 0.05$) that of the control group. The post mortem pathology of the *E. coli* challenged chickens were airsacculitis, fibrinopurulent perihepatitis, fibrinopurulent pericarditis and peritonitis. These typical clinical symptoms were similar to the enrofloxacin treated group and the non treated group, but the lesions of airsacculitis, fibrinopurulent pericarditis, and fibrinopurulent perihepatitis were less in the treatment group. Also, the lesion scores of airsacs, pericardium and livers of chickens in group 1 was statistically significant less than ($p < 0.05$) that of the control group (Table 1).

Table 1 Pathological parameters of chickens infected with *E. coli* and *P. multocida*.

<i>E. coli</i> infection			Lesion scores from <i>E. coli</i> infection			<i>P. multocida</i> infection		
Group	Morbidity	Mortality	Airsac ^x	Pericardium ^y	Perihepatic ^z	Group	Morbidity	Mortality
1	0/10 ^a	0/10 ^a	1.1±0.99 ^a	0.8±0.79 ^a	0 ^a	1	0/7 ^a	0/7 ^a
2	0/10 ^a	3/10 ^{a,b}	2.2±1.32 ^{a,b}	1.2±0.63 ^{a,b}	0.3±0.67 ^b	2	0/7 ^a	1/7 ^a
3	1/10 ^a	5/10 ^b	2.7±1.70 ^b	1.7±0.48 ^b	0.3±0.48 ^b	3	0/6 ^a	6/6 ^b

^xAir sac lesions were scored on a scale of from 0 to 4.

^yPericardial lesions were scored on a scale of from 0 to 2.

^zPerihepatic 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% ($p < 0.05$)

Efficacies of enrofloxacin treatments against *P. multocida* infection: The chickens in groups 1 and 2 which received enrofloxacin immediately and 6 hrs after challenged with *P. multocida* revealed less morbidity and mortality comparing to the positive control group. The mortality of chickens in group 1 and 2 was statistically significant less than ($p < 0.05$) that of the positive control group. All chickens in the positive control group were died. The post mortem pathology of the *P. multocida* challenged chickens were generalized hemorrhage at the various muscles especially heart and thigh muscle. The hemorrhage also found at the coronary fat. All of chickens died within one day after the challenge showed typical lesions of hemorrhage. The typical clinical symptoms were similar to the enrofloxacin treated group and the non-treated group, but the hemorrhage was less in the treatment group (Table 1).

Discussion

In the experiment, chickens were challenge with *E. coli* serotype O78 produced 50% mortality and 10% morbidity in the positive control group. The mortality rate in the experiment is much higher than that of the field cases. In the field cases, the mortality level is 0.25% in the primary and increase to 1% after infected for 5 day and can rise up to 10% (Shane et al., 1981; Wray et al., 1996). In the experimental model, 5% mortality was found after challenge with less than 10⁴ CFU of *E. coli*, but it increased to 50% when the challenge dose was increased to 10⁶ CFU (Dunnington et al., 1991). This data is according to our infection model because the high mortality was observed among the infected, non-medicated birds.

Death losses from fowl cholera in chickens usually occur in laying flocks, because birds of this age are more susceptible than younger chickens. Chickens less than 16 weeks of age generally are resistant to the fowl cholera infection. In the *P. multocida* infection model used in this experiment produced 100% mortality in non medicated birds. This level is much higher than the 50% mortality in the field that may occur by high challenge dose (10⁶ CFU). Under experimental conditions, 90-100% of mature chickens exposed by swabbing the palatine cleft may die within 24-48 hrs. The mortality was 35-45%, depending on the strain of *P. multocida* used, but only 10-20% usually dies within a 2 weeks period when exposed by contact with infected birds (Pritchett et al., 1930).

In both experiments showed that the immediately enrofloxacin treatment and 6 hrs treatment after infection of *E. coli* and *P. multocida* were not significantly reduce pathological lesions and mortality; indeed, there was a trend for lower mortality and fewer pathological lesions in the bird was treated with enrofloxacin immediately after infection than in the bird was treated with enrofloxacin 6 hrs later. The general lesions developed by inoculation APEC or bacteria-free culture filtrates into the air sac. Airsacculitis occurs within 1.5 hrs. Bacteremia and pericarditis develop within 6 hrs and most mortality occurs during the first 5 day (Derosa et al., 1992). Thus the bird was treated with enrofloxacin 6 hrs after *E. coli* infection had tend to reduce lesion and mortality less than the bird was treated with enrofloxacin immediately because the lesion had occurred before receiving drug. In the *P. multocida* model the bird was treated with enrofloxacin immediately infection had not significant lower mortalities than the infected birds that treated with enrofloxacin 6 hrs after infection. The result of this study also accorded to the findings of Chansiripornchai (2007) that Chickens received antibiotics 1 hr before challenges, immediately after challenges or 1 hr after challenges. The result revealed that enrofloxacin has efficacy for prevention and treatment of fowl cholera. No death chickens had been found in these 3 enrofloxacin treated groups. However, FCR of the chickens received antibiotics before the challenges tended to be better than those of chickens received antibiotics immediately after challenges or 1 hr after challenges. The current experiment revealed the efficacy of enrofloxacin to treat colibacillosis and fowl cholera, the results may cause by the merit of rapid tissue penetration of enrofloxacin. A time to maximum (T_{max}) of enrofloxacin (Baytril®) in serum, lung and muscle is 0.5, 2 and 2 hrs, respectively. Also, a maximum tissue concentration (C_{max}) of enrofloxacin (Baytril®) in serum, lung and muscle is 0.77, 1.76 and 1.75 µg/ml or µg/g, respectively (Franz, 2006). The T_{max} of enrofloxacin is faster than the incubation period of colibacillosis and fowl cholera around 24 hrs (Chansiripornchai, 2007; 2009). A minimum inhibition concentration of the *E. coli* isolate is 0.5 µg/ml (data not shown), the level is still lower than the C_{max} level of enrofloxacin in serum, lung and muscle.

In conclusion, the pathological lesions and mortality of the *E. coli* infected birds which immediately treated with enrofloxacin after infection had not significant lower than those of the infected

birds that treated with enrofloxacin 6 hrs after infection. The mortality of the *P. multocida* infected birds with immediately treated with enrofloxacin infection had not significant lower than those of the infected birds that treated with enrofloxacin 6 hrs after infection. But the treatment of enrofloxacin would provide the less morbidity, mortality, clinical findings or pathological findings compared to the non-treatment group.

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