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Full Length Research Paper

Comparative efficacy of doxycycline and flumequine against experimentally induced colibacillosis in broiler chicks

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This study sought to determine comparative efficacy of doxycycline and flumequine for the treatment of Colibacillosis, which is an acute septicaemic disease caused by pathogenic *Escherichia coli*. This disease has illness coming on in the range widely, death rate is relatively high and some are up to more than 30%, have caused the enormous economic losses. The birds were infected intra-peritoneally with approx. 3×10^8 colony forming units per 0.25 ml of Enterotoxigenic *E. coli* (ETEC) and the infection developed within twelve hours. Chickens in group A1 were given doxycycline via the drinking water at a dose of 10 mg/kg body weight for 5 days, while group B1 was treated with flumequine at a dose of 12 mg/kg body weight for 5 days. The trial lasted for 9 days and then the surviving chickens were sacrificed. Doxycycline reduced the number of deaths and the severity of the clinical symptoms. In contrast, flumequine slightly influenced the mortality; however, it delayed death and reduced the severity of clinical symptoms. Present data indicate that doxycycline is highly effective for the treatment of experimental *E. coli* in chickens. The present study is of great importance for prescribing best effective drug against colibacillosis to avoid getting resistance to antibiotics.

Key words: Colibacillosis, doxycycline, flumequine, broilers.

INTRODUCTION

Inspite of the scientific development, poultry industry is still in the grip of various diseases of bacterial, viral, fungal and parasitic origin. Among the bacterial diseases, colibacillosis is one of the most frequently encountered problems. It is an acute septicaemic disease in intensively raised birds, caused by *Escherichia coli* (*E. coli*) and characterized by pansystemic involvement and great economic losses (Anjum, 1997). The disease causes high morbidity and mortality throughout the life span of poultry from an egg to an adult bird and constantly results in huge economic losses (Barnes and Gross, 1997; Ewers et al., 2003). In the past few years, both the incidence and severity of colibacillosis have increased rapidly and current trends indicate that it is prevail continue and has even greater problem in the poultry industry (Altekruse et al., 2002; Blanco et al, 1997).

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed et al., 1993; Watts et al., 1993). *E. coli* may be sensitive to many antibiotics. The use of doxycycline for the treatment of experimentally induced colibacillosis in broilers with doxycycline through the drinking water was effective and the achieved therapeutic effects were similar to those of tetracycline and of flumequine (Goren et al., 1988). In comparison with amoxicillin the recovery is faster with flumequine, which remains the best treatment of Colibacillosis (Mogenet et al., 1997).

Antimicrobial therapy is an important tool in reducing the

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Table 1. Vaccination schedule for different diseases.

S/no.	Age of birds	Vaccine type	Route of administration
1.	3 days	Laosta NDV	Eye drop
2.	12 days	Gumboro vaccine	Eye drop
3.	16 days	Hydropericardium vaccine	Inj. S/C 0.3 ml
4.	19 days	Lasota NDV	Drinking water
5.	22 days	Gumboro vaccine	Drinking water

the enormous losses in the poultry industry caused by *E. coli* infections (colibacillosis). However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Blanco et al., 1997). There usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Witte, 1998; Keyes et al., 2000).

The increasing use of antibiotics for prophylactic, therapeutic and nutritive purposes in veterinary medicine creates a potentially powerful selective pressure for the spread of antibiotic resistance. So the development of bacterial resistance to an antibiotic is one of the unfortunate results of therapeutic use. In order to carry out antibacterial therapy on a rational basis, the clinician needs to have an accurate and reliable guidance regarding which antibiotics can be effectively used, to provide this guidance the present project was designed to carry out the biological trials in parallel with an assessment of antibiotic sensitivity. The current study was designed to check the efficacy of two different antibiotics that is, doxycycline (Tetracycline) and flumequine (Quinolone) against experimentally induced colibacillosis in broiler chickens. This will guide veterinarian regarding the selection of drugs at proper time, minimizing the risk of mortality. The study is mainly focusing to find the sensitivity and efficacy of available drugs both *In-vitro and In- vivo* conditions and to recommend the best drug against colibacillosis to avoid getting resistance to antibiotics.

MATERIALS AND METHODS

Housing, feeding, management and grouping of Broilers

One hundred and fifty, day old broiler chicks were reared in Bio-park at the University of Malakand. These chicks were vaccinated against different diseases during rearing see Table 1. Water and feed were freely available to all the chicks.

At the age of 28 days, one hundred birds were selected from the 150 reared birds. The selected hundred birds were divided into two major groups that is, group A and B. Each group was then further divided into two sub groups that is, A1 and A2 and group B into B1and B2, each comprising 25 birds.

E. coli strain and inoculation

Enterotoxigenic *E. coli* (ETEC) strain was provided by poultry pathology section of Veterinary Research Institute Peshawar. The pathogenic *E. coli* was cultured and characterized by using the method described by Arenas et al. (1999). After finding out the viable cell count, the broth was diluted to have approximately $3x10^8$ bacteria per 0.25 ml and was used for inducing the infection. The diluted broth culture of pathogenic strain of *E. coli* having $3x10^8$ of bacteria per 0.25 ml was inoculated to all groups intra-peritoneally as described by Arenas et al. (1999). All the groups were kept under keen observation for the development of clinical signs of *E. coli* infection. The clinical signs appeared with in twelve hours after the inoculation of *E. coli*.

Treatment

After the appearance of clinical signs, group A1 was medicated with doxycycline at the dose rate of 10 mg/kg b.wt in drinking water for 5 consecutive days, while A2 was kept as un-medicated control. To the group B1, Flumequine was administered at the dose rate of 12 mg/kg b.wt in drinking water for 5 consecutive days while B2 was kept as un-medicated control.

Postmortem examination

The morbidity, mortality and postmortem lesions were noted. The mortality in the infected chicks started and recorded at 24, 48 and 72 h after the inoculation of *E. coli*. The mortality after medication in each group was recorded and the efficacy of the 2 different drugs was compared. Mortality percentage after medication was calculated as follows:

$$\text{Mortality rate (\%)} = \frac{\text{Number of birds died during treatment X 100}}{\text{Total No. of birds at the beginning of the treatment}}$$

RESULTS

The *E. coli* was obtained from the Veterinary Research Institute Peshawar and was further sub-cultured for identification and to conduct various biochemical tests. By culturing the respective pathogenic strain, following data was obtained.

Cultural and colony characteristics

The colonies developed on Mac Conkey's agar were pin pointed, smooth, glossy and translucent and were rose pink in color. The size of the colony varied from 2 - 3 mm in diameter after 24 h of incubation at 37°C. The colonies developed on nutrient agar were dome shaped, round,

Table 2. Sensitivity pattern of various antimicrobials against *E. Coli*.

S/no.	Antimicrobial drug	Highly sensitive	Quite sensitive	Moderate sensitive	Resistant
1	Amoxicillin	-----	-----	++	-----
2	Doxycycline	-----	+++	-----	-----
3	Enrofloxacine	++++	-----	-----	-----
4	Flumequine	-----	-----	++	-----
5	Gentamycin	-----	+++	-----	-----

convex, colorless and smooth. The size of colonies varied from 1 - 2 mm in diameter after 24 h of incubation at 37°C. In nutrient broth, a slimy deposit was developed in the bottom of the tube which was slight pellicle after 24 h of incubation and by shaking the tube. A uniform turbidity appeared in the tube. On Eosin Methylene Blue agar, the colony developed after 24 h of incubation at 37°C were 2 - 3 mm in diameter and exhibited greenish metallic shine by the reflected light and dark purple centers by transmitted light.

Staining and motility

The smears were stained with gram's staining and microscopy was performed. All the isolates were Gram negative rods and motile.

Biochemical reactions

Acid and gas were produced by the fermentation of various sugars like glucose, lactose and sucrose within one day incubation at 37°C. Methyl red test was performed which was positive for all isolates while no hydrogen sulphide was produced.

Antibiogram of *E. coli*

The five different drugs that is, Doxycycline, Flumequine, Gentamycin, Amoxicillin and Enrofloxacine discs were used for antimicrobial susceptibility test for *E. coli* strain. The sensitivity pattern of *E. coli* strain against the aforementioned antimicrobial drugs is summarized in Table 2.

Pathogenicity of *E. coli*

The purified strain of *E. coli* was injected intra-peritoneally in 5 chicks and another 5 chicks were kept as control group. The inoculated group of chicks was kept under observation till the appearance of clinical signs. The clinical signs appeared with in twelve hours after the inoculation of *E. coli* and mortality started within 16 - 24 h. The clinical signs observed were rise in temp, in-appetince, dullness, depression with closed eyes. Postmortem lesions of the dead chicks showed slight congestion of liver and heart and whitish inflammatory fluid accumulation in thoracic and peritoneal cavities. The stained impression smear prepared from heart blood and liver of the dead chicks was examined for the presence of organisms. The organism showed typically morphological and staining reaction which confirmed the presence of *E. coli*.

Anti microbial trails in experimental broilers

For *in-vivo* antimicrobial trails, on appearing the signs, to sub-group A1, doxycycline was administered and sub-group A2 was kept as un-medicated control. Same protocol was adopted for the group B. On appearing the clinical signs after twelve hours of inoculation, Flume-quine was administered to sub-group B1 and B2 was kept as un-medicated control.

Clinical signs

The clinical signs developed after twelve hours in all the inoculated groups. Mortality in all the groups started after 16 h of inoculation. The clinical signs and gross pa-thological lesions observed are given below. Postmortem findings for all the groups have been mentioned in Table 3.

Determination of antibiotics efficacy

After the appearance of clinical signs of colibacillosis the sub- group A1 was treated with Doxycycline at the dose rate of 10 mg/kg b.wt while sub-group A2 was kept as un-medicated control to check the doxycycline efficacy against *E. coli* infection in poultry. In the same way the diseased broilers in sub-group B1 was treated with Flumequine at the dose rate of 12 mg/kg b.wt and the sub-group B2 was kept as un-medicated control to check the flumequine efficacy against *E. coli* infection in poultry. Efficacy of doxycycline and Flumequine were compared by comparing the mortality rates in both medicated and un-medicated sub-groups.

Mortality rate

After initiation of doxycycline to sub group A1, the mortality

Table 3. Gross pathological lesions of *Colibacillosis* in all the groups.

Organs affected	Postmortem findings		
	24 h post infection	48 h post infection	72 h post infection
Heart	Slight pericarditis	Pericarditis	Pericarditis.
Intestine	Mild congestion.	Mild congestion	Mild enteritis.
Kidney	----------	Slight congested.	Slight congested.
Liver	Necrotic and hemorrhagic	Necrotic and hemorrhagic	Necrotic and hemorrhagic
Lung	Slight congested.	Congested.	Congested.
Spleen	Slightly congested	Slight congested.	Slight enlarged and congested.

rate was 56%. In sub-group A2, mortality recorded throughout the experimental period was 76%. The mortality rate in un-medicated group was high as compared to medicated group as shown in Figure 1. In contrast the observed mortality rate for flumequine treated group (sub group B1), the mortality rate was 65%. While in un-medicated (sub-group B2) the recorded mortality throughout the experimental period was 79%. In the same way the mortality rate in un-medicated group was high as compared to medicated group as shown in Figure 1. In the similar way the mortality rate is higher in flumequine treated group (B1 = 65%) as compared to doxycycline treated group (A1 = 56%). It shows that doxycycline is more effective against Colibacillosis as compared to flumequine.

DISCUSSION

In the present study all the broilers were similar in gaining the infections and showing the pathological lesions and mortality percentage. The primary lesions of experimentally induced colibacillosis were pericarditis and the pericardial fluid became progressively more fibrinous up-to 72 h post infection and the inflammatory lesions spread to adjacent tissues of lungs and liver. The experimentally produced lesions of *E. coli* infections were similar to those of natural infection. The postmortem lesions of the chicks died post infection had necrotic foci in the heart muscles, pericardial sac was thickened and pale color gelatinous exudate was present. These findings have also been supported by the findings of Jiang et al. (2005). It has been documented that there were primary lesions in birds died due to colibacillosis included airsacculitis and pericarditis with occasional findings of perihepatitis.

The present results of gross pathological lesions in colibacillosis are also in correlation with the necropsy findings in ostriches (Cooper, 2005). In present study, there was slight spleenomegaly and also there was mild enteritis after 72 h of infection which is also been reported by Jiang et al. (2005). Same results for pathology of colibacillosis have also been published by Saif, reporting that colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *E. coli* (APEC), including colisepticemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), cellulites (inflammatory process), swollen-head syndrome, peritonitis, salpingitis, osteomyelitis/synovitis (turkey osteomyelitis complex), panophthalmitis, and omphalitis/yolk sac infection (Saif, 2003).

Today, *E. coli* is linked to a wide range of clinical diseases of poultry, such as yolk sac infection, air sac disease, bacteriaemia, acute septicaemia, salpingitis, peritonitis, swollen head syndrome, cellulitis, enteritis, arthritis, omphalitis and coligranulomatosis, affecting chickens, turkeys and ducks (Rosenberger et al., 1985; Gross, 1991; Gross, 1994) . These all findings are in correlation with the results of present study.

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed et al., 1993; Watts et al., 1993). *E. coli* may be sensitive to many antibiotics. However, isolates of *E. coli* from poultry are frequently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over a long period (e.g., tetracyclines) (Allan et al., 1993; Blanco et al, 1997; Watts et al., 1993). The *E. coli* was found much sensitive to doxycycline as compared to flumequine, used in present experiment both *in-vivo* and *in-vitro* conditions. The absence of acute deaths in the chickens medicated with doxycycline, the appearance of clinical signs and lesions were less severe than those found in chickens which were kept un-medicated control. These findings are indicative of the effectiveness of the treatment used. Same results have also been documented after comparison of efficacy of doxycycline, chlortetracycline and linco-spectinomycin against *E. coli* infection in young chickens (George et al., 1977). This effectiveness has also been supported by the results of Goren et al., by proving that doxycycline is effective against *E. coli* infection in poultry (Goren et al., 1988). Same results have been mentioning that doxycycline prevents mortality and reduced *E. coli* lesions in broiler chickens (Velkers et al., 2005) . In view of our results, we can conclude that treatment of *E. coli* infection in chickens with doxycycline medicated through drinking water (10 mg/kg b.wt.) prevents the disease, reducing the severity of clinical signs and lesions.

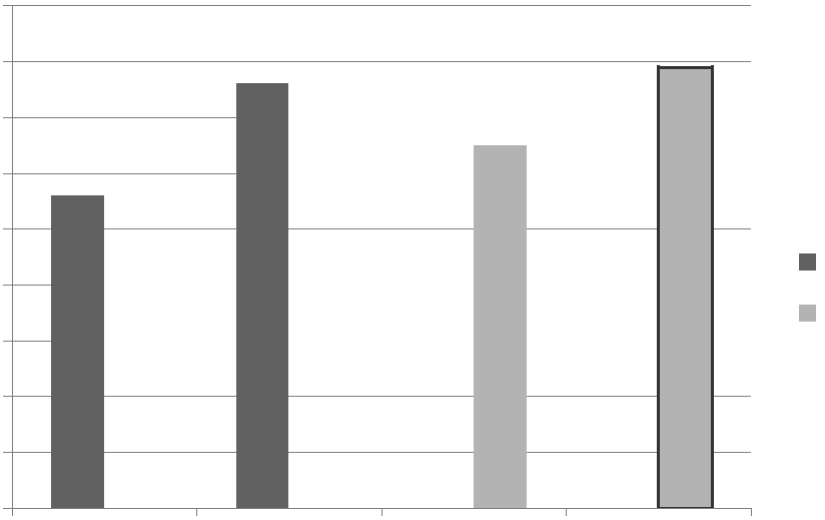

Figure 1. Mortility rate befor and after medication with Doxycycline and Flumequine. Group A1 was medicated with dose rate of 10 mg/kg b.wt of doxycycline, grope A2 was un-medicated control for A1, group B1 was medicated with 12 mg/kg b.wt of flumequine and grope B2 was un-medicated control for B1. In the same way the mortality rate in un-medicated group was high as compared to medicated group and also mortality rate is high in flumequine treated group (B1 = 65%) as compared to doxycycline treated group (A1 = 56%).

Mortality rate in the chickens medicated with flumequine was high, clinical signs and lesions were more severe than the chickens medicated with doxycycline. These results are pinpointing of the inefficiency of the treatment used. From the present results, it has been concluded that *E. coli* is more sensitive to doxycycline as compared to flumequine. Similar results have observed after isolating quinolone-resistant *E. coli* strains from poultry in Saudi Arabia (Khac et al., 1996). The present results have also been justified by Salehi and Bonab, according to them, *E. coli* showed 94% resistance to flumequine (Salehi et al., 2006). The findings that. *E. coli* showed more resistance to flumequine as compared to doxycycline also validates our results (Webber and Piddock et al., 2001; Van-den- Bogaard et al., 2001). *E. coli* resistance to flumequine is also supported by Garau et al. (1999) as he suggested that the high prevalence of fluoroquinolone- resistant avian *E. coli* in the stools of healthy humans in their area (Barcelona, Spain) could be linked to the high prevalence of resistant isolates in poultry and pork.

Conclusion

The present study was designed to check the efficacy of two commercially available antibiotics against colibacillosis in broiler chickens. In present study, after inoculation of pathogenic *E. coli*, general clinical signs of colibacillosis such as rise in temp, in-appetince, dullness, depression with closed eyes etc were observed. Gross pathological lesions were also undertaken after performing the postmortem of infected birds, showing lesions of lung, liver, spleen, intestine, kidney and heart. After appearance of clinical signs, antibiotic medication was made to infected birds. It was found that doxycycline is better to control the colibacillosis as compared to flumequine. Doxycycline reduced the mortality rate in comparison with that of flumequine as mortality rate was still high after flumequine administration to infected birds. The present work will help the veterinarian in diagnosing and prescribing the suitable drug for treating colibacillosis in broiler chicks.

RECOMMENDATION

Broiler chicks are susceptible to numerous diseases of bacterial, fungal or parasitic origin. For some diseases afflicting boilers, control is mainly based on bio-security. Diseases normally result in significant losses to producers and in order to maintain a healthy and profitable enterprise, producers must implement, with assistance from the local veterinary authority, comprehensive, practical and effective methods of health management and preventative medicine. Clearly much research needs to be carried out to gather more information on diseases in ratites, including gaining information of the regulations

for meat after drug administration for a particular ailment.

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