

Effect of Oral Treatment Flumequine on an Experimental Colibacillosis on Chicken of Flesh (Algeria)

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Abstract: Two quinolones, flumequine and enrofloxacin (For comparison) were administered in two modes of drinking interval (03 hours and 24 hours of watering) in treatment of experimental colibacillosis by inoculation of a highly pathogenic serotype of *Escherichia coli* (O78K80) (using a main suspension adjusted to 10⁹ CFU / ml) on two broiler strains. Symptom score, mortality and lesional score (which define the clinical criteria established for this study) were higher in treated drinking lots on 24h and particularly more pronounced in the ISA 15 animal strain. The antibiotic molecule, the mode of administration and animal strain seems to play a role in the effectiveness of treatment. Distributed over 3h of watering, flumequine remains an effective treatment in avian colibacillosis.

Key words: Oral Treatment • Fluméquine • Quinolones • Expérimental Colibacillosis • Clinical Criteria • Broilers

INTRODUCTION

The *Escherichia coli* infections are responsible for avian colibacillosis, a complex syndrome which are characterized by the airsacculitis, the polyserositis, septicemia and other extra-intestinal disorders mainly in chickens, turkeys and other avian species. It is through several experimental studies that the pathogenicity of *E. coli* has been detected in poultry indicating the growing importance of this pathogen agent in poultry breeding [1 - 6].

In broilers, the *Escherichia coli* infections are often associated with these serotypes *O1:K1*, *O2:K1* and *O78:K80* [3 - 9], causing significant economic losses in the poultry industry [3 - 8, 10 - 12].

In avian therapy, the quinolones and specifically the flumequine, prove to be the most effective and represent a reference treatment of an avian colibacillosis [10, 13 - 15]. That is why we tested two quinolones (Flumequine and enrofloxacin) based on the mode of

administration and duration of treatment (03h/24h and 24h/24h of watering) on two broiler strains (Identified strain: ISA15 and unidentified strain: farm's chicken) in the treatment of an experimental avian colibacillosis [4, 13].

Through this experimental study, we want to evaluate the therapeutic efficacy of these two quinolones administered orally, according to their watering duration on two broilers strains and to define the therapeutic regimen best suited for flumequine in the treatment of avian colibacillosis.

MATERIALS AND METHODS

Animals and Housing: The experiment was conducted on identified broiler strain (ISA 15) and unidentified (Farm's chicken), brought up on a litter of wooden shavings and subjected to natural lighting and static ventilation. Hoppers and siphon-like drinking troughs in appropriate number complete the equipment of breeding [4, 10, 13, 16].

Allotement: The allotment was made 4 days before the incubation (D-4). The subjects were divided in 6 experimental groups for every animal origin at the rate of 10 subjects / m².

- 1st and 2nd lot of 20 subjects for each inoculated and treated with the fluméquine on 03h/24h and 24h/24h watering respectively,
- 3rd and 4th lot of 20 subjects for each inoculated and treated with enrofloxacin on 03h/24h and 24h/24h watering respectively,
- 5th and 6th lot of 10 subjects for each as control (Positive control=T+, inoculated and untreated and negative control=T-, uninoculated and untreated respectively) [4, 13].

Inoculums and Inoculation: The experiment was carried out using a collection of highly pathogenic strain of *Escherichia coli* (from a main suspension adjusted to 10⁹ CFU / ml) obtained from Veterinary Regional Laboratory of Constantine. The inoculums of serotype O78:K80 was prepared in the same day of use by diluting the suspension in buffer media PBS (Phosphate Buffer Saline) at pH of 7.4 [13 - 16, 17].

The inoculation was performed at D0, on subjects of 21 days old, by deep intramuscular injection into the breast muscle of a dose diluted in 1/5 (Selected dose) in a volume of 0,1ml [4, 13, 16, 18].

Treatment: Depending on whether the mode of treatment of drinking 03h/24h or 24h/24h watering, the dose of flumequine (Fluméquine 10% in.) and enrofloxacin (Baytril® 10% po) was calculated based on the weight of the bird and the amount of water consumed per day to allow the ingestion of a therapeutically effective dose. Treatments are introduced in drinking water 48 hours after inoculation (D+2) [4, 13, 16]. The duration of treatment was 3 days for enrofloxacin (D+2 to D+4) and 3 to 5 days for flumequine (D+2 to D+4 or D+2 to D+6) according to changes in symptoms [4, 13].

Determination of clinical criteria: The study protocol is based on the following clinical criteria [10, 13, 16] :

Symptomatic Score: all observed symptoms from the 21st day are collected and classified according to the degree of their evolution with a score ranging from 0 to 5 and also according to the affected animal strain [13].

Mortality: Throughout the period of the experimental farm, mortality is regularly raised at a rate of: once a day (Morning) from the 1st the 20th day and twice a day (Morning and evening) from the 21th to 38th day [13].

Lesional Score: The lesions observed in the experiment are collected and classified according to the degree of their evolution with a score ranging from 0 to 6 and also according to the affected animal strain [13].

Consumption of Water and Food: The quantities of water and food consumed are raised every day throughout the period of breeding experimental. These quantities recorded allow to appreciate the appetite of animals and therefore, their health status [13].

Animal Weight: in the experimental breeding, the weight is raised every day. It allows to appreciate the growth of animals and establishing the therapeutic dose for treatment of experimental colibacillosis [13].

RESULTS

Symptomatic Score: Prostration is the first symptom observed in all inoculated subjects. It represents the essential element on which it relied to establish the symptom score. There is also, of locomotion disorders that affect both animal strains. Symptoms evolve as nervous disorders that are represented by abnormal wearing of the head and convulsions observed only in the ISA 15 strain (Table 1).

Mortality:

There Are Two Peaks of Mortality: A first peak 24 hours after installation chicks in the breeding building; a second peak 24 hours after inoculation of the pathogen. Mortality occurs in all inoculated batches and mainly affects the ISA 15 strain. It is more important for the groups receiving 24-hour treatment of drinking.

Lesional Score: Lesions begin with a congestion of the abdominal viscera, which in a first time, affects the liver and spleen and affects both animal strain. Lesions evolve in the ISA 15 strain only in the form of a fibrin deposit on the liver and a urate deposit in the ureters (Table 2).

Table 1: Evolution of symptoms of experimental colibacillosis by animal strain

| Score | Observed symptoms | Animal strains |
|-------|---|----------------|
| 0 | No symptoms | ISA 15 and |
| 1 | Chicken is prostrate, standing and easy moves in the solicitation | Range chicken |
| 2 | Chicken is prostrate, standing or lying and difficult moves in the solicitation | |
| 3 | Chicken is prostrate, lying and not moves in the solicitation | |
| 4 | Chicken is prostrate, lying and presents an abnormal wearing of head | ISA 15 |
| 5 | Chicken is prostrate, lying and presents convulsions | |

Table 2: Evolution of lesions of experimental colibacillosis by animal strain

| Score | Observed lesions | Animal strains |
|-------|--|----------------|
| 0 | No lesions | ISA 15 and |
| 1 | Abdominal viscera slightly congested and shiny | Range chicken |
| 2 | Severe congestion of the liver | |
| 3 | Severe congestion of the liver and spleen | |
| 4 | Severe congestion of the liver, spleen and kidneys | ISA 15 |
| 5 | Hypertrophy and severe congestion of the liver with thick fibrinous deposit covering the liver | |
| 6 | Severe congestion of the kidneys with a deposit of urate (ureters whitish) | |

Weight-Consumption of Water and Food: Subjects exhibit a significant growth delay following an average consumption of food and water especially for the groups receiving 24-hour treatment of drinking.

DISCUSSION

The results obtained from clinical criteria established confirm the interest of the treatment delivery on 3-hours compared to continuous mode (24 hour). Indeed, symptomatic score, mortality and lesional score are higher in the treated groups over 24 hours and particularly more pronounced in the ISA 15 strain [13].

The symptomatic score reveals the presence and severity of nerve disorders represented by convulsions which are not described so far in an avian colibacillosis. The observed symptoms range from the simple prostration to the marked prostration, titubation, paralysis and finally convulsions. The subjects with convulsions register a highest symptomatic score [13].

The lesional score established, allows to evaluate the extension of the lesions and the target organs. Contrary to what has been reported in the literature, there is no respiratory disorder, digestive (Intestinal) or cardiac ; by cons, there is a nervous system disorder which is expressed through the observed symptoms of prostration which evolve toward a convulsion condition. The lesions are mainly concentrated on the liver with an attack or not of spleen but also on the urinary tract ; indeed, all subjects rated at 5, present hypertrophy and severe congestion of the liver with thick fibrinous deposit

covering the liver (Table 2) and those rated at 6, present a severe congestion of the kidneys with a deposit of urate in the ureters. All of these lesions are observed in the strain of ISA 15 only, from where a higher mortality rate among these latter strain compared to range chickens [13].

The severely ill subjects escape treatment, which clearly explains the low consumption of water and food as well as growth retardation observed, hence the necessity to establish a treatment over a very short period (3 hours of watering) to ensure of the treatment effectiveness in the beginning of disease progress, contrary to what has been reported in the study of Lezzar [13] and Mogenet *et al.* [16].

We arrived at the same reflection made by Bensari [10], Bensari [11], Lezzar [13] and Mogenet *et al.* [16], namely that « Inoculation by deep intramuscular injection of *E. coli* O78 K80 triggered a rapidly chaging of colibacillosis». In the addition to the inoculation route, the dose of the inoculum and the pathogenicity of the strain appear to be the main determinants. This pathogenicity is explained by the fact that O78 K80 is an immunosuppressive pathogen agent [14] and which acts on the young birds males who are predisposed to the disease and, consequently, to an immunosuppressive pathogen [19].

This colibacillosis seems to be related to another factor, that of the animal strain ; indeed, it is best expressed in the ISA15 strain that reaches a high lesional score (6) and in a more attenuated in chickens farmer, which seems more resistant [13].

The mode of administration of the antibiotic is another criterion of appreciation since all of treated groups on 24 hours showed a high mortality rate compared to that of the groups treated on 03 hours [13]. According to Mogenet *et al.* [16], Bezille and Borne [20] and Borne [21] this is explained by a high plasmatic concentration of flumequine (x2) which is reached 04 hours after the start of the treatment on 03 hours watering [13].

CONCLUSION

In this study, we could establish the effect of oral treatment of flumequine on an experimental colibacillosis on chicken on flesh. It was found that the symptomatic score, mortality and lesional score (Defining the established clinical criteria) were higher in the treated groups on 24 hours and particularly more pronounced in the ISA 15 strain and this, some is the molecule of antibiotic administered.

The molecule of antibiotic (Flumequine or enrofloxacin) and its mode of administration (On 03 hour of watering) contribute to the success of the treatment. "Distributed on three hours of watering, the flumequine remains a reference treatment in avian colibacillosis."

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REFERENCES

1. Dho-Moulin, M. and J.M. Fairbrother, 1999. Avian pathogenic *Escherichia coli* (APEC). Vet Res. 1999 Mar-Jun, 30(2-3): 299-316.
2. Vandemaele, F., A. Assadzadeh, J. Derijcke, M. Vereecken and B.M. Goddeeris, 2002. Avian pathogenic *Escherichia coli* (APEC). Tijdschr Diergeneesk. 2002 Oct 1, 127(19): 582-8.
3. Ahmed, H.A., H.M. Mekky and G.M. El-Sadek, 2012. Studies on Vaccination of Turkey Against *Escherichia coli* Infection. Global Veterinaria, 8(6): 601-604.
4. Lezzar, N., C. Bensari, A. Lezzar, F. Smati, K. Benlabeled, C. Bentchouala, H. Laouar and D. Satta, 2015. Effect of the Administration of Flumequine by Oral Route on the Resistance of *Escherichia coli* Against Quinolone During an Experimental Colibacillosis on Chicken of Flesh (Algeria). Global Veterinaria, 14(1): 17-22.
5. Khedr, M.M.S., F.R. El-Seedy, S.M. Shafei, W.S.A. Shell, S.S. El-Mahdy and M.A. Sadek, 2015. Preparation and Evaluation of Combined Inactivated Vaccine Against Salmonellosis and Colibacillosis in Chickens. Global Veterinaria, 14(2): 205-210.
6. Gadallah, F.M., S.S. Salama and F.R. El-Seedy, 2015. Preliminary Study on Bacterial Strains Used in the Preparation of Polyvalent Inactivated Vaccine Against Chronic Respiratory Disease in Chickens. Global Veterinaria, 14(3): 287-291.
7. Ewers, C., T. Janssen and L.H. Wieler, 2003. Avian pathogenic *Escherichia coli* (APEC). Berl Munch Tierarztl Wochenschr. 2003 Sep-Oct, 116(9-10): 381-95.
8. Aggad, H., Y.A. Ammar, A. Hammoudi and M. Kihal, 2010. Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens with Colibacillosis. Global Veterinaria, 4(3): 303-306.
9. Mellata, M., M. Dho-Moulin, C.M. Dozois, R. Curtiss III, B. Lehoux and J.M. Fairbrother, 2003. Role of avian pathogenic *Escherichia coli* virulence factors in bacterial interaction with chicken heterophils and macrophage. Infection and Immunity, Janvier, 71(1): 494-503.
10. Bensari, C., 1999. Essai de standardisation d'un modèle de colibacillose aviaire expérimentale. Maitrise Es-Sciences Vétérinaires, Ecole Nationale Vétérinaire de Lyon (France).
11. Bensari, C., 2009. Reproduction expérimentale d'une colibacillose chez le poulet. Comparaison de l'efficacité d'une Fluméquine et d'une Amoxicilline par rapport à une Enrofloxacin de référence dans le traitement de cette pathologie. Thèse de Doctorat en Sciences Vétérinaires, Institut des Sciences Vétérinaires, Université Constantine1, Constantine (Algérie).
12. Yogaratnam, V., 1995. Analysis of the causes of high rates of carcass rejection at a poultry processing plant. The Veterinary record, 137: 215-217.

13. Lezzar, N., 2006. Influence d'un traitement oral à la flumequine sur la résistance aux quinolones des souches d'*Escherichia coli* dans la flore fécale du poulet de chair. Thèse de Magister en Sciences Vétérinaires, Institut des Sciences Vétérinaires, Université Constantine1, Constantine (Algérie).
14. Lecoanet, J., 1992. Colibacilloses Aviaires. In Manuel de Pathologie Aviaire, édition Maison Alfort, pp: 237-240.
15. Stipkovits, L., 1988. Studies on the Efficacy of Baytril in Chicks after Experimental Infection with *Mycoplasma gallisepticum* and *E. coli*. *Vet. Med. Rev.*, 59: 103-107.
16. Mogenet, L., P. Bezille, J. Guyonnet and H. Karembe, 1997. Comparaison de la fluméquine (Flumisol) à l'amoxicilline (Vetrimoxin poudre orale) dans deux modes d'administration par voie orale, en traitement de la colibacillose du poulet : approche pharmacodynamique et clinique. *Revue Méd. Vét.*, 148(10): 793-804.
17. Arp, L.H., 1985. Effect of antibodies to type 1 Fimbriae on clearance of fimbriated *Escherichia coli* from the blood stream of turkeys. *Am. J. Vet. Res.*, December, 46(12): 2644-2647.
18. Millemann, Y., C. Mouline, J.P. Lafont and E. Chaslus-Dancla, 2005. Bacteraemia assays in chickens as a model for the evaluation of the virulence of *Salmonella enterica* serovars Typhimurium and Enteritidis strains. *Revue Méd. Vét.*, 156 : 70-76.
19. Turqi, S., 2000. Protectotyping of avian Infectious Bronchitis Virus-Importance and applications. *Poultry Middle East and North Africa*, 22nd Year Nbr., 155, Nov.-Dec. pp: 11-13.
20. Bezille, P. and P.M. Borne, 1996. Clinical evaluation of the effects of the timing of flumequine administrations on an experimental colibacillosis in poultry. *Proceedings of the 20th WPSA Congress*, New-Delhi, Inde, 2-5 septembre, 1996: 358-359.
21. Borne, P.M., 1995. Application concrete de la notion d'antibiotique concentration-dépendant: utilisation de Flumisol® dans le traitement des affections bactériennes des volailles. *Recueil des conférences des Rencontres Internationales de Production Avicole*, Nantes, France, 4 Octobre 1995: 19-35.