

DANOFLOXACIN AS AN EFFICIENT ANTIMYCOPLASMAL AGENT

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Abstract

The efficacy of Danofloxacin in treatment and prophylaxis of experimentally infected chicks was tested. One hundred Hubbard chicks were divided into five groups of twenty each. First group was infected at 15 days old with virulent strain of *Mycoplasma gallisepticum* (MG) S-6 then, treated with Danofloxacin (50ppm) one day after the appearance of the symptoms. Second group took a prophylactic dose for three days then, it was challenged with S-6, followed by treatment after 24 hours from the appearance of symptoms. Third group was dosed with Danofloxacin prophylactically without infection for 7 days and considered as residue group. Fourth group was used as negative control (uninfected, untreated). Fifth group was positive-control (infected, untreated).

The results revealed that the second group (prophylactic then infected) gave more body weight and internal organs weight gain than the first (Infected then treated) and third (residue) groups which both gave nearly same results.

Clinical symptoms were severe in the fifth and first groups accompanied with high mortality rate (80, 25%), respectively. Re-isolation of *M.gallisepticum* was successful in first (infected, treated), second (infected, prophylactic) and fifth (positive-control) groups.

Haemagglutination-inhibition (HI) titer was high in the first group during the first week after treatment, then, lowered by the second week. Meanwhile, HI titer of the second group was lower in first and second weeks after treatment than the first group, while, the residue and negative-control groups (3,4) showed a very high titer by the second week.

INTRODUCTION

For many years, workers have reported numerous attempts to treat mycoplasmosis in chickens and turkeys with various drugs and antibiotics. No substance has yet been found which completely could eradicate the pathogenic

organisms from all birds. Clinical improvement, better weight gains and feed conversion are often accomplished, and the majority of secondary bacteria, etc., were eliminated; but frequently *M.gallisepticum* remained latent in the treated birds, ready to infect fresh susceptible birds or to re-invade the original birds when their resistance or immunity became again lowered (Newnham, 1963). The problem of drug resistance also comes into the picture. *M.gallisepticum* became resistant to streptomycin extremely rapidly (Domermuth, 1960 and Osburn *et al.* 1960), and also to the tetracyclines which are often included at a low level in some proprietary diets (Newnham, 1963). However, some resistance could be detected due to chromosomal mutation (Jordan, 1981, christainsson and Mardh, 1983).

So, many efforts are required to overcome the problem of drug resistance and this can be done by using new antimicrobials. Fluoroquinolones are a relatively new class of potent synthetic agents, with a broad spectrum antibacterial activity, including strain resistant to many other antibacterial agents (Neer, 1988, Vansaene and Percival, 1991 and Guay, 1992). The informations about the in vitro and/or in vivo activity of Danofloxacin against *M.gallisepticum* are limited (Jordan *et al.*, 1993, Bradbury *et al.*, 1994).

The present work aimed to study the efficacy of Danofloxacin with regard to:
-The control and treatment of the infected chicks.
- Effect on gain weight, feed conversion, gross lesions, reisolation of and antibodies to *M.gallisepticum*.

MATERIALS AND METHODS

Antimicrobial agent : Danofloxacin was obtained as a pure powder from Pfizer Ltd. Company and was used as oral droppings (50 ppm).

Chicks : One hundred, Hubbard broiler chicks were obtained at one day old from Middle East Poultry Company. All birds were examined by clinical and laboratory methods according to Jordan and Kulasegram (1968) to be sure that they were free from Mycoplasma. At age of two weeks, the chicks were weighed individually and allocated at random into five groups of twenty birds. Each group had approximately the same average weight.

Mycoplasma strain : Virulant strain of *Mycoplasma gallisepticum* (S-6) was kindly supplied by Prof. Dr. Aly El-Dbeedy, Director General, Animal Health Research Institute, Giza.

Culture Medium : PPLO medium was prepared according to Hayflick (1965).

Infection : 0.1 ml (containing 10^9 CFU) of *M.gallisepticum* (S-6) was sprayed into groups 1, 2 and 5 only. Chicks of groups 3 and 4 were sprayed with sterile diluents.

Haemagglutination - inhibition test (HI) : It was used as described by Meszaros (1964).

Experimental Design : One hundred chicks were divided into five groups:

1. First group: was infected with virulent strain of (S-6) and treated orally with Danofloxacin in drinking water (50 ppm) after 24 hours of starting symptoms of Mycoplasma for four days, then, measuring the efficacy of Danofloxacin by changes in body weight, mortality rate, clinical symptoms, HI antibodies, and re-isolation of *M.gallisepticum*.
2. Second group: An attempt was made to protect or prevent Mycoplasmosis in experimental chicks by giving the tested chicks Danofloxacin for three days, and after 24 hours the chicks were challenged with (S-6) then, treated for another four days to measure the efficacy as done with the first group.
3. Third group: the residual effect of Danofloxacin was studied by giving it for seven days, then, slaughtering the birds after other seven days.
4. Fourth group: was used as untreated negative control.
5. Fifth group: It took the infection and was left without any treatment as positive control. The experimental design was summarized in Table 1.

Table 1. Summary of the experimental design for testing Danofloxacin Efficacy in chicks.

Group	Design	Age/Day	Experiment
1	Infected, then Treated	15 19	Infected with (S-6). Treated orally, Daanofloxacin (50 ppm)
2	Prophylactic, Infected, then Treated.	12 15 19	Orally, Danofloxacin (50 ppm). Infected with (S-6). Treated, other 4 days.
3	Residue	15	Orally, Danofloxacin (50 ppm/7 days).
4	Uninfected, untreated	15	Negative - Control.
5	Infected, untreated	15	Infected with S-6 (Positive - Control).

RESULTS

Danofloxacin was tested experimentally in 100 chicks to study its effect in the control and treatment of *M. gallisepticum* infection. The results of the experiment revealed more body weight and internal organs gain in the third and fourth group 690-710g (Residue and negative control groups), respectively. The body weight of the second group which took Danofloxacin before infection was higher than the first group which did not take it before infection (620, 515 g), respectively. The fifth infected group showed the least body weight (390 g). Similar findings were noticed on the mean weight of liver, kidneys and spleen. Table 2 summarized the changes in mean body weight and internal organs during three weeks period.

Clinical symptoms included congestion and oedema of lungs, turbidity of air-sacs and tracheal exudate. The signs were severe in the positive control group (5th) and the infected, then, treated one (1st) accompanied with mortality rate (80 and 25%), respectively. Neither symptoms nor deaths were noticed on the residue and negative-control groups 3rd and 4th.

The Haemagglutination-inhibition titer of the third and fourth groups were negative. The first group showed higher HI titer starting from the first week, then, lowered after second week of treatment (1:256 then 1:128, respectively). The titer in second prophylactic group (infected and treated) was lowered in the second week of treatment (1:128 then 1:64), respectively.

Table 2. Changes in mean body weight and internal organs in chicks tested for Danofloxacin efficacy.

Group	Mean Body weight / g			Mean Internal Organs / weight / g								
	Week after treatment			Liver			Kidney			Spleen		
	1st	2d	3d	1st	2d	3d	1st	2d	3d	1st	2d	3d
1	195	396	515	5.7	11.4	12	2	4.7	5.1	0.1	0.5	0.9
2	307	456	620	9.8	14.7	14	3.2	4.6	6.2	0.4	0.9	1.1
3	326	480	690	10.3	15	17.5	4.4	5.8	6.9	0.6	0.9	1.3
4	466	650	710	11	16.2	19.7	4.6	6.1	6.9	0.9	1.2	1.5
5	170	285	390	3.9	5	6.1	1.8	3.5	0.2	0.1	0.35	0.9

Re-isolation of *Mycoplasma gallisepticum* was successful in the first, second and fifth groups (infected then, treated; prophylactic then treated and positive control groups). Negative re-isolation results were obtained from third and fourth groups (residue and negative). Summary of these data was given in Table 3.

Table 3. Changes in clinical symptoms, mortality, HI titer, and reisolation of *M.gallisepticum* in chicks groups with Danofloxacin.

Group	Clinical Signs	Mortality (Total 20)		HI titer (after treatment)*				Re-isolation of Mg (Total 7 Birds) After treatment	
				First week		Second week		1st week	2d week
				Min.	Max.	Min.	Max.		
1	++	5	25	1:128	1:256	1:64	1:128	6	4
2	+/-	2	10	1:64	1:128	1:32	1:64	4	2
3	-	0	0	1:8	1:16	1:8	1:16	0	0
4	-	0	0	1:8	1:16	1:8	1:16	0	0
5	+++	16	80	1:256	1:512	1:512	1:1024	7	7

* Positive = 1: 64 and above (Meszaros, 1964).

DISCUSSION

Recently, a number of new Quinolone antibacterial drugs have been synthesized which showed improved anti-Mycoplasma activity over Quinolone compounds that were available hitherto (Hannan *et al.* 1989). Jordan *et al.* (1993) compared the efficacy of Danofloxacin and Tylosin in the control of *M.gallisepticum* infection in chicks, and found that the control of clinical signs and mortality was better, and more weight gain was obtained with Danofloxacin at 21 days after infection. Bradbury *et al.* (1994) carried out similar evaluation using Danofloxacin, a new Quinolone antimicrobial drug, against *M.gallisepticum* and *M.synoviae* and found that they were susceptible to it with minimal inhibitory concentration from 0.008 to 0.5 ug/ml.

Our results indicated that, protection based on the survival of chicks proved the efficiency of Danofloxacin in the control of mycoplasmal infection. As shown in Table 2, the mean body weight of treated group (1) was 515 g, prophylactic group (2) 620g and reached 690 g in residue group (3). The untreated groups (4, 5) reached 710 g for the negative-control and 390 g for the positive-control group. The same results were noticed regarding the mean weight of the internal organs (liver, spleen and kidneys). The oral administration of Danofloxacin (50 ppm) minimized the depression in weight due to Mycoplasma infection, and reduced the severity of secondary infection. Similar observations were noticed by Gale *et al.* (1967) and Levisohn *et al.* (1980) who found that birds which were infected and treated with Tylosin and Tiamin were heavier than the untreated ones. Kempf *et al.* (1992) studied the efficacy of Danofloxacin in the therapy of experimental mycoplasmosis in chicks and found that, treatment with Danofloxacin and Tylosin

significantly decreased mortality rate and increased weight gain compared with un-medicated birds. Similar findings were reported by Tanner *et al.* (1993) and Migaki *et al.* (1993) revealed that the decrease in both the frequency of *M.gallisepticum* re-isolation and serconversion that occurred following Danofloxacin treatment indicated rapid elimination of organisms from the system.

Haemagglutination-inhibition titer in group 1 (infected, treated) and 2 (prophylactic, treated), as well as, the positive-control (infected, untreated) were positive. Similar findings were noticed by Fahey and Crawley, 1955 and Newnham (1963). There is generally considerable individual variation in response among birds, towards both infection and HI antibody titer level. This experiment gave good picture of this variation, and of the misleading interpretation that can be obtained if only the clinical aspect of respiratory mycoplasmosis is considered. Even when birds appear clinically normal, the pathogens may still be found in the respiratory tract, but HI titers should give an indication of past or present infection. These results agreed with those of Fahey and Crawley (1955), Heishman *et al.* (1962) and Newnham (1963) who reported the lowering of HI titers in infected birds by administration of tetracycline in diet.

Mycoplasma gallisepticum was re-isolated from live birds till the end of experiment (groups 1,2,5). Thus, although treatment of infected birds resulted in better weight gain compared with those for untreated stock, flock infection still remained, and under the condition of the experiment the drug was not mycoplasmacidal. However, therapy for a period longer than three days or repeated treatment might have reduced the number of the carriers. Furthermore, the immune system was relatively immature during the period of the experiment. These findings are in agreement with those of Newnham (1963), Jordan *et al.* (1978), Jordan and Knight (1984), Migaki *et al.* (1993) and Tanner *et al.* (1993).

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عقار الدانو فلوكساسين كمضاد قوي للميكوبلازما

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تم اختيار كفاءة الدانو فلوكساسين فى علاج ووقاية كتاكيت تم بها العدوى التجريبية. ولهذا الغرض تم استخدام عدد مائة كتكوت من نوع هابرد وتم تقسيم هذه الكتاكيت الى خمس مجموعات متساوية بعد ١٥ يوم من بداية التجربة.

تمت عدوى المجموعة الاولى بعثرة ضارية للميكوبلازما جاليسبتكم من نوع اس - ٦ والتي اعطيت عقار الدانو فلوكساسين بمعدل ٥٠ جزء فى المليون وذلك بعد يوم واحد من ظهور الاعراض، والمجموعة الثانية تم إعطاؤها نفس الجرعة لمدة ثلاثة ايام كوقاية تم بعدها اجراء اختبار التحدى والعدوى بعثرة الميكوبلازما أس - ٦ وتم بعدها العلاج باربع وعشرين ساعة من ظهور الاعراض، والمجموعة الثالثة تم إعطاؤها عقار الدانو فلوكساسين دون اجراء العدوى وذلك لمدة سبعة ايام وكانت هذه بمثابة مجموعة المتبقيات، والمجموعة الرابعة كانت بمثابة ضابط سلبى بمعنى عدم العلاج او العدوى التجريبية والمجموعة الخامسة تم اعتبارها بمثابة الضابط الايجابى بمعنى العدوى مع عدم العلاج.

وقد أظهرت النتائج ان الدواجن التى أعطيت جرعات وقائية من الدواء ثم اجريت عليها العدوى التجريبية ظهر فيها زيادة وزن الجسم وكذلك وزن الاعضاء الداخلية بالنسبة لغيرها من المجموعات. مما هو جدير بالذكر ان الاعراض الاكلينيكية كانت شديدة فى الدواجن التى كانت بمثابة الضابط الايجابى وكذلك الدواجن التى تم بها العدوى التجريبية وأعطيت عقار الدانو فلوكساسين بعد ٢٤ ساعة من ظهور الاعراض وقد كانت نسبة النفوق ٨٠ ، ٢٥٪ على التوالي فى المجموعتين، وقد تم عزل ميكروب الميكوبلازما جاليسبتكم فى الدواجن التى تمت بها العدوى ثم أعطيت الدواء وكذلك الكتاكيت التى بها العدوى التجريبية وأعطيت الجرعات الوقائية وكذلك الدواجن التى اعتبرت كضابط ايجابى.

أما بالنسبة لمعدل تلازن الدم الاحباطى فقد كان عاليا فى المجموعة الأولى وذلك خلال الاسبوع الاول بعد اعطاء الدواء ثم انخفض المعدل فى الاسبوع الثانى. بينما فى المجموعة الثانية كان منخفضا خلال الاسبوع الاول والثانى بعد اعطاء الدواء. أما فى مجموعتى المتبقيات والضابط السلبى فقد كان معدل التلازن منخفضا فى الاسبوع الثانى.