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RESIDUES OF QUINOLONE GROUP OF ANTIBIOTICS IN TABLE EGGS (*)

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(With 4 Tables)

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قياس المضادات الحيويه (مجموعه الكينولون) فى بعض بيض المائدة بالاسواق

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كثر فى الاونه الاخيرة استعمال المضادات الحيويه بطريقه غير سليمه قد تؤدى الى ظهور بقاياها فى بيض الدواجن حيث تكمن خطورة ذلك ليس فقط فى اكتساب الميكروبات الضارة مناعة ضد هذه المضادات الحيويه ولكن ايضا فى ظهور بعض امراض الحساسيه بين المستهلكين لبيض هذه الدواجن خصوصا بين الاطفال . وقد تم اجراء هذا البحث لتقدير اقل تركيز يمكن تعيينه فى البيض لبعض هذه المضادات الحيوية (الكينولون) والكشف عن تواجد بقايا المضادات الحيوية وهى الإنروفلوكساسين- الفليموكوين. كما تم دراسه مدى تواجد بقايا المواد المثبطه فى البيض المتداول فى اسواق محافظات الاسكندريه والبحيرة وكفر الشيخ وقد تبين ان اقل تركيز أمكن تقديره فى البيض لكل من المستحضرين المستخدمين هو ٠,٠٢٢ ، ٠,٠٩٦ ميكروجرام لكل مللى على التوالى وقد وجد ان ميكروب الاشريشيا القولونى هو اكثر حساسيه لتحديد اقل تركيز من هذين المستحضرين من الميكروبات الاخرى وهى الزائفه المتالقه والعصويه الرقيقه والكليبسيلا الصدريه . وقد تم تقدير كميه بقايا المضادات الحيويه باستخدام ٧٥ دجاجه بياضه ومقسمه الى ثلاثه مجموعات منها مجموعه تضم ٢٥ دجاجه كمجموعه ضابطه وتم اضافه كل من الإنروفلوكساسين والفليموكوين بجرعات علاجيه الى ماء الشرب للمجموعه الأولى والثانيه على التوالي بنسبه ١٠ملليجرام لكل مللى جرام لكل كيلو جرام من وزن الطائر على التوالى لمدة ثلاثة ايام متتاليه ووجد ان الانروفلوكساسين يستمر وجوده فى البيض اربعه ايام فى البياض حتى اليوم الثانى من اخر يوم للعلاج اما فى حاله الفليموكوين لوحظ ان بقايا المستحضر تستمر خمسه ايام ووجد ان المواد المتبقيه فى البيض لمدة المتداول فى الاسواق فى هذه المحافظات توجد بنسب متفاوته وان ارتفعت فى البحيرة وكفر الشيخ عنها فى محافظه الاسكندريه وهذا وينصح بضرورة

إقرار إشتراطات خاصه عند استعمال هذه المضادات الحيويه وضرورة الكشف الدورى على البيض المتداول فى الاسواق للتأكد من خلوة من بقايا المواد الضارة لما لها من خطـــورة لا يستهان بها على صحه المستهلكين.

SUMMARY

This study was carried out for determination of the minimal inhibitory concentration of enrofloxacin and flumequine giving inhibition zone by using microbiological assay technique and determination of the withdrawal time of enrofloxacin and flumequine after oral application as well as the incidence of inhibitory substance in table eggs marketed in Alexandria, Behera and Kafr El-Aheikh governorates. The MICS of enrofloxacin using E.Coli, P.fluorecens ,K pneumoniae and B. subtilus as test organisms were 0.022, 0.12, 0.054 and 0.096 µg / ml while MICS OF flumequine using the same test organismes were 0.04, 0.17, 0.096 and. 022µg /ml respectively. Enrofloxacin residues could be detected at the 2nd day of oral adminstration and last for 2 days in albumen, and 4 days after last dose of application, while flumequine last for 3 days in albumen and 2 days in yolk after the last dose of application. The inhibitory substances in the examined egg samples perchased from Alexandria, Behera and Kafr El-Sheikh governorates were,70, 62 and 24%;78,82 and 16% and 84,64 and 48% of brown, white and Balady table eggs respectively.

Key Words: *Quinolone, antibiotics table eggs.*

INTRODUCTION

Antibiotics are widely used in poultry farms not only for treatment and control of infectious diseases but also as feed additives for growth promotion. The extensive use of these drugs in laying hens leads to appearance of the problem of drug residues as a result of excretion of such drugs in the eggs during and after treatment which have harmful effect on the consumers. So it is necessary to know how much and for how long a drug will continue to be secreted in eggs after hens are treated. Nowadays, flouroquinolone group (e.g. flumequine, enrofloxacin,...) is one of the most prevalent antibiotics used in poultry farms. Enrofloxacin is a new flouroquinolone chemotherapeutic agent (Brown,1996). It has a broad spectrum activity against Gram positive and Gram negative bacteria as well as Mycoplasma and some anaerobic pathogens (Watts et al., 1993 and

Nakamura, 1995). Flumequine is a relatively new synthetic antibacterial drug especially active against Enterobacteriaceae and in veterinary field it is recommended for treatment of E. coli, Salmonellae and Pasteurellae infections of poultry (Giebel et al., 1982 and Duplay and Chomel, 1983).

MATERAL and METHODS

I. Detection of the minimal inhibitory concentration:

- The reference substances: were enrofloxacine (Pt. Nr. R 177-1, Bayer and flumequine (Bremer pharma GmbH, Germany).
- The test organisms: were E.Coli strain, Pseudomonas fluorescens and Klebsiella pneumoniea which were obtained from Medical Research Institute, while Bacillus subtilis (BGA) was obtained from Animal Health Research institute.
- The Spore suspension: of the Bacillus subtilis (BGA) was prepared according to Pastors, (1988), then the non-spore forming organism suspension for E. Coli Pseudomonas fluorescens and Klebsiella pneumoniae were also prepared
- Agar diffusion test: (Bogaerts and Walf, 1980),E. Coli strain and standard agar II were used. Samples of egg white and egg yolk were applied by using a well technique (Coretti, 1961).
- The standard curve: of flumequine and enrofloxcin was made .
- Double fold serial dilution of the standard enrofloxacin and flumequine solutions were prepared in sterile bidistilled water, 100 µL from each dilution were applied in a well. made in previously prepared agar plate. The plates were left for 1 hour at room temperture then incubated at 30°C for 18 hours. The inhibition zone was measured from the edge of the well to the beginning of bacterial growth. 1-2mm inhibition zone was considered negative. The test repeated at different pH 6.o, 7.2 and 8.o for detection of the effect of pH on the efficacy of enerofloxacin and flumequine.

II. Experimental

Seventy five clinically healthy laying hens of Lohman Selected leghorn of 3o weeks age were used in this study and classified into three groups as follows:

a) The first group:A total of 25 laying hens were supplied with 3 successive oral doses enrofloxacin (pt.Nr.R177-1, Bayer) at a

concentration of 10 mg/kg for determination of enrofloxacin residues (withdrawal time) in eggs.

b) The second group: A total of 25 laying hens received 3 successive oral doses of flumequine (Bremen pharma, Germany) at a concentration of 12 mg/kg for determination of flumequine residues.

c) A total of 25 laying hens were used as a group.

III. Screening:

A survey of 450 eggs samples purchased from different localities in Alexandria, Behera and Kafr El-Sheikh markets (150 each of native and 300 of foreign breeds) and examined for possible occurrence of inhibitory substances using agar diffusion technique. Bacillus subtilis (BGA) was used as a test organism and standard II agar as a test medium (Pastoors, 1988).

RESULTS

Are presented in tables 1-4.

DISCUSSION

I.Minimal inhibitory concentration (MICS):

The MICs of enrofloxacin using E. coli, P. fluorescens, K. pneumoniae B. subtilis as test organisms were 0.022,0.12,0.054 and 0.096 µg / ml at pH7.2, respectively.While the MICs of flumequine using the same test organisms were 0.04, 0.17, 0.096 and 0.22 µg / mL at pH 6, respecively (Table 1). E. coli as a test organism is more senseitive for determination of enrofloxacin and flumequine residues in egg contents than the other abovementioned organisms and this selection of E. coli as a test organism agreed with Zehl, (1989) Prescott and Yielding, (1990) Samaha et al., (1991) and Khodary and Abd El-Latif, (1997).

II. Withdrawal time:

Enrofloxacin was firstly detected in egg white at the 2nd day of the oral administration at a level of 0.96 µg/ml and also be detected during the period of application (3 successive oral doses of 10 mg/kg body weight) as well as 2 days after the last dose of application at a level of 0.3 µg/ ml. Enrofloxacin could be detected in egg yolk at the

2nd day after the last dose of application at a level of 0.07 µg/ml and continued for 3 days (Table 2).

Flumequine residues were firstly detected in egg white at a level of 1.59 µg/ml at the 2 nd-day of appliccation and could be detected for 3 days after the last dose. While it was detected in egg yolk at the 4 th day after the last dose of application and continued for 2 days (Table 3). These observations are supported by the hypothesis of the development of egg in poultry (Strukie, 1965) who stated that during the development of the egg, the period of deposition of albumin and yolk was between 12-24 hours 1-10 days, respectively before laying.

III) Incidence of ihibitory substances in table eggs:

The inhibitory substances in the examined egg samples purchased from Alexandria markets were detected in 70, 62 and 24% of brown, white and balady table eggs, respectively. The incidence of inhibitory substances in table eggs of foreign breeds (brown and white - shelled eggs) and balady breeds collected from different localities at EL-Behera and Kafr- El-Sheikh Goverortes were 78,82 and 16%, respectively in El-Behera while in Kafr El-Sheikh were 84, 64, and 48% respectively (Table 4). Lower incdence of inhibitory substances in egg contents were obtained by El-Rashedy (1978), El- Bassiony (1985), Ahmed et al (1987) and Gad El Rab (1989).

The incidence of residues in the egg albumin was higher than in the yolk. These results are in accordance with those reported by Roudaut (1993). The low incidence of antibacterial agents in the examined balady eggs may due to the high resistance of these native breeds of chickens to diseases and consequently the little use of antibacterial agents and also may be due to feeding on rations free from growth promoters.

Suggestions to make assurance that eggs will be free from any antibiotic residues were discussed.

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Table (1) : Minimal inhibitory concentrations of enrofloxacin and flumequine (μg / ml)

Antibacterial	pH	Escherichia coli	Pseudmonas Fluorescens	Klebsiella Pneumoniae	Bacillus subtilis
Enrofloxacin	7.2	0.022	0.12	0.054	0.096
	6.0	0.040	0.17	0.096	0.220
Flumequine					

Table (2) : Levels of enrofloxacine residues in eggs of laying hens during and after treatment :

Days		Albumen		Yolk	
		Inhibitory zone	Enrofloxacin	Inhibitory zone	Enrofloxacin
		(mm)*	(μg / ml)	(mm)	(μg / ml)
Days during application	1	2	0.00	0	0.00
	2	11.5	0.96	0	0.00
	3	11	0.91	0	0.00
	4	11	0.91	0	0.00
Days after application	5	5	0.31	6	0.07
	6	2	0.00	4	0.03
	7	2	0.00	3	0.01
	8			2	0.00
	9			2	0.00

* Average values

Table (3) : Levels of Flumeqiune residues in eggs of laying hens during and after treatment :

Days		Albumen		Yolk	
		Inhibitory zone (mm)*	Enrofloxacin (μg / ml)	Inhibitory zone (mm)	Enrofloxacin (μg / ml)
Days during application	1	2	0.00	0	0.00
	2	13	1.59	0	0.00
	3	12	1.43	0	0.00
	4	13	1.59	0	0.00
	5	10	1.12	0	0.00
Days after application	6	6	0.48	0	0.00
	7	2	0.00	6	0.20
	8	2	0.00	4	0.07
	9			2	0.00

* Average values

Table (4) : Incidence of antibacterial agents in table eggs :

Type of egg	No. of eaxmined samples	positive samples						Albumin						Yolk					
								positive samples						positive samples					
		A		B		K		A		B		K		A		B		K	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Borwn-shelled	50	35	70	39	78	42	84	35	70	39	78	42	84	11	22	15	30	38	76
White-shelled	50	31	62	41	82	32	64	31	62	41	82	32	64	11	22	11	22	14	28
Balady	50	12	24	8	16	24	48	12	24	8	16	24	48	7	14	4	8	8	16

A = Alexandria governorate
B = El-Behera governorate
K = Kafr El-Sheikh governorate