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**STUDY OF WITHDRAWAL PERIOD OF SOME
ANTIBIOTICS AND SULFA DRUGS IN MILK OF
TREATED DAIRY BUFFALOES**
(With 7 Tables)

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دراسة عن تواجد بقايا بعض المضادات الحيوية والسلفا
فى لبن الجاموس الحلاب المعالج

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تم حقن ٥ مركبات من المضادات الحيوية والسلفا (كل على حدة) فى ١٠ حيوانات من الجاموس الحلاب (متماثلة فى السن والوزن ومرحلة الحليب) بمزرعة كلية الطب البيطرى جامعة قناة السويس . وقد تبين انه بحقن المستالون بالضرع، سلفاديميدين ٣٣.٣٪ بالوريد، بانن تيراميسين بالعضل، ستربتونسيد بالعضل وكذلك بوضع اقراص التيراميسين داخل الرحم لوحظ تواجد بقايا المضادات الحيوية فى اللبن لمدة ١٥٦ ساعة، ١٠٨ ساعة، ٦٠ ساعة، ٢٠ ساعة، ٢٢ ساعة من زمن حقن هذه المضادات على التوالى. علاوة على ذلك فقد اثبتت الدراسة بقاء بعض اثار هذه المضادات فى البان ٦٠٪ من الحيوانات المعالجة لفترة اطول من الفترة المقررة سواء بالنشرة المرفقة او من خلال التوصيات الطبية البيطرية المعروفة . كذلك اثبتت الدراسة وجود توافق جيد ($r=0.92$) بحساب معامل الارتباط بين كل من اختبار ال بى آر (BR test) واختبار الديلفو (Delvotest-P)

SUMMARY

Ten apparently healthy, lactating dairy buffaloes (relatively similar in age, weight and stage of lactation) at the farm of Faculty of Veterinary Medicine, Suez Canal University were used for each drug. They were injected with Mastalone intramammary, sulfadimidine 33.3% I/V, panteramycin I/M, streptopencid I/M and terramycin tablets intrauterine. The obtained results reveal that the withdrawal period were 156 h and 132 h for quarter and

composite milk, respectively, 108 h, 60 h, 120 h and 72 h for the drugs used, respectively. About 60 % of treated buffaloes produced antibiotic residues in their milk beyond the end of recommended withdrawal times. A good agreement (92.99 %) was detected between the Delvotest-P and BR test.

INTRODUCTION

Antibiotics and sulfa drugs are widely used in dairy cows for the treatment and prevention of mastitis and other infections through various routes of injection. Such uses have led to the contamination of milk and milk products with antibiotics and to their accumulation in animal tissues (Mielach et al., 1987; Seymour et al., 1988 and El-Kohly et al., 1994).

Improper milk discard times and/or multiple dosing with the same or another product can create drug residue problems. In addition, residues can occur after proper adherence to withdrawal period (Katz, 1982). The Food and Drug Administration (1976) considered antibiotic contaminated milk a type of adulteration. Such adulteration can be minimized by exclusion of contaminated milk from the general milk supply by limiting the quality of antibiotic in each preparation to be used for mastitis therapy, and by a requirement that a warning against the use of milk from recently treated animals be placed on the preparation.

The presence of antibiotics in milk even in minute quantities has created numerous problems in the dairy industry causing high economical losses and for the public health hazard of consumers (Mercer, 1975; Gerald, 1981; Brady and Katz, 1988 and Walton, 1988).

The need for qualitative and quantitative tests to detect antibiotic residues and inhibitors in milk has long been recognized. Therefore, the development of analytical methods of low levels of antibiotic residues in milk is necessary. Recently, modified Delvotest-P and BR test has been used and evaluated by several investigators (Oliver et al., 1990).

The purpose of this study is to determine the withdrawal period of some common preparations used in Egypt, frequency of treated dairy cows exceeded the recommended withholding periods, and the agreement between the Delvotest-P and BR test.

MATERIALS and METHODS

Animals

Ten, apparently healthy, lactating dairy buffaloes nearly of the same age, weight and stage of lactation, at the farm of the Faculty of Veterinary

Medicine, Suez Canal University were used for each drug. Mid stream quarter milk samples were collected from each animal before treatment and examined to ensure that the produced milk is free from inhibitory substances.

Drugs and administration

Five of the most common antibiotics and sulfa drug preparations used in the treatment of dairy animals in Egypt were chosen for this experiment. The drugs were used according to the route of injection as a maximum dose as recommended in the instruction pamphlets. The drugs injected were mastalone (Pfizer) intramammary, sulphadimidine sodium 33.3% solution (Khaira Pharmaceuticals & Chemical Ind. Co.) I/V, panterramycin (Pfizer) I/M, streptopencid (Cid) I/M and terramycin tablets (Pfizer) by intrauterine route

Sampling and detection of antibiotic residues

Individual sample of mid stream of right fore-quarter milk, as well as quarter and composite milk samples in case of intramammary infusion from every animal were collected after 12 h following the last-dose of treatment. Subsequent samples were conducted every 12 h until having two successive negative results. Each milk sample was tested for antibiotic residues using Delvotest-P (Gist - Brocades, Delft, Holland), and BR test ampoules (Enterotox Laboratorium, Germany) according to the methods instructed in the kits.

RESULTS and DISCUSSION

The data recorded in Table 1 show that the antibiotic residues in milk after intramammary treatment with mastalone preparation (of no recommended withdrawal period) were persisted in treated quarter milk for 156 h after last dose of treatment. While, the withholding time in composite milk was decreased and reached to 132 h. Also, the results point out that the number of positive samples for residues decreased as the time after treatment increased. The obtained results nearly agreed with those obtained by Moustafa (1980) and Egan and Conner (1980), while lower results were obtained by Aboel-Naga et al. (1973). The intramammary treatment and prophylaxies of mastitis is considered the primary source of antibiotic residues in milk. Mol (1975) estimated that intramammary treatment of lactating animals accounted for 63% of residues, while dry cow therapy was responsible for 9% of residues.

The excretion periods of sulfadimidine (of no recommended withholding period) in milk of treated buffaloes was 108 h after last - dose of treatment. The results also show that all samples (100%) gave positive

results at 84 h, while 60% and 30% of samples showed positive results at 96 h and 108 h, respectively (Table 2). The obtained results are in agreement with those reported by Saring (1975), while lower excretion time was recorded by Fechner et al. (1974) and Moustafa (1980).

Table 3 reveals that oxytetracycline residues persisted in milk samples of I/M treated buffaloes with panterramycin of recommended withdrawal periods (60 h) for 60 h post-treatment. Residues could be detected in all milk samples (100%) of treated buffaloes at 36 h, then 90% and 60% of the samples reacted positively at 48 h and 60 h, respectively, after last-infusion. This finding agreed with the results of Oliver et al. (1990). Higher retention time was recorded by Vyhalek (1971) and Moustafa (1980), while lower results were reported by Hogh and Rasmussen (1972) and Noums et al. (1985).

It is evident from the results recorded in Table 4 that the residues in milk of treated buffaloes with streptopencid (of no recommended withdrawal period) could be persisted for 120 h following last - treatment. The results also point out that 100% of samples contained antibiotic residues at 48 h, then decreased gradually to reach 20% of the samples at 120 h. These findings nearly coincided with those obtained by Oliver et al. (1990). Higher withdrawal period was obtained by El-Sherbini and El-Sayed (1993), while Folkesad and Thomasson (1977) recorded lower withholding period.

Oxytetracycline residues in milk of treated buffaloes intrauterine with terramycin tablets (of no instructed withholding period) was persisted for 72 h after last - treatment. All milk samples (100%) contained residues at 48 h, while the residues persisted in 80% at 60 h and in 30% at 72 h (Table 5). These results suggest that intrauterine infusion by antibiotics can result in residues in milk after intrauterine treatment. These results support the findings of Oliver et al. (1984) and Oliver et al. (1990), while Kaneene et al. (1986) recorded lower retention times.

Several studies have shown that the maximum retention times of antibiotic residues in milk after intrauterine treatment of lactating animals were influenced by many factors. The time after parturition, where prolonged retention times was observed in the early postpartum phase (1 - 20 day), due to the less rate of absorption of the drug from the non involuted uterus than late postpartum. Cows that were in their first lactation had significantly lower retention time of the drug than others, because in older cows the endometrium may have a large amount of connective tissue (scars) which will interfere with proper blood supply resulting in slow absorption of the drug, and therefore longer retention period. The severity of metritis did not

influence the maximum retention time of oxytetracycline (Righter et al., 1975 and Kaneene et al., 1986).

Out of the five antibiotic preparations used in the experiment, only panterramycin (Pfizer) has recommended withdrawal period. According to the general recommendation, milk of treated animals should be withheld from the bulk for at least 72 h following last-treatment (A.P.H.A., 1985 and Atherton and Newlander, 1987), therefore, the obtained withdrawal period were compared. Results represented in Table 6 indicate that 30 (60%) of treated buffaloes produced antibiotic residues in their milk beyond the end of recommended withdrawal time in spite of the animals treated according to the pamphlet directions.

It was recorded that the persistence of antibiotic residues in milk could be related to several factors such as the dose and frequency of administration which plays an important role in prolonging the withdrawal period (Seymour et al. 1988), milk production at time of treatment, type of antibiotic used, type of vehicle used in antibiotic formulations and the disease state of the animal (Mercer et al. 1970). Mercer (1975) stated that most animal drugs approved before 1962 have not definitive data on withdrawal times, this included the penicillin. This may be the reason for the persistence of the drug beyond its specified withdrawal time. Furthermore, current methods for detecting antibiotic residues in milk are significantly more sensitive than methods used in past. Thus, milk discard times may not be long enough for some of the antibiotics used currently for treatment of diseases affecting dairy animals.

It is evident from the results represented in Table 7 that a good agreement (92.99%) was detected between the Delvotest-P and BR test used in this experiment. Delvotest-P was found to be agreed well (94.7%) with the *B.stearothermophilus* disc assay method (Oliver et al. 1990). Therefore, Delvotest-P and BR test should be used because they are promoted as on-farm methods for detecting antibiotic residues in milk, sensitive, fairly rapid and simple to be use.

Our results concluded that whatever the mode of antibiotic administration, the residues may be secreted in milk of treated animals. Such milk should be disposed separately for a sufficient withdrawal time and not be used either for consumption or manufacturing. Milk of dairy animals treated intramammary should be discarded until the treated quarters showed negative results. Delvotest-P and BR test should be used as a farm tests for detection of antibiotic residues in milk.

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Table (1): Withdrawal periods of Mastalone in milk of intramammary treated buffaloes.

Withdrawal periods (h)	Methods of residue detection	+ Ve treated Quarter milk		+ Ve whole milk	
		No.	%	No.	%
12	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
24	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
36	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
48	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
60	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
72	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
84	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
96	Delvo	10	100.0	10	100.0
	BR	10	100.0	10	100.0
108	Delvo	10	100.0	9	90.00
	BR	10	100.0	10	100.0
120	Delvo	9	90.00	5	50.00
	BR	10	100.0	7	70.00
132	Delvo	7	70.00	1	10.00
	BR	7	70.00	3	30.00
144	Delvo	2	20.00	-	-
	BR	4	40.00	-	-
156	Delvo	2	20.00	-	-
	BR	1	10.00	-	-
168	Delvo	-	-	-	-
	BR	-	-	-	-

Table (2): Withdrawal period of sulfadimidine 33.3 % in milk of intravenous treated buffaloes using B.R. test.

Withdrawal period (h)	Positive samples	
	No. of animals	%
12	10	100.0
24	10	100.0
36	10	100.0
48	10	100.0
60	10	100.0
72	10	100.0
84	10	100.0
96	6	60.00
108	3	30.00
120	-	-
132	-	-

Table (3): Withdrawal period of panteramycin in milk of intramuscularly treated buffaloes.

Withdrawal periods (h)	Methods of residue detection	Positive samples	
		No. of animals	%
12	Delvo	10	100.0
	BR	10	100.0
24	Delvo	10	100.0
	BR	10	100.0
36	Delvo	10	100.0
	BR	10	100.0
48	Delvo	7	70.00
	BR	9	90.00
60	Delvo	3	30.00
	BR	6	60.00
72	Delvo	-	-
	BR	-	-
84	Delvo	-	-
	BR	-	-

Table (4): Withdrawal period of streptopencid in milk of intramuscularly treated buffaloes.

Withdrawal periods (h)	Methods of residue detection	Positive results	
		No. of animals	%
12	Delvo	10	100.0
	BR	10	100.0
24	Delvo	10	100.0
	BR	10	100.0
36	Delvo	10	100.0
	BR	10	100.0
48	Delvo	10	100.0
	BR	10	100.0
60	Delvo	8	80.00
	BR	7	70.00
72	Delvo	6	60.00
	BR	6	60.00
84	Delvo	4	40.00
	BR	5	50.00
96	Delvo	2	20.00
	BR	2	20.00
108	Delvo	2	20.00
	BR	2	20.00
120	Delvo	2	20.00
	BR	2	20.00
132	Delvo	-	-
	BR	-	-
144	Delvo	-	-
	BR	-	-

Table (5): Withdrawal period of terramycin in milk of intrauterine treated buffaloes.

Withdrawal periods (h)	Methods of residue detection	Positive results	
		No.of animals	%
12	Delvo	10	100.0
	BR	10	100.0
24	Delvo	10	100.0
	BR	10	100.0
36	Delvo	10	100.0
	BR	10	100.0
48	Delvo	9	90.00
	BR	10	100.0
60	Delvo	7	70.00
	BR	8	80.00
72	Delvo	3	30.00
	BR	2	20.00
84	Delvo	-	-
	BR	-	-
96	Delvo	-	-
	BR	-	-

Table (6): Persistence period (h) of administrated antibiotics and sulpha drugs in relation to recommended withdrawal period.

Drug	Route	recommended withdrawal period		No. of animals tested	h	%
		Hours	Source			
1-Mastalone intramammary		72	G.R.	10	+48	20
					+60	30
					+72	20
					+84	10
					+96	10
2-Sulphadimidin I/V		72	G.R.	10	-12	40
					+24	30
					+36	30
3-Panterramycin I/M		60	Lable	10	-24	10
					-12	30
					0	60
4-Strepopenicid I/M		72	G.R.	10	-24	30
					-12	10
					+12	50
					+48	10
5-Teramycin tablets Intrauterine		48	G.R.	10	0	20
					+12	50
					+24	30

h : Hours of persistence minus the recorded withholding time

% : Percentage of animals

+ : Hours beyond the recorded withdrawal period

- : Hours lower the recorded withdrawal period

0 : Compatible results

G.R.: General recommendation

Table (7): Agreement % between Delvotest-P and BR test for detection of antibiotic residues in milk

Drug	Route	Delvo + BR +	Delvo + BR-	Delvo - BR +	Delvo - BR-	% of agreement	P-value
1-Intramammary	mastalone	108	2	4	26	95.71	0.000
2-Intramuscular							
a.Panterramycin		39	1	6	24	90.00	0.001
b.Streptopencid		59	3	3	55	95.00	0.001
3-Intrauterine	terrarmycin	46	3	4	27	91.25	0.000
Total		210	9	17	132	92.99	0.005

P-value < 0.05 means if the two tests applied on larger numbers of samples has also the same results obtained