

## DETERMINATION OF WITHDRAWAL TIME OF SOME ANTIMICROBIALS USED FOR DAIRY COWS

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### ABSTRACT

*As the massive use of antibiotics for treatment and prophylaxis in the dairy cows, the antibiotic residues may occur in milk during the withholding periods or after withholding periods. So the control of antibiotic residues is necessary to protect the reputation of milk as healthy food and to prevent exposure of the consumer to risk of drug residues. The present study was conducted for determination of the withdrawal periods of some common antibiotics (Amoxicillin IM, Oxytetracycline IM, Gentamicin S/C, Ierrexline<sup>®</sup> (cephalexin + kanamycin) intramammary and chlorotetracycline intrauterine) used in dairy cows by different routes of administration by using Delvotest - p and Brilliant Black Reduction test (BR test).*

*The results revealed that most antibiotic residues in milk of treated cows by different routes persist beyond their specified withdrawal periods. The Delvotest -P is effective and sensitive for detection of antibiotic residues than BR test.*

*It could be concluded that the withholding period of each drug preparation must be assigned on the inclusive pamphlet. Moreover, any lactating cow treated with the antibiotic should be tested at the end of the recommended discard period and positive cows should be retested every 24 hrs until they test negative. Minimizing illegal drug residues in milk requires cooperation between farmers, veterinarians, dairy industry and regulations.*

### INTRODUCTION

Antimicrobial agents are used extensively for treatment of dairy cows suffering from mastitis, endometritis, pneumonia and other diseases by various routes through injection (I/M, I/V and S/C) intramammary and intrauterine infusion, topically on the skin and orally in the feed and water. Accordingly, antibiotic residues may occur in milk (Allison 1985, Mitchell et al., 1988 and Choma et al., 1999).

The health risks arising from such residues include possible pharmacological-toxicological (teratogenicity, carcinogenicity and mutagenicity), microbiological (favouring resistant of pathogenic microorganisms in the intestine) and immunopathological (allergies) effects. In addition, the presence of antibiotic residues even at minimum levels in milk inhibit growth of lactic acid and this interfere with milk product processing such as cheese manufacturing (Prato, 1997, Zeng et al., 1998 and Hillerton et al, 1999). So heavy responsibility is placed on the veterinarians and live stock producers to observe the period for withdrawal of a drug from milk prior marketing to assure that illegal concentrations of drug residues in milk not occur. The United States Food and Drug Administration (FDA) limits the quantity of antibiotic in drug products and requires label instruction explained products usage and withdrawal times (Sundlof et al., 1995). The WHO / FAO guidelines for antibiotic residues in milk for human consumption limit most residues to <0.2 ppm (IDF, 1991).

Concerns have recently been expressed that residue limits especially for antibiotics are too often set at or around the limits of analytical determination rather than on the basis of toxicology or risk assessment (Telling 1990). Nevertheless, the public health risks of antibiotics and their metabolites in food are difficult to define and the presence of violative levels of residues in food is illegal and subjected to financial penalties in many countries (Prescott and Baggot 1988).

Accordingly, the demand for reliable, simple, sensitive, rapid and low cost methods for residues analysis are needed in the farm, in processing plants and in regulatory laboratories (Telley 1999).

The objective of this study was to determine the withdrawal periods of some commonly used antimicrobials for lactating cows by different routes of administration and frequency of treated cows exceeding the recommended withholding periods.

### **Materials and Methods**

**Animals :** Ten clinically normal lactating cows nearly of the same age and stage of lactation were used for each drug. Complete health and medication records were available for each cow.

**Drugs :** Amoxicillin (Trioxyl LA)<sup>®</sup> Univet - Ireland (15mg/kg B.W injected IM), oxytetracycline (Terramycin LA)<sup>®</sup> Pfizer (20 mg/kg B.W. injected IM), Gentamycin (gentagect 10%)<sup>®</sup> Franklin, Ireland (2mg/kg B.W injected S/C), terrexine<sup>®</sup> (Cephalexin 200mg + kanamycin sulphate 100,000 IU in 10gm syringe) Univet, Holland intramammary and chlorotetracycline (Terramycin Tablet)<sup>®</sup> Pfizer (2gm intramammary). The drugs were used according to dose, route of administra-

tion and withholding periods as recommended in the instruction pamphlets.

#### **Collection of milk samples:**

Samples were collected 24 hrs before drug administration and examined to ensure that the produced milk is free from inhibitory substances. After drug administration, milk samples were collected twice daily after last dose as individual quarter milk samples or as whole udder composite samples and continued until no evidence of antimicrobial activity was found in any sample for two consecutive days. Collected samples were held at 0-4°C in ice box and delivered to laboratory for testing within 24 hrs.

#### **Detection of Antimicrobial residues:**

The collected samples were tested for microbial inhibitory substances using each of the following methods.

##### **- Delvotest -p (Van Os et al., 1975)**

Kit was obtained from Gist Brocades laboratory, Delft - Holland.

##### **- Brilliant Black Reduction test (BR) (Knaack and Tolle 1969).**

Kit was obtained from Enterotox laboratories, Germany.

The instruction provided with each test kit were followed exactly.

## **RESULTS AND DISCUSSION**

The withdrawal periods of amoxicillin with recommended withdrawal time 7 days after last dose are summarized in Table (1). All milk samples are positive for amoxicillin residues on 7th day post treatment by both Delvotest -p and B R test. While, 80 - 40% of samples showed positive reaction 24 and 72 hrs, respectively after the recommended discard time. Only one sample showed amoxicillin residues after 240 hrs. The obtained results were nearly similar to those obtained by **Holstege et al., (2002)**. Meanwhile, **Anderson et al., (1996)** reported that even at extralabel dosage of 22 mg / kg of amoxicillin IM, milk residues were >10 ppb (the FDA tolerance value) were not detected beyond the label milk withholding time for amoxicillin (96 hrs). This difference may be related to frequency of drug dosages, type of vehicle, body weight, stage of lactation and method of analysis.

The results of withdrawal time of Oxytetracycline Inj. IM at dose level of 20mg /kg B.W. with recommended withholding period 7 days after last dose, analyzed by the two methods were tabu-

lated in Table (2). All samples were test positive after 168 hrs from last dose. While, 90, 70, 40, 20% of samples react positively at 180, 194, 204 and 216 hrs, respectively after last dose. The obtained results were not compatible with that of **Rule et al., (2001)** and **Payne et al., (2002)** in goats. They indicated the presence of oxytetracycline residues in milk of goats for 3 days after last dose. This difference may be due to species variation.

The data recorded in Table (3) showed that the antibiotics residues in milk after Intramammary infusion with terredne with label milk withholding times (96 hours) were persisted in treated quarter milk for 120 hrs after last dose, while, the residues in the whole udder composite milk were persisted for 96 hrs. Nearly similar findings were obtained by **Chagonda and Ndikuwera (1989)**. They detected antibiotic residues for up to 8 days in milk of cows infused Intramammary with dispolac R 4B. **Booth (1982)** reported that 92% of antibiotic residues in milk was likely to be due to the use of intramammary infusion (61% lactational and 31% dry cow) for the treatment of mastitis, injection accounted for 6% of contamination incidents and 2% were due to other causes. Moreover, **Kitagawa et al., (1988)** and **Heller et al (2000)** reported that the intramammary treatment of mastitis is considered the primary source of antibiotic residues in milk.

It is evident from the results recorded in Table (4) the residues of gentamicin in milk of treated cows (Inj. S/C. at 2 mg / kg BW) with recommended withdrawal period of 3 days after last dose. Residues could be persisted for 72 hrs after last dose for all samples then decreased gradually to reach 20% of samples after 120 hrs. This result agreed with that of **Shalkh and Allen (1985)**.

In case of the intrauterine infusion of chlorotetracycline (Terramycin Tablet)<sup>TM</sup> the antibiotic residues were not detected beyond the label milk withholding time of the drug (72 hrs) Table (5). These results support the findings of **Carson and Breslyn (1996)** and **Kancene et al., (1986)** reported that residues in milk caused by intrauterine treatment with tetracycline, dihydrostreptomycin, benzyl penicillin, oxytetracycline and sulphamethazine had been detected for 24 - 48 hrs after last dose.

Our results revealed that most of the antibiotics used in this study were persisted in the milk beyond their specified withholding periods. This may be related to the minimum concentration of antibiotic detected by Delvotest -p (0.004 to 0.005 units/ml), meanwhile, many antibiotics withholding times were established with tests capable of detecting no less than 0.02 units /ml. Consequently, many treated cows may produce antibiotic positive milk at the end of the approved discard period **Seymour et al., (1988a)**.

**Seymour et al., (1988b)** recorded that the persistence of antibiotic residues in milk over its

label withdrawal times could be related to dose and frequency of administration. Mercer et al., (1970) and Paige (1994) stated that the type of antibiotic, type of vehicle used in antibiotic formulation, body weight and diseased state of animals all affect the persistence of residues beyond their withholding periods. Moreover, McEwen et al, (1991) reported that in case of the extralabel use of drugs (e.g. different species, increased dosage, different routes of administration) the withdrawal times are difficult or impossible to be determined in these situations.

Regarding to the effectiveness and sensitivity of the two methods for detection of antibiotic residues, the Delvotest- p gave extra-positive results cases for all drugs used beyond those detected by BR test. More or less likely results have been reported by MacCoulay and Packard (1981) and Abdel Hakiem and El- Kosi (2000) who found greater number of positive detection of antibiotics in milk samples by Delvotest -P than B. subtilis and B. stearothermophilus disc assay methods.

It could be concluded that the control of antibiotic residues is necessary to protect the reputation of milk as healthy food and to prevent exposure of the consumer to risks of drug residues. This occur by the following measures. Clearly identifying the treated animal. Antibiotics treatment of dairy animals should be carried out only under veterinary advise. The withholding time for each drug intended for dairy animals must be assigned on the inclusive pamphlet. any lactating cows treated with antibiotics should be tested at the recommended milk discard period and positive cows should be retested every 24 hrs until they test negative.

Also cows that have been treated longer than the recommended or with higher doses or with combination of drugs should be tested before milk is shipped.

**Table (1): Withdrawal period of amoxicillin in milk of intramuscularly treated cows (15 mg / kg B.W).**

Withdrawal period (h)	No of animals	Delvotest -P		Brilliant Black Reduction Test	
		No of positive samples	Percent %	No of positive samples	Percent %
12	10	10	100	10	100
24	10	10	100	10	100
36	10	10	100	10	100
48	10	10	100	10	100
60	10	10	100	10	100
72	10	10	100	10	100
84	10	10	100	10	100
96	10	10	100	10	100
108	10	10	100	10	100
120	10	10	100	10	100
132	10	10	100	10	100
144	10	10	100	10	100
156	10	10	100	10	100
168	10	10	100	10	100
180	10	9	90	8	80
192	10	8	80	8	80
204	10	7	70	6	60
216	10	4	40	3	30
228	10	2	20	1	10
240	10	1	10	-	-
252	10	-	-	-	-
264	10	-	-	-	-

**Table (2): Withdrawal period of oxytetracycline (terramycin I.A) in milk of intramuscularly treated cows (20 mg / kg B.W).**

Withdrawal period (h)	No of animals	Delvotest - P		Brilliant Black Reduction Test	
		No of positive samples	Percent %	No of positive samples	Percent %
12	10	10	100	10	100
24	10	10	100	10	100
36	10	10	100	10	100
48	10	10	100	10	100
60	10	10	100	10	100
72	10	10	100	10	100
84	10	10	100	10	100
96	10	10	100	10	100
108	10	10	100	10	100
120	10	10	100	10	100
132	10	10	100	10	100
144	10	10	100	10	100
156	10	10	100	10	100
168	10	10	100	9	90
180	10	9	90	8	80
192	10	7	70	7	70
204	10	4	40	3	30
216	10	2	20	1	10
228	10	-	-	-	-
240	10	-	-	-	-

**Table (3): Detection of Withdrawal period of terrexine preparation (caphalexin 200 mg and Kanamycin 100,000 IU) in quarter and whole milk udder samples of intramammary treated cows.**

Withdrawal period (h)	No of animals	Delvotest-P				Brilliant Black Reduction Test			
		(+ve) treated quarter milk		(+ve) whole milk		(+ve) treated quarter milk		(+ve) whole milk	
		No	Percent	No	percent	No	percent	No	percent
12	10	10	100	10	100	10	100	10	100
24	10	10	100	10	100	10	100	10	100
36	10	10	100	10	100	10	100	10	100
48	10	10	100	10	100	10	100	10	100
60	10	10	100	10	100	10	100	10	100
72	10	10	100	10	100	10	100	10	100
84	10	7	70	8	80	7	70	8	80
96	10	5	50	2	20	4	40	1	10
108	10	4	40	-	-	3	30	-	-
120	10	2	20	-	-	1	10	-	-
132	10	-	-	-	-	-	-	-	-
144	10	-	-	-	-	-	-	-	-



Table (4): Withdrawal period of gentamicin in milk of subcutaneously treated cows (2 mg / kg B.W).

Withdrawal period (h)	No of animals	Delvotest-P		Brilliant Black Reduction Test	
		No of positive samples	Percent %	No of positive samples	Percent %
12	10	10	100	10	100
24	10	10	100	10	100
36	10	10	100	10	100
48	10	10	100	10	100
60	10	10	100	10	100
72	10	10	100	10	100
84	10	7	70	8	80
96	10	6	60	7	70
108	10	3	30	4	40
120	10	2	20	1	10
132	10	-	-	-	-
144	10	-	-	-	-

Table (5): Withdrawal period of chlorotetracycline in milk of intrauterine treated cows (2 gm single dose).

Withdrawal period (h)	No of animals	Delvotest-P		Brilliant Black Reduction Test	
		No of positive samples	Percent %	No of positive samples	Percent %
12	10	10	100	10	100
24	10	10	100	10	100
36	10	10	100	10	100
48	10	8	80	8	80
60	10	6	60	7	70
72	10	2	20	3	30
84	10	-	-	-	-
96	10	-	-	-	-

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الملخص العربي

قياس فترة سحب الدواء لبعض مضادات الميكروبات المستخدمة للأبقار الحلابة  
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نظراً لزيادة استخدام المضادات الحيوية للأغراض الوقائية والعلاجية وخاصة للأبقار الحلابة وخطورة متبقيات هذه الأدوية على صحة الإنسان وصناعة الألبان فقد أجريت هذه الدراسة للكشف عن مدة سحب بعض المضادات الحيوية شائعة الاستخدام فى ألبان الأبقار الحلابة بعد إعطائها بطرق مختلفة وذلك باستخدام اختبار Delvotest-p and BR test . وقد أثبتت الدراسة تواجد متبقيات معظم المضادات الحيوية من اللبن لمدة أطول من المدة المقررة لسحب الدواء وكان اختبار Delvotest-P أكثر فاعلية وحساسية من اختبار BRtest فى الكشف عن متبقيات المضادات الحيوية، وأنتهى البحث إلى ضرورة إتخاذ إجراءات وقائية لترشيد استخدام المضادات الحيوية فى الحيوانات الحلوب وعدم استخدام اللبن للاستهلاك الأدمى أو فى صناعة الألبان إلا بعد إنتهاء وجود المتبقيات الدوائية الغير مسموح بها، وضرورة التعاون الكامل بين المزارعين والأطباء البيطريين ومصانع الألبان والجهات الرقابية للحد من بقايا العقاقير الدوائية الغير مسموح بها قانوناً فى الألبان ومنتجاتها.