

MINIMUM CONCENTRATION OF PENICILLIN RESIDUES IN COW MILK AFTER INTRAUTERINE APPLICATION

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Summary

The residues of antibiotics in milk are commonly determined by qualitative biological detection methods. However, among all possibilities of quantitative determination of antibiotic residues in milk, the method of preparing of milk dilutions can be used, along with any qualitative biological detection method (Vukovic, 2005).

Purpose of sample dilutions is to obtain values between last ambiguous or positive and the first negative result. One way to indicate concentration of antibiotic residues in milk is minimal concentration, which represents the basis for determination of approximate, medium, variable and exact concentration of antibiotic residues in milk.

In total, 16 milking cows, clinically in good health condition, East Friesian sort, are divided into two equal groups. First group of cows was treated with 400.000 i.u. each, and second group with 800.000 i.u. each. The milk samples of treated cows were examined on the presence of penicillin residues with resazurin test, with *Str. termophilus* as test microorganism, diluted with sterilised skimmed milk, and obtained results were statistically analysed. The retaining period of penicillin residues in milk, minimum concentration, minimal secreted quantities and minimal percentage of dosage secreted by milk after intrauterine application of penicillin, were monitored.

Key words: milk, penicillin, residues, minimal concentration

Penicillin is a broad-spectrum antibiotic, and period of presence of residues in milk and concentration after intrauterine application of milking cows may vary (Adams, 1995; Jezdimirovic, 2000). Presence and concentration of penicillin residues were examined using resazurin test and specific quantitative method (Vukovic, 2005). Sensitivity level of the above mentioned method for detection of penicillin residues in milk is 0.08 - 0.09 i.u./ml (ambiguous result) and 0.1 i.u./ml (positive result) (Vukovic, 1991, 2005). The minimal concentration of penicillin residues was calculated on the basis of samples dilution and obtaining the last questionable or positive result and the sensitivity level of the used method for penicillin residues (Vukovic, 2005).

Materials and methods

The examination was carried out on 16 cows East Friesian sort, clinically in good health condition, body mass 450-500 kg, age 3-5 years and daily milking capacity 10-15 l.

In total, 16 experimental animals were divided into two equal groups. First group of cows was treated with 400.000 i.u. each, and second group with 800.000 i.u. each.

The experimental animals were intrauterine treated with the Jugocilin ad us. vet. (Galenika, Beograd), composed of (as declared): 600.000 i.u. procaine benzyl penicillin and 200.000 i.u. potassium benzyl penicillin (hereinafter referred to as: penicillin).

Collective milk samples from all quarters from treated cows were acquired and stored in refrigerator until processing. Samples were taken before treatment, and in same periods: 12, 24, 36, 48, 60, 72, 84, 96 hours after application. Milk samples were taken until occurrence of two negative results in a row (Table 1).

For detection, resazurin test was used, with *Str. termophilus* as test microorganism, bacterial culture 18 h old, diluted 1:1 in sterilized skimmed milk. Volume of 1 ml of water solution of resazurin (0.5g/L in distilled water) is added.

10 ml of milk sample were pasteurized for 5 min., at 80°C, and cooled on room temperature. Then, 1 ml of prepared test microorganism and 1 ml of resazurin solution were added. After shaking, sample was incubated 2 hours, at 45°C. After 1 to 1.5 hours of incubation, samples were shaken 3 times (upside down). After 2 hours of incubation, interpretation of the results was done: white color – negative result (-); azure blue or purple color – positive result (+); color between azure blue and white – ambiguous result (+/-). Milk sample was examined on the presence of antibiotic residues for excluding possible presence of inhibitory agents. Ambiguous and positive results were diluted with sterilized skimmed milk until acquiring highest dilution which results in ambiguous or positive result. As negative control, sterilized skimmed milk without inhibitory substances, were used. As positive control, sterilized skimmed milk contaminated with 0.01 i.u. penicillin/ml, were used, and 0,08 and 0,09 penicillin/ml for ambiguous results.

Statistical analysis

Calculation method (Vuković et al. 2005):

$$K_{\min} \geq R_a \times n$$

was used for calculation of minimal penicillin concentrations from diluting samples values.

K_{\min} – Minimal concentration

R_a – lowest value for positive and ambiguous results

n – detection limit for positive and ambiguous results

Results are statistically analyzed.

Results and discussions

The clearance of penicillins residues in the milk of experimental cows is variously long, in dependence of applicable penicillin quantity. After intrauterine application of low dose, penicillin residues in milk were detected in 6 cows (75%),

but in experiment with higher dose, the penicillin residues were detected in the milk of all treated cows (100%). The clearance of penicillin residues is described in *Table 1*.

Table 1.

Comparative review of penicillin residues delay in the milk of cow after intrauterine administration of 400.000 i.u. and 800.000 i.u.

The clearance of penicillin residues in the milk			
400.000 i.u.		800.000 i.u.	
cow mark	hours after test	cow mark	hours after test
1.	48	11.	48
2.	-	12.	60
3.	36	13.	60
4.	60	14.	72
5.	-	15.	48
6.	24	16.	36
7.	36	17.	48
8.	36	18.	48
\bar{X}	30.0		52.75
SD	21.03		10.99
CV	70.1%		20.8%
lv	0-60		36-72

The clearance for penicillin residues was 30.0 hours (0-60 variation interval), after applying dose of 400.000 i.u. per cow, and average 52.75 hours (36-72 variation interval) after applying dose of 800.000 i.u. per cow. This difference is statistically significant.

After intrauterine administration of 400.000 i.u. per cow, penicillin residues in milk were detected in 6 out of 8 cows. Thereby, after 12 experimental hours (the first milking), the average minimal concentration of penicillin residue was 0,158 (0-0.40) i.u./ml of milk, but average total secretion of penicillin in the milk of cows was minimum 866.25 (0-1.750) i.u. per milking, as average minimum of 0.216 % (0-0.437) of received dose. During the experiment, values of the observed parameters have been scaling down and after 60 hour of the experiment (the fifth milking), minimal concentration was 0.005 (0-0.04) i.u./ml of milk average, and minimal average secretion of penicillin was 25.0 (0-200) i.u. per milking. This is average minimum of 0.006 (0-0.05) percent of received dose (*Table 2*).

Table 2.
Penicillins residues secreting by milk after intrauterine administration
in dose of 400.000 i.u.

cow	Parameter	400.000 i.u. penicillin					Total
		Time after test (hours)					
		12	24	36	48	60	
1.	Kmin (i.u./ml) M	0.14	0.18	0.10	0.02		0.44
	i.u./milking dose	6.000	7.000	6.000	7.000		
	%	840	1.260	600	140		2.840
		0.21	0.315	0.15	0.035		0.71
2.	Kmin (i.u./ml) M	-	-				-
	i.u./milking dose	5.000	7.000				
	%	0	0				0
		0	0				0
3.	Kmin (i.u./ml) M	0.25	0.08	0.04			0.37
	i.u./milking dose	7.000	8.000	7.000			
	%	1.750	640	280			2.670
		0.437	0.16	0.07			0.667
4.	Kmin (i.u./ml) M	0.30	0.30	0.25	0.04	0.04	0.93
	i.u./milking dose	5.000	6.000	5.000	6.000	5.000	
	%	1.500	1.800	1.250	240	200	4.990
		0.375	0.45	0.312	0.06	0.05	1.247
5.	Kmin (i.u./ml) M	-	-				-
	i.u./milking dose	5.000	6.000				
	%	0	0				0
		0	0				0
6.	Kmin (i.u./ml) M	0.06	0.02				0.08
	i.u./milking dose	4.000	6.000				
	%	240	120				360
		0.06	0.03				0.09
7.	Kmin (i.u./ml) M	0.40	0.30	0.06			0.76
	i.u./milking dose	5.000	7.000	5.000			
	%	2.000	2.100	300			4.400
		0.5	0.525	0.075			1.1
8.	Kmin (i.u./ml) M	0.12	0.08	0.01			0.21
	i.u./milking dose	5.000	7.000	5.000			
	%	600	560	50			1.210
		0.15	0.14	0.012			0.302
\bar{X}	Kmin (i.u./ml)	0.158	0.117	0.057	0.007	0.005	0.34
	i.u./milking dose	866.25	810.0	310.0	47.5	25.0	2.058.75
	%	0.216	0.202	0.077	0.012	0.006	0.514

Legend:

X - average value

Kmin - minimal concentration of penicillin residues

M - milk yield per milking

Minimum secretion concentration of penicillin residues, totally average was 0.34 (0-0.93) i.u./ml of all milking quantity per cow and minimum quantity of penicillin secretion was approximately 2.058.75 (0-4.990) i.u. of all milking quantity per cow. This is a minimum average quantity of 0.514 (0-1.247) percent of received penicillin dose (*Table 2.*)

After intrauterine application of 800.000 i.u. penicillin per cow, the evidence of residues was detected with different delay and concentration in milk of all treated cows. After 12 experimental hour (the first milking), minimum concentration of penicillin residues was average 0.408 (0.12-0.70) i.u./ml of milk, but after 72 hour of experiment, that values became lower with average minimum 0.010 (0-0.08) i.u./ml of milk (the sixth milking). After 12 hour (the first milking), average secret in milk of some cows was minimum 1977.5 (720-3.000) i.u. per milking, as average minimum 0.247 (0.090-0.375) percent of received penicillin dose. In further period of experiment, minimum quantities i.u. and percents per milking have been scaling down, to 72 experimental hours (the sixth milking), when they became average minimum 60.0 (0-480) i.u. per milking, as average minimum of 0.007 (0-0.060) percent of received dose (*Table 3.*)

Table 3.
Secretion of penicillin residues in milk after intrauterine administration of 800.000 i.u. penicillin

cow	Parameter	800.000 i.u. penicillin						Total
		Time after test (hours)						
		12	24	36	48	60	72	
11.	Kmin	0.40	0.45	0.12	0.06			1.03
	(i.u./ml) M	6.000	7.000	6.000	7.000			
	i.u./milking	2.400	3.150	720	420			6.690
	dose %	0.300	0.394	0.090	0.052			0.836
12.	Kmin	0.35	0.40	0.25	0.12	0.06		1.18
	(i.u./ml) M	5.000	6.000	5.000	6.000	5.000		
	i.u./milking	1.750	2.400	1.250	720	300		6.420
	dose %	0.219	0.300	0.156	0.090	0.037		0.802
13.	Kmin	0.60	0.60	0.20	0.14	0.02		1.56
	(i.u./ml) M	5.000	6.000	5.000	6.000	5.000		
	i.u./milking	3.000	3.600	1.000	840	100		8.540
	dose %	0.375	0.450	0.125	0.105	0.012		1.067
14.	Kmin	0.70	0.45	0.35	0.20	0.16	0.08	1.94
	(i.u./ml) M	4.000	6.000	4.000	6.000	4.000	6.000	
	i.u./milking	2.800	2.700	1.400	1.200	640	480	9.220
	dose %	0.350	0.337	0.175	0.150	0.080	0.060	1.152
15.	Kmin	0.50	0.15	0.08	0.01			0.74
	(i.u./ml) M	5.000	6.000	5.000	6.000			
	i.u./milking	2.500	900	400	60			3.860

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	dose %	0.312	0.112	0.050	0.007			0.481
16.	Kmin	0.12	0.08	0.02				0.22
	(i.u./ml) M	6.000	8.000	6.000				
	i.u./milking	720	640	120				1.480
	dose %	0.090	0.080	0.015				0.185
17.	Kmin	0.35	0.14	0.14	0.08			0.71
	(i.u./ml) M	4.000	6.000	4.000	6.000			
	i.u./milking	1.400	840	560	480			3.280
	dose %	0.175	0.105	0.070	0.060			0.410
18.	Kmin	0.25	0.10	0.04	0.02			0.41
	(i.u./ml) M	5.000	6.000	5.000	6.000			
	i.u./milking	1.250	600	200	120			2.170
	dose %	0.156	0.075	0.025	0.015			0.271
\bar{X}	Kmin	0.408	0.296	0.150	0.078	0.030	0.010	0.97
	(i.u./ml)	1977.5	1853.7	706.2	480.0	130.0	60.0	5.207.5
	i.u./milking	0.247	0.232	0.088	0.060	0.016	0.007	0.650
	dose %							

Legend

X - average value

Kmin - minimal concentration of penicillin residues

M - milk yield per milking

The average minimal penicillin residues concentration in all quantity of dairy cows was 0.97 (0.22-1.94) i.u./ml of all milk per cow, for whole period of experiment. Minimal quantity of penicillin secretion was 5.207.5 (1.480-9.220) i.u. for total milk quantity per cow, as average minimum of 0.650 (0.185-1.152) percent of received penicillin dose, for total period of experiment, too (*Table 3*).

The Penicillin residues were found in milk of 75% cows treated with 400.000 i.u. and in milk of 100% cows treated with 800.000 i.u. The results are not in agreement with the findings of Mc Clary (1984) who, after intrauterine application of 1.000.000 i.u. per cow, detected the penicillin residues in a milk of 3 cows (10% of 30 treated cows) after 12 experimental hours, and in a milk of all treated cows after treatment with 1.500.000 and 2.000.000 i.u. penicillin.

Also, the findings are in disagreement with previous results (Prouty, 1961), who did not found the penicillin residues in a milk after intrauterine application of 5.000 i.u./kg body weight. According to our findings, the penicillin residues were detected in average 30,0 hours (0-60) after application 400.000 i.u. per cow, as for average 52.75 (36-72) hours after application of 800.000 i.u. per cow. The results are in disagreement with results of Prouty (1961) and Uhlig (1973). Prouty detected penicillin residues in milk 36-46 hours after intrauterine application of 10.000 i.u./kg body weight. Uhlig found secretion of penicillins residues only 48 hours after applying intrauterine dose of 5.000.000 i.u. per cow.

Otherwise, Vukovic (2005) detected penicillin residues in milk during 72 hour of experiment after applying intrauterine dose of 400.000 i.u. per cow and 800.000 i.u. per cow after 82 hour. It represents one milking more than it was in our experiment results.

Clearance of penicillin residues in the milk were similar with Haaland's results (1984). According to these findings, the penicillin given in 1.500.000 i.u. intrauterine dose per cow has been secreted in milk after 60-84 hours.

Applying 400.000 i.u. dose per cow, the most value of average minimal penicillin residues concentration in our experiment was 0,158 and the least was 0.005 i.u./ml of milk. After application of 800.000 i.u. dose per cow, the most value was 0.408 and the least 0.010 i.u./ml of milk. These differences are statistically significant ($P < 0.001$).

The findings are different from Uhlig's results (1973). This author found penicillin residues concentration from 0.01 to 0.8 i.u./ml of milk, after applying intrauterine dose of penicillin of 5.000.000 i.u. per cow.

The dose and time influence after applying intrauterine dose of penicillin of 400.000 and 800.000 i.u. per cow, indicate that average minimal concentration of penicillin residues (i.u./ml of milk) and total secretion of penicillin in the milk of some cows (i.u. per milking) are higher, when applying higher tested dose, in all analyzing period, as totally, as in all quantity of individual milking.

This difference reached the grade of high statistic importance ($P < 0,001$). In both of tested dose, the average minimal concentrations, average minimal secretion of penicillin in the milk by individual milking and average minimal percent of penicillin secretion of received dose, had higher values after 12 experimental hour (first milking) and then they scaled down in time function till the end of the experiment.

Summary, although differences in total average concentrations and minimal average quantities of secreted penicillin in all milking individual have higher statistical significancy, average values of minimal dose is 0.514 (0-1.247) percents for 400.000 i.u. dose per cow, relative to the minimal average 0.650 (0.185-1.152) percent for 800.000 i.u. per cow, which is not statistically significant ($P > 0.005$).

In accessible data indicated parameters for comparing did not found.

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