Biotechnology in Animal Husbandry 23 (5-6), p 77 - 85 , 2007 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.06

RESIDUES OF PENICILLIN IN THE MILK AFTER INTRAUTERINE TREATMENT OF LACTATING COWS**

Vujadin Vuković[1*], Anka Kasalica [2]

[1]Institute of Veterinary Medicine of Serbia, Auto put 3, 11077 New Belgrade, Serbia
[2]Dairy Institute, Auto put 3, 11070 New Belgrade, Serbia
* Corresponding author: e-mail: jpszavod@infosky.net
**Original scientific paper

Abstract: The penicillin is a broad-spectrum antibiotic, active against a large number of Gram-positive and Gram-negative microorganisms. A total of 14 cows (East Friesian sort) were divided into two equal experimental groups, and one group was administered a dose of 400.000 IJ/ i.u./cow, and the order group 800.000 IJ/ i.u./cow penicillin. The penicillin residues in milk was determined using of Delvo test SP method with *B. stearothermophilus* var. *calidolactis,* as the test mikroorganism. In the first group, the average duration of the presence of its residue in milk of treated cows was 37,7 (0-60) hours. After the intrauterine dose of 800.000 I.U./cow, this time period was an average of 53,8 (36-72) hours. The concentrations of penicillin residues at the intrauterine treatment dairy cows were determinined an average of 0,003-0,143 I.U./ml milk for dose of 400.000 I.U./cow, and an average of 0,008-0,325 I.U./ml milk for dose of 800.000 I.U./cow. Total excretion of penicillin residues per cow were an average of 1.288,2 (0-2.538) I.U./cow for dose of 400.000 I.U./cow, and an average of 6.149,1 (1.452-15.330) I.U./cow for dose of 800.000 I.U./cow.

Key words: Penicillin, cow, milk, residues, milking, dose

Introduction

The healthy lactating dairy cows have been treated intrauterine of penicillin in dose of 400.000 or 800.000 I.U./cow. The folowing intrauterine therapy of dairy cows penicillin residues enter to milk (*Adams*, 1995; *Mercer* 1978; *Jezdimirović*, 2000). Concentrations of the penicillin residues are proved maintly by biological methods using sensitive species of the tested mikroorganisms and specific quantitative assay methods (*Vuković*, 1999).

mikroorganisms and specific quantitative assay methods (*Vuković*, The sensitivity level of the Delvo test SP method for penicillin in milk is 0,006 I.U./ml (positive finding)(*Vuković*, 1991; *Vuković*, 2001)

Materials and methods

The trials were carried out in the total of 14 cows of an farm. The all cows was intrauterine (i.u.) treated with the Jugocillin ad us.vet. (contens declared: 600.000 I.U. procain benzilpenicillin and 200.000 I.U. potassium benzilpenicillin) with a single dose of penicillin. The total number of 7 cows treated with the penicillin in dose of 400.000 I.U./cow (No. cows 1-7), while the remaining 7 cows were treated with the dose of 800.000 I.U./cow (No. cows 8-14). The daily milk yield of all cows was between 11.000-15.000 ml/day (between 4.000-8.000 ml/milking for dose of 400.000 I.U./cow, and between 5.000-8.000 ml/milking for dose of 800.000 I.U./cow), body weight ranging between 450 and 500 kg, age between 3-5 years.

Milk samples were taken at milkings the total yield per cow. Samples of the milk were taken in the periods of 12, 24, 36, 48, 60, 72 and 84 hours after a single administration of the drug. The all samples were kept at + 4^{O}**C** until assay. After the intrauterine tretment with doses of 400.000 or 800.000 I.U. penicillin/cow, elimination of residues in the milk of the 14 examinated cows was studied used Delvo test SP method with *Bacillus stearothermophilus* var. *calidolactis*, as the test mikroorganism and and specific quantitative assay method. Formule

$$X = a \times b$$

was applied on the positive and questionable results from the Delvo test SP (*Vuković*, 1991), where:

X - unknown concentration of penicillin in ml of milk (I.U./ml);
a - sensitivity level of applied method for penicillin residues (0,006 I.U./ml);
b - maximal value of diluted examined milk in which existence of penicillin residues were could be proved (ml).

The limits of sensitivity of the Delvo test SP method for the detection of penicillin residues in milk is 0,006 I.U/ml (positive finding), and 0,004 I.U. (questionable finding) (*Vuković*, 1991; *Vuković*, 2001).

Milk samples were taken at milkings the total yield per cow.

Results and discussion

The concentration and persistence of penicillin residues in the milk

samples following intrauterine treatment were determined by qualitative and quantitative assay methods. Results of the two divide experiments are show in Table 1, 2 and 3.

1. The time of eliminated of penicillin residues in milk samples after dose of 400.000 and 800.000 I.U./i.u./cow

The milk samples in this experiment showed detectable penicillin levels. After dose of 400.000 I.U./cow (no. cow 5), penicillin residues could not be detected in milk.

Folowing tretment of penicillin in dose of 400.000 IJ/i.u./cow, according to this results the penicillin residues were being eliminated in the milk samples of tested cows for 0-60 hours, which, on the average means 37,7 hours, after the treatment. The six of the milk samples showed detectable penicillin levels in 12 and 24 hours after treatment. Penicillin residues could not be detected in milk samples one cow (no. cow 5) in all time of the experiment. Penicillin residues in the milk samples were detectable and measurable longest 24 hours (no. cow 2), 36 hours (no. cows 1 and 3) and 48 hours (no. cow 7), after treatment. In cows no. 4 and 6, penicillin residues were found in the milk samples longest 60 hours after treatment. These assay methods did not detect penicillin in the all milk samples after this time (Table 1).

Table 1. The time eliminated of penicillin residues in the milk after intrauterine doses of 400.000 and 800.000 I.U./cow.

The time eliminated of penicillin residues in the milk			
After dose of 400.000 I.U./cow		After dose of 800.000 I.U./cow	
No. cow	Hours after treatment	No. cow	Hours after treatment
1.	36	8.	60
2.	24	9.	36
3.	36	10.	48
4.	60	11.	72
5.	0	12.	72
6.	60	13.	48
7.	48	14.	36
average	37,7	average	53,8

Folowing treatment of penicillin in dose of 800.000 I.U./i.u./cow, according to this results the penicillin residues were being eliminated in the milk of tested cows for 36- 72 hours, which, on the average means 53,8

hours, after the tretment. Penicillin residues in the milk were detectable and measurable for 12, 24 and 36 hours after treatment by all cows. For 48 hours penicillin residues could not be detected in two milk samples (no. cows 9 and 14), for 60 hours in milk samples yet two cows (no. cows 10 and 13),and for 72 hours in milk sample yet one cows (no. cow 8).

Penicillin residues in the milk sample were detectable and measurable longest 72 hours (no. cows 11 and 12).

These assay methods did not detect penicillin residues in the all milk samples after this time (Table 1).

2. Concentration of penicillin residues in the milk samples after dose of 400.000 I.U./i.u./cow

Intrauterine application of the penicillin resulted in the milk samples concentrations with a maximum of about 0,300 I.U./ml for 12 hours after treatment of the drug in dose of 400.000 I.U./cow (no. cow 6). The minimum detectable milk concentration of penicillin residues was 0,006 I.U./ml (no. cows 3, 4 and 7) in the special time after treatment (Table 2).

The concentrations of penicillin residues at the intrauterine treatment dairy cows, for all samples, were determinined by 0-0,300 I.U./ml milk, an average of 0,143 I.U./ml milk for 12 hours after the treatment, by 0-0,120 I.U./ml milk, an average of 0,055 I.U./ml for 24 hours after the treatment, by 0-0,060 I.U./ml milk, an average of 0,016 I.U./ml milk for 36 hours after the treatment and by 0-0,018 I.U./ml milk for 48 and 60 hours after the treatment, an average of 0,004 I.U./ml milk for 48 hours after the treatment and an average of 0,003 I.U./ml milk for 60 hours after the treatment (Table 2).

The total excretion penicillin residues per milking were an average of 784,2 (0-1.500) I.U./milking for 12 hours after the treatment, an average of 382,8 (0-960) I.U./milking for 24 hours after the treatment, an average of 78,0 (0-240) I.U./miliking for 36 hours, an average of 28,2 (0-108) I.U./milking for 48 hours and an average of 16,3 (0-90) I.U./milking for 60 hours after the treatment (Table 2).

Total excretion penicillin residues in the milk per cow in all time were an average of 1.288,2 (0-2538) I.U./cow. The percentes of dose (400.000 I.U./cow) which were eliminated in the milk treated cows were an average of 0,322 (0-0,634) % of dose (Table 2).

Table 2. Results quantitative of penicillin residues in the milk cows after intrauterine treatment in dose of 400.000 I.U./cow.

No. cow	Sign	Concentration of penicillin residues in the milk (I.U./ml) Hours after treatment						The total (I.U./cow)	% of dose
		12	24	36	48	60	72		
1.	A	0,240	0,060	0,012	0	0	0		
	B	6.000	7.000	6.000	7.000	6.000	7.000		
	C	1.440	420	72	0	0	0	1.932	0,483
2.	A	0,060	0,012	0	0	0	0		
	B	6.000	8.000	6.000	8.000	6.000	8.000		
	C	360	96	0	0	0	0	456	0,114
3.	A	0,042	0,030	0,006	0	0	0		
	B	5.000	6.000	5.000	6.000	5.000	6.000		
	C	210	180	30	0	0	0	420	0,105
4.	A	0,180	0,042	0,060	0,006	0,006	0		
	B	4.000	7.000	4.000	7.000	4.000	7.000		
	C	720	294	240	42	24	0	1.320	0,330
5.	A	0	0	0	0	0	0		
	B	5.000	7.000	5.000	7.000	5.000	7.000		
	C	0	0	0	0	0	0	0	0
6.	A	0,300	0,120	0,024	0,018	0,018	0		
	B	5.000	6.000	5.000	6.000	5.000	6.000		
	C	1.500	720	120	108	90	0	2.538	0,634
7.	A	0,180	0,120	0,012	0.006	0	0		
	B	7.000	8.000	7.000	8.000	7.000	8.000		
	C	1.260	960	84	48	0	0	2.352	0,588
average	A	0,143	0,055	0,016	0,004	0,003	0		
	B	5.428	7.000	5.428	7.000	5.428	7.000		
	C	784,2	382,8	78,0	28,2	16,3	0	1.288,2	0,322

A- penicillin concentration (I.U./ml)
A- koncentracija penicilina (i.j./ml)
B- milk yield (ml/milking)
B- mlečnost (ml/muži)
C- total ecretion (I.U./milking)
C- ukupno izlučeno (i.j./muži)

3. Concentration of penicillin residues in the milk samples after dose of 800.000 I.U./i.u./cow

The all milk samples in this experiments showed detectable penicillin levels after dose of 800.000 I.U./cow.

Higgest penicillin concentration in the milk samples (0,600 I.U./ml) were showed at the 12 hours after treatment (no. cow 12). The minimum

detectable milk concentration of penicillin residues was 0,006 I.U./ml (no. cow 14) in the special time after treatment (36 hours after treatment) (Table 3).

Table 3. Results quantitative of penicillin residues in the milk cows after intrauterine treatment in dose of 800.000 I.U./cow.

No. cow	Sign	Concentration of penicillin residues in milk (I.U./ml) Hours after treatment							The total (I.U./cow)	% of dose
		12	24	36	48	60	72	84		
8.	A	0,240	0,300	0,180	0,090	0,018	0	0		
	B	6.000	8.000	6.000	8.000	6.000	8.000	6.000		
	C	1.440	2.400	1.080	720	108	0	0	5.748	0,718
9.	A	0,300	0,120	0,012	0	0	0	0		
	B	6.000	7.000	6.000	7.000	6.000	7.000	6.000		
	C	1.800	840	72	0	0	0	0	2.712	0,339
10.	A	0,150	0,180	0,060	0,030	0	0	0		
	B	6.000	7.000	6.000	7.000	6.000	7.000	6.000		
	C	900	1.260	360	210	0	0	0	2.730	0,341
11.	A	0,540	0,360	0,240	0,180	0,090	0,030	0		
	B	7.000	8.000	7.000	8.000	7.000	8.000	7.000		
	C	3.780	2.880	1.680	1.440	630	240	0	10.650	1,331
12.	A	0,600	0,480	0,540	0,330	0,090	0,030	0		
	B	7.000	8.000	7.000	8.000	7.000	8.000	7.000		
	C	4.200	3.840	3.780	2.640	630	240	0	15.330	1,916
13.	A	0,300	0,300	0,210	0,012	0	0	0		
	B	5.000	6.000	5.000	6.000	5.000	6.000	5.000		
	C	1.500	1.800	1.050	72	0	0	0	4.422	0,552
14.	A	0,150	0,096	0,006	0	0	0	0		
	B	5.000	7.000	5.000	7.000	5.000	7.000	5.000		
	C	750	672	30	0	0	0	0	1.452	0,181
average	A	0,325	0,262	0,179	0,091	0,028	0,008	0		
	B	7.000	7.285,7	7.000	7.285,7	7.000	7.285,7	7.000		
	C	2.052,8	1.956,0	1.150,2	726,0	195,4	68,5	0	6.149,1	0,768

A- penicillin concentration (I.U./ml)
A- koncentracija penicilina (i.j./ml)
B- milk yield (ml/milking)
B- mlečnost (ml/muži)
C- total ecretion (I.U./milking)
C- ukupno izlučeno (i.j./muži)

The concentrations of penicillin residues in the milk samples that were related and peak concentrations were obtained at the 12 hours after the

treatment of the drug, an average of 0,325 (0,150-0,600) I.U./ml milk. The concentrations for 24 hours after the treatment were determinined by 0,096-0,480 I.U./ml milk, an average of 0,262, for 36 hours after the treatment an average of 0,179 (0,006-0,540) I.U./ml milk, for 48 hours were determined an average of 0,091 (0-0,330) I.U./ml milk and for 60 hours after the treatment, an average of 0,028 (0-0,090) I.U./ml milk. The minimum detectable of the milk concentrations was an average of 0,008 (0-0,030) I.U./ml milk in the 72 hours after the treatment (Table 3).

The total excretion of penicillin residues per milking were an average of 2.052,8 (750-4.200) I.U./milking for 12 hours after the treatment, an average of 1.956,0 (672-3.800) I.U./milking for 24 hours after the treatment, an average of 1.150,2 (30-3.780) I.U./milking for 36 hours, an average of 726,0 (0-2.640) I.U./milking for 48 hours, an average of 195,4 (0-630) I.U./milking for 60 hours after the treatment, and an average of 68,5 (0-240) I.U./milking for 72 hours after the treatment (Table 3).

The total excretion of penicillin residues in milk per cow in all time were an average of 6.149,1 (1.452-15.330) I.U./cow. The percentes of dose (800.000 I.U./cow) which were eliminated in milk treated cows were an average of 0,768 (0,181-1,916) % of dose (Table 3).

Pursuant to the findings, the milk levels of penicillin residues were determined in six by seven cows (85,7%) after intrauterine under tested of dose and at the all cows (100%) after intrauterine high tested of dose. These results are not in accordance with the data reported by *Mc Clary* (1984) for intrauterine dose of 1.000.000 I.U. penicillin/cow. This results showed detected of penicillin in the milk 3 by 30 treated cows in 12 hours after treatment.

The time of eliminated of penicillin residues in milk samples folowing intrauterine dose of 400.000 I.U./cow were detectable for average 37,7 (0-60) hours after treatment. Detectable of the time were prolonged for the dose of 800.000 I.U./cow, average 53,8 (30-72) hours. The presented data comply with *Haaland* et all (1984) which have also reported detectable of the time by 60-84 hours after intrauterine dose of 1.500.000 I.U. penicillin/cow.

Acording to the findings, concentrations of penicillin residues in the milk were average 0,003-0,143 I.U./ml for dose of 400.000 I.U./cow, and average 0,008-0,325 I.U./ml for dose of 800.000 I.U./cow. These results are mostly in accordance with the data reported by *Uhlig* (1973) for intrauterine dose of 5.000.000 I.U. penicillin/cow which was detectable penicillin residues in concentrations by 0,1-0,8 IU/ml.

Conclusions

The milk levels of penicillin residues were determined in six by seven cows (85,7%) after intrauterine under tested of dose, and at the all cows (100%) after intrauterine high tested of dose.

The time of eliminated of penicillin residues in milk samples folowing intrauterine dose of 400.000 I.U./cow were detectable for average 37,7 (0-60) hours after the treatment. Detectable of the time were prolonged for the dose of 800.000 I.U./cow, average 53,8 (30-72) hours. The mutual differences observed are reach the level of statistical significance.

The concentrations of penicillin in milk samples were related and were related proportional to dose. Treatment with a dose of 400.000 I.U./cow resulted in significantly lower penicillin residues levels in milk samples than after the intrauterine treatment with dose of 800.000 I.U./cow.

Total excretion of penicillin residues per milking and average total excretion per cow were related proportional to dose and move average by 16,3-784,2 I.U./milking after dose of 400.000 I.U./cow. This values were average 68,5-2.052,8 IJ/milking after dose of 800.000 I.U./cow.

Calculation of percentage of dose penicillin excreted in milk samples showed high average value for dose of 800.000 I.U./cow (0,768 % of dose). After dose of 400.000 I.U./cow, this result is 0,322 % of doses.

REZIDUE PENICILINA U MLEKU POSLE INTRAUTERINOG TRETMANA MUZNIH KRAVA

V. Vuković, A. Kasalica

Rezime

Penicilin je antibiotik širokog spektra delovanja, aktivan protiv velikog broja Gram-pozitivnih i Gram-negativnih mikroorganizama. Ukupno 14 krava (Istočno Frizijska rasa) podeljeno je u dve jednake eksperimentalne grupe, i jedna grupa je dobila dozu od 400.000 i.j. penicilina/i.u./kravi, a druga grupa od 800.000 i.j. penicilina/i.u./kravi. Za određivanje rezidua penicilina u mleku tretiranih krava korišćen je Delvo test SP sa B stearothermophilus var. calidolactis, kao test mikroorganizmom. U prvoj

grupi, u mleku tretiranih krava, rezidue su se zadržavale prosečno 37,7 (0-60) časova. Posle intrauterine doze od 800.000 i.j./kravi, to vreme je iznosilo prosečno 53,8 (36-72) časova. Pri tome, koncentracija rezidua penicilina iznosila je prosečno 0,003-0,143 i.j./ml mleka za dozu od 400.000 i.j./kravi, i prosečno 0,008-0,325 i.j./ml mleka za dozu od 800.000 i.j./kravi. Ukupno izlučene rezidue penicilina kravi iznosile su prosečno 1.288,2 (0-2.538) i.j./kravi za dozu od 400.000 i.j./kravi, a prosečno 6.149,1 (1.452-15.330) i.j./kravi za dozu od 800.000 i.j./kravi.

Ključne reči: penicilin, krava, mleko, rezidue, muža, doza

References

ADAMS, R.H. (1995): Veterinary Pharmacology and Therapeutics. 7. edition, Iowa State Univ. Press/Ames, Iowa.

HAALAND, M.A. et all (1984): Antibiotics residues in milk after i.u. infusion. Vet. Med. and small Animal Clinical, 79 (3), pp. 382-386.

JEZDIMIROVIĆ, M. (2000): Veterinary farmacology, Faculty of Veterinary Medecine, Belgrade.

MERCER, H.D., TESKE, R.H. & LONG, P.E. (1978): Drug residues in food animals. J. vet. Pharmacol. Therap., 1, 119-128.

MC CLARY, D.G. (1984): B. stearothermophilus disc assay detection of penicillin in milk of dairy cows after postestrual i.u. infusion. Am.J. of Vet. Resear. 45 3, pp. 416-419.

UHLIG, A. (1973): Excretion of various penicillines in milk after i.u. administration to cows. Inagural Diss. Tierarztliche, Munchen, 332.

VUKOVIĆ, V. (1999): Determination of penicillin residues in milk by using the modified resasurine methode. 2. Slovenian Congres, with International Participation «Milk and dairy products», Portorož, Slovenija, Book of abstracts, str.65

VUKOVIĆ V. (2001): Examination of sensitivity level methods Delvo and Resasurine on presence of penicillin and oxytetracycline residues in milk. Vet.. J., 55, pp. 55-59.

VUKOVIĆ, V. (1991): Residue resorption and secretion of penicillin, streptomycin and oxytetracycline in milk after intrauterine administration. Doctoral thesis, Faculty of Veterinary Medecine, Belgrade.