

Verification of Suitability of Selected Detection Systems for Estimating Antibiotic Residues in Goat's Milk

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Abstract

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The objective of the paper was to verify the convenience of the application of three standardized detection systems: disk diffusion method, Delvotest SP and Penzym S 100 to control the antibiotic residues in goat's milk (β -lactam antibiotics and cephalosporins, aminoglycosides, tetracyclines, macrolides and others). It has been found that despite of certain specificity of goat's milk versus cow's milk the values of the majority of detection limits mutually correspond approximately to 90 %. The sensitivity of tests manifested itself in the following order: Penzym S 100 > Delvotest SP > disk diffusion method (the sensitivity was even several times lower). Inasmuch as the treatment of mastitis is carried out by using β -lactam antibiotics and cephalosporins, the above-indicated rapid methods (especially Penzym S 100 and Delvotest SP) can be recommended for the routine purposes of accomplishing a rapid hygienic control of goat's milk.

Keywords: goat's milk; disk diffusion method; Delvotest SP; Penzym S 100; antibiotics

Goat's milk and its products belong to eatables of the valuable nutritive value. In recent time, an interest in its consumption has been revived in connection with a change in the life style of some groups of the population, with orientation to the healthful alimentation, and with growing occurrence of allergic diseases associated with food consumption.

Goat's milk has increasingly been used by infants and children with an allergy to cow's milk (MANN 1999). The former milk is more easily digestible because its caseins differ by the composition of amino acids from lacteal caseins of cow's milk.

Until now, goat breeding in Slovakia has been connected with home consumption of goat's milk and meat. First of all, at the end of the eighties and at the beginning of the nineties attention was focused on large-scale goat breeding which was associated not only with greater interest in goat's meat, dairy products and wool on the side of consumers but also with an easier possibility of importing the high-quality breeding material as well as breeding and processing technologies. Since 1989, the number and production of goats have increased. At present, however, about 98% of goats are owned by private farms where they are bred mainly for the production of milk and for the production and export of various kinds of cheese (as

much as 90% of goat cheese is exported to the Czech Republic, Hungary, Austria and Arabic countries).

The government and Ministry of Agriculture of the Slovak Republic pay great attention to the goat breeding sector. The Conception of Goat Breeding Development was approved by the Council of the Slovak Ministry of Agriculture in 1993 and it was updated in 1996. The conceptions are specified as for the livestock population, production, technical equipment, personnel, requirements for feeds and veterinary service. The goat breeding sector is also financed from the state budget of the Slovak Ministry of Agriculture (in accordance with the resolution of the Ministry registered under the number 307/1997-100 of 18th February, 1997) (MARGETÍN & MICHALÍK 1998).

The raw milk quality is characterized by the complex of qualitative indices, from among which the hygienic and sensory quality, chemical composition and occurrence of undesirable (xenobiotic and inhibitory) substances have a priority position. The latter substances involve natural inhibitors, all chemical compounds employed in the primary agricultural production (especially in the sanitation measures), veterinary drugs for treatment of mastitis, preservation substances and mycotoxins.

The most frequent reason for the occurrence of inhibitors in goat's milk is an extended application of veteri-

nary drugs (HOFERICOVÁ 1999), failure to observe protective terms (ZENG *et al.* 1996) and the incorrect mode of sanitation.

Differences between cow's and goat's milk suggest that analytical tests used for detection of antibiotics in cow's milk are not always suitable for assessment of goat's milk. This antagonism can be explained by natural inhibitors contained in the cow's milk lipidic fraction which lead to the induced resistance to antibiotics (KLINGER & ROSENTHAL 1997). The occurrence of xenobiotic substances in eatables is generally controlled by the Food Codex of the Slovak Republic (1996) and the detection of inhibitors in goat's milk is carried out according to the valid legislative cow's milk requirements included in STN 57 0531 (1995) until the acceptance of individual legislative EU regulations. Inasmuch as the data concerned with the problem of determination of suitable applications of screening tests for goat's milk (ZENG *et al.* 1998) are almost absent in the available literature, the objective of this work was to evaluate and compare some detection limits of the most frequently used antibiotics (β -lactam antibiotics and cephalosporins, aminoglycosides, tetracyclines, macrolides, others) estimated by means of three different methods, i.e. the classical disk diffusion method and the rapid methods represented by Delvotest SP and Penzym S 100. A model experiment permitted us to assess the sensitivity

and convenience of selected detection systems commonly applied in the diagnostics of milkers afflicted by mastitis, and also for the control of goat's milk.

MATERIAL AND METHODS

Material

An experiment was conducted on goat's raw milk from the private farm at Miloslavov. Hand milking was used to obtain milk for the experiment, and mixed milk was obtained from 5 to 6 goats on average (white short-wooled breed). Milk samples were collected from March to April in 2000 (approximately at 1 to 2 week intervals). The milk (defatted during 5 min at 5000 rev/min on the MPW-210 laboratory centrifuge, produced by Mechanika Precyzyjna, Poland) was processed for the purpose of analyses within 10 h after its collection; it was kept at 5°C by that time. According to STN 57 0531 (1995), before the analyses it was thermally treated in water bath at 100°C for 30 min and then maintained at 7°C for not longer than for 7 days.

Model samples were prepared from separated goat's raw milk without the presence of inhibitors which was artificially fortified with antibiotics at the above-indicated concentration ranges. The milk without antibiotics was used as a control.

For the estimation of detection limits in goat's milk the following antibiotics and concentration ranges (from 6 to 10 concentrations in each antibiotic) were used:

Penicillin G	(Biotika Slovenská Ľupča, SR)	(0.001–0.1 IU/ml)
Amoxicillin	(Léčiva, Prague, CR)	(0.002–0.9 µg/ml)
Oxacillin	(Lek d.d. Ljubljana, Slovenia)	(0.005–0.8 µg/ml)
Ampicillin	(Biotika, Slovenská Ľupča, SR)	(0.001–0.2 µg/ml)
Cefuroxime	(Glaxo, Greenford, England)	(0.02–1.0 µg/ml)
Cefotaxime	(Roussel Paris, France)	(0.02–1.0 µg/ml)
Cefamandol	(Lilly France S.A. Fegersheim, France)	(0.001–0.9 µg/ml)
Cefazolin	(Lilly France S.A. Alcobendas, Madrid, Spain)	(0.002–0.5 µg/ml)
Cefquinome	(Hoecust Roussel Vet GmbH, Wiesbaden, Germany)	(0.01–2.0 µg/ml)
Cefoperhazone	(Pfizer GmbH, Karlsruhe, Germany)	(0.01–1.8 µg/ml)
Ceftazidime	(Glaxo, Greenford, UK)	(0.2–10.0 µg/ml)
Streptomycin	(Biotika, Slovenská Ľupča, SR)	(5.0–20 µg/ml)
Neomycin	(Biotika, Slovenská Ľupča, SR)	(0.3–5.0 µg/ml)
Chlortetracycline	(Biotika, Slovenská Ľupča, SR)	(5.0–30.0 µg/ml)
Erythromycin	(Biotika, Slovenská Ľupča, SR)	(0.9–6.0 µg/ml)
Trimethoprim	(Biotika, Slovenská Ľupča, SR)	(1.0–9.0 µg/ml)
Bacitracin	(Biotika, Slovenská Ľupča, SR)	(1.0–15.0 µg/ml)

Bacterial Strain

Bacillus stearothermophilus var. *calidolactis* C953 (received from the collection of microorganisms in the Research Institute of Veterinary Medicine in Bratislava), propagated on GTK agar before its application and subsequently in the liquid substrate of the same composition (produced by Imuna, Šarišské Michaľany, SR). After 24 h of incubation at $64 \pm 1^\circ\text{C}$ the inoculum contained about 10^8 cells per 1 ml.

Methods (STN 57 0531, 1995)

1. Disk diffusion method (DDM)

The principle of the method: The paper disk (Whatman 1, A 12 mm) soaked with the sample examined is placed on the surface of the agar nutrient medium containing *Bacillus stearothermophilus* var. *calidolactis*. The incubation, when the tested strain is growing, results in the opacity of the agar medium. If the investigated sample contains substances inhibiting the growth of the tested strain, clear zones are formed around the disk. The size of the inhibition zones depends on the concentration and type of microbials present in the investigated sample. The sensitivity of the method corresponds to the concentration of 0.0025 IU penicillin in 1 ml of milk. (Inhibition zones are indicated in mm, the positive zone is read off at the size above 1 mm.)

2. Delvotest SP (Gist Brocades, The Netherlands)

The standard diffusion test Delvotest SP is sensitive especially to penicillins and sulfonamides. Its sensitivity corresponds to the concentration range of penicillin from 0.003 to 0.005 IU/ml and to that of sulfadimidine from 0.3 to 0.8 µg/ml.

The principle of the method: Delvotest SP combines the principle of agar diffusion tests with a colour change of the indicator in consequence of the active metabolism of the tested microorganism in the absence of the inhibitor. The sample investigated is batched into microtitration plates filled with the agar nutrient medium containing *Bacillus stearothermophilus* var. *calidolactis*. The incubation ($64 \pm 1^\circ\text{C}/2.5\text{--}3$ h), when the tested strains is growing, leads to a colour change of the pH indicator from blue-violet to yellow. If the sample examined contains substances inhibiting the growth of the tested strain, the colour of the indicator will remain blue-violet.

3. Penzym S 100 (Hersteller UCB Bioproducts, Belgium)

The principle of the test: The method is based on the reaction of DD-carboxypeptidase with β-lactam antibiotics (a two-step procedure: 7.5 min + 15 min/47°C). If milk does not contain any microbials, the enzyme will remain fully active, which will show itself in the pink colour. In the opposite case, the enzyme will be totally inactivated and no colour reaction will occur. The results can be read off within 20 min.

4. Calculation methods (ECKSCHLAGER *et al.* 1980)

The second-degree polynomial regression according to the equations:

$$y = a_0 + a_1x + a_2x^2 \quad \text{and} \quad y = a + bx + cx^2 + d/x$$

where: x – antibiotic concentration

y – size of the inhibition zone

RESULTS AND DISCUSSION

Table 1 summarises the detection limits of antibiotics determined experimentally by means of three simultaneously used methods (DDM, Delvotest SP and Penzym S 100) in 3 to 4 parallel analyses.

The analytical curves of selected representatives of individual groups of antibiotics involving amoxicillin (β-lactam antibiotics), cephalosporins (cefuroxime, cefquinome), neomycin (aminoglycosides), chlortetracycline (tetracyclines), erythromycin (macrolides), trimethoprim (others) detected by DDM are illustrated in Fig. 1.

In estimating the detection limit for penicillin by means of DDM (Table 1) the lower sensitivity of the applied bacterial strain to the above-indicated antibiotic present in goat's milk (as much as 0.005 IU/ml) was found. The de-

Table 1. Detection limits of selected antibiotics tested by various methods

Antibiotic	Detection limits (µg/ml)		
	DDM ($n = 4$)	Delvotest SP ($n = 3$)	Penzym S 100 ($n = 3$)
Penicillin G*	0.005	0.002	0.003
Amoxicillin	0.100	0.006	0.003
Oxacillin	0.500	0.006	0.010
Ampicillin	0.090	0.002	0.001
Cefuroxime	0.300	0.030	0.030
Cefotaxime	0.300	0.030	0.050
Cefamandol	0.200	0.006	0.002
Cefazolin	0.200	0.009	0.003
Cefquinome	0.500	0.080	0.020
Cefoperhazone	0.600	0.020	1.000
Ceftazidime	5.500	0.400	0.300
Streptomycin	7.000	**	**
Neomycin	0.600	**	**
Chlortetracycline	7.000	**	**
Erythromycin	1.200	**	**
Trimethoprim	2.500	**	**
Bacitracin	5.000	**	**

DDM – disk diffusion method

* expressed in IU/ml

** nonanalyzed

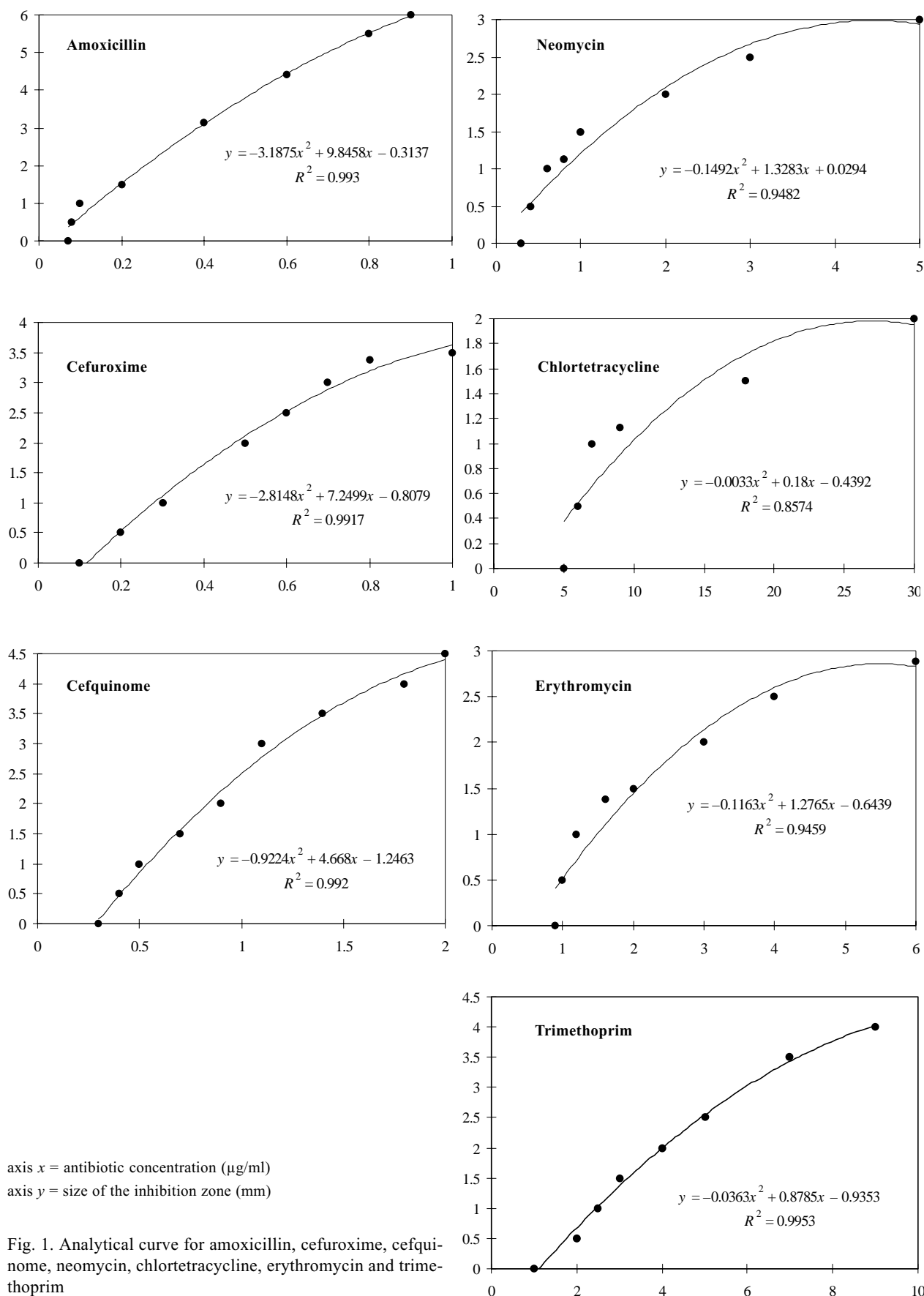


Fig. 1. Analytical curve for amoxicillin, cefuroxime, cefquinome, neomycin, chlortetracycline, erythromycin and trimethoprim

tection limits achieved by means of Delvotest SP and Penzym S 100 are substantially lower (0.002 and 0.003 IU per ml) and correspond to the limits determined for cow's milk (YAMANI *et al.* 1998; HOZOVÁ *et al.* 1998).

In evaluating the detection limits of amoxicillin the above-indicated rapid methods appeared to be more sensitive by two orders (0.006 and 0.003 µg/ml) in comparison with DDM (0.100 µg/ml). All estimated detection limits are, however, comparable to the cow's milk data (SUHREN *et al.* 1996). The analytical curve for amoxicillin is illustrated in Fig. 1.

The results obtained by estimation of oxacillin and ampicillin present in goat's milk suggest much higher sensitivity of Delvotest SP (0.006 and 0.002 µg/ml) and Penzym S 100 (0.010 and 0.001 µg/ml) than in the case of DDM (0.500 and 0.090 µg/ml); however, they are comparable to data achieved by means of the rapid methods for cow's milk (YAMANI 1998; SUHREN & REICHMUTH 1998).

Comparison of the detection limits of cephalosporin antibiotics showed to be very important because they are very often employed in the clinical treatment of goats afflicted by mastitis (they are efficient mainly against *Streptococcus agalactiae*).

The sensitivity of methods being compared within one method for cefuroxime and cefotaxime is almost identical: DDM (0.300 and 0.300 µg/ml) (Table 1, Fig. 1), Delvotest SP (0.030 and 0.030 µg/ml) and Penzym S 100 (0.030 and 0.050 µg/ml). In comparing the estimated detection limits in goat's milk for cefuroxime with data indicated in the literature (HOZOVÁ *et al.* 1998) almost ten times higher sensitivity of Delvotest SP and Penzym S 100 to the above-indicated antibiotic in goat's milk was ascertained.

The detection limits for cefamandol achieved by means of DDM (0.2 µg/ml) (Table 1), Delvotest SP (0.006 µg per ml) and Penzym S 100 (0.002 µg/ml) correspond to detection limits estimated for cow's milk (HOZOVÁ *et al.* 1998), the most sensitive being Penzym S 100.

In comparing the detection limits for cefazolin 10 to 30 times higher sensitivity of Delvotest SP (0.009 µg/ml) and Penzym S 100 (0.003 µg/ml) was found in comparison with DDM (0.200 µg/ml) (Table 1, Fig. 1) and in the case of cefquinome the sensitivity of rapid methods was 5 to 25 times higher than that of DDM.

In appraising the detection limits for cefoperazon the achieved values varied considerably. The most sensitive was Delvotest SP, then DDM and finally Penzym S 100 (1 µg/ml), which showed to be the least sensitive method for this only antibiotic.

In comparing the detection limits for ceftazidim 10 to 15 times higher sensitivity of the rapid methods (Delvotest SP: 0.4 µg/ml; Penzym S 100: 0.3 µg/ml) was found in comparison with DDM (5.5 µg/ml).

The values of detection limits of aminoglycosides (streptomycin, neomycin), tetracyclines (chlortetracycline),

macrolides (erythromycin) and of other antibiotics (trimethoprim, bacitracin) were established only by means of the disk diffusion method. Delvotest SP was not used for technical reasons (lack of ampoules) and the test Penzym-test S 100 is sensitive only to β-lactam antibiotics. The results achieved for streptomycin (7.0 µg/ml), neomycin (0.6 µg/ml), chlortetracycline (7.0 µg/ml), erythromycin (1.2 µg/ml), trimethoprim (2.5 mg/ml) and bacitracin (5.0 µg/ml) are more or less well comparable with data indicated by ZENG *et al.* (1998) and, at the same time, with detection limits for these antibiotics determined in cow's milk by identical methods (RYŠÁNEK & SCHLEGELOVÁ 1995; HOZOVÁ *et al.* 1998). The analytical curves for neomycin, chlortetracycline, erythromycin and trimethoprim is demonstrated in Fig. 1.

The results obtained from the aforesaid detections reveal that the rapid methods Delvotest SP and Penzym S 100 are several times more sensitive than the classical disk diffusion method and thereby also more suitable for the control of goat's milk in dairy practice. The values of the majority of detection limits determined by these methods correspond to about 90% of those assessed for cow's milk and therefore based on our experience it is possible to claim that no special legislation for estimating antibiotics in goat's milk is necessary.

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Súhrn

HOZOVÁ B., MINAROVIČOVÁ Ľ. (2001): **Overenie vhodnosti použitia vybraných detekčných systémov pri stanovení rezíduí antibiotík v kozom mlieku.** *Czech J. Food Sci.*, **19**: 207–212.

Cieľom práce bolo overenie vhodnosti použitia troch štandardizovaných detekčných systémov: diskovej difúznej metódy, Delvotestu SP a Penzymu S 100 na kontrolu rezíduí antibiotík v kozom mlieku (β -laktámové a cefalosporíny, animoglykozidy, tetracyklíny, makrolidy, ostatné). Zistilo sa, že testované detekčné systémy sú dostatočne citlivé, a to v poradí: Penzym S100 (β -laktámové antibiotiká a cefalosporíny) > Delvotest SP > disková difúzna metóda a preto ich možno odporučiť aj pre rutinné účely na rýchlu hygienickú kontrolu kozieho mlieka.

Kľúčové slová: kozie mlieko; disková difúzna metóda; Delvotest SP; Penzym S 100; antibiotiká

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