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STUDY ON THE PRESENCE AND RATE OF CONTAMINATION WITH ANTIBIOTICS AND SULPHONAMIDES OF RAW COW MILK

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Abstract. It has been studied the presence and the rate of contamination of raw cow milk with antibiotics and sulphonamides in the region of Plovdiv. It has been found that from the tested total number of 1895 samples – 1436 (75.6%) are positive for the presence of residues from antibiotics and sulphonamides, from which: 702 dairy farms at the regional territory and 732 samples are from private farmers. Of these samples 486 (80.6%) are from milk of cows with subclinical mastitis and 246 (77%) – from cows with clinical mastitis. The number of the positive samples of cow milk for presence of residues of antibiotics from the penicillin and tetracycline series with an extent of the inhibitor zone up to 20 mm is bigger – respectively 165 and 97 samples, comparing to those with an extent of the zone in the limits between 40–50 mm – 52 samples for penicillin and 21 for tetracycline. It shows that in most cases the quarantine time limits are strictly considered by the private farmers, when they deliver milk from cows treated with antibiotics with prophylactic or medicative purposes, but not always and not in full, which is proved by the waste quantity of milk – 12 366 t for the studied period.

Keywords: contamination, antibiotics, sulphonamides, cow milk.

AIMS AND BACKGROUND

The livestock absorb, excrete, take down and accumulate fallen into their organisms foreign substances (antibiotics, sulphonamides, heavy metals, pesticides, etc.), which can be found as residues in the animal products[1,2].

The antibiotics create a serious problem. They sensibilise the living organism and cause eczema, asthma and some other allergic diseases[3,4]. According to Katz[3], 5 % of the people are sensitive to penicillin and 20-25% – to streptomycin. The residues, got into the organism along with the food, inhibit or kill the beneficial microflora, which is necessary for the process of product maturing or worsen the technological characteristics of the product as well.

As a result of the wide and very often uncontrollable use of medicines (antibiotics and sulphonamides) there is a risk to fall into the food products, obtained from the treated animals[3-5].

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959

The milk contains residues of antibiotics and sulphonamides, when the cows are treated with them for different diseases and especially for mastitis, in which they are mainly applied intramammarily.

It is found that the milk until 72th day after the last treatment contains antibiotics and it is not good for consumption and processing. Because of that, it is absolutely necessary the quarantine time limits to be considered for each single kind of antibiotics[2,6,7].

It is established that in the presence of penicillin of 1 mg/cm^3 milk or streptomycin – 5 mg/cm^3 the process of fermentation of Bulgarian youghurt and white brined cheese is considerably delayed. The time for making yoghourt and turning into cheese is prolonged, compared with the control sample, which does not contain antibiotic residues. The produced milk products usually do not meet the respective quality, as in most cases, they are not good for consumption by human being, leading to big economical losses from the wasted production[2,8,9].

The control of the food products with biologically active substances is a part of the general problem for environment preservation and for ecologically clean food products.

The aim of the following paper is to study the presence and the rate of contamination of raw cow milk with residues of antibiotics and sulphonamide, in order to decrease the economic losses from the waste products and to reduce the risk from consumption of non-qualitative milk and milk products.

EXPERIMENTAL

In this study, 1895 samples were tested: out of them, 832 samples from 8 dairies in the region of Plovdiv (town of Stambolijski, villages of Trud, Manole, Tzalapitza, Graph Ignatievo, town of Kritchim – for production of yellow cheese and Kurtovo Konare – for youghurt) and Tzaratzovo; 318 milk samples from cows with clinical mastitis; 607 – with subclinical mastitis and 139 samples – from healthy cows of private farmers.

Proof of the presence of antibiotics from the penicillium series. According to the Bulgarian state standard (BSS) 6688-74, it was used the method of agar-diffusion and the test-organism *Bac. stearothermophilus* var. *calidulactis* – C_{953} (Ref. 10).

Into 100 ml agar of Miler–Hinton 1 ml bacterial suspension with density of 40 million-cells/ml agar was added and poured into Petri dishes. Aluminum rings with volume of 1 cm^3 and d = 0.9 mm were put above the prepared food. The tested milk sample (10 ml) was warmed up to 80-85°C for 5 min to destroy the side microflora; after that, the milk was cooled to 42°C. A quantity of 0.1 cm^3 from it was put in the rings (3 in number), placed on the apexes of an equilateral triangle. The Petri dishes were cultivated at temperature of 55°C for 2.5 to 5 h. Then, it was followed the appearance or absence of light (inhibitor) zone and its size, according

960

to the quantity of antibiotics in the tested sample. We counted as positive the samples with a size of the inhibitor zone 13 and above 13 mm.

Proof of antibiotics from the tetracyclin series. According to the BSS 1223-75 and test-microorganism *Bac. subtilis* – L_2, suspension from which (1 ml) was added to Hotinger agar cooled to 55°C and it was poured into Petri dishes. The cultures were put at temperature of 37°C for 24 h. The preparation of the raw milk samples and the results counting were the same as by antibiotics from the penicillin series.

Proof of the presence of sulphonamides. Mueller–Hinton agar was used with adding of 1% solution of trimetroprim and test-microorganism – *Bac. stearothermophilus* var. *calidulactis* – C_{953}. The sample preparation for analysis and the results counting were the same as by antibiotics.

The study was conducted for the period of 1998 to August 2000.

Positive samples, mostly with the extent of the inhibitor zone up to 20 mm were included, in order to find out the limit, from which the significant changes in the quality of the produced milk product begin, reflecting on their suitability for consumption and realisation.

RESULTS AND DISCUSSION

The results from conducted studies are presented in Table 1. From the tested total number of 1895 samples – 1436 (75.6%) are positive for the presence of residues of antibiotics and sulphonamides, from which: 702 samples (87.5%) are from mixed cow milk from the included 8 dairy farms from the region of Plovdiv. From them, 382 samples (62.4%) are positive for antibiotics from the penicillin series, 231 (37.6%) for antibiotics from the tetracycline series, and 89 samples (10.6%) – for sulphonamides. The percentage and number of the positives cow milk samples submitted by private farmers are significantly high, i.e. 486 samples (80%) are positive among the cows with sublinical mastitis and 246 (77%) from cows with clinical mastitis.

In most cases, the private farmers do not consider the accepted quarantine time limit for use of antibiotics and sulphonamides, which gives the opportunity for their presence in the total milk leading to worsening of its nutritive value and technological qualities. It is expressed in two major directions:

(i) From the one side, the milk, the human does not allow containing residues of antibiotics and sulphonamides for consumption, because there is a danger of allergic reactions, nevertheless, that in most cases it is sterilized. It is found by us and other authors[1,6,8,9], that the temperature of 80-85°C for 1-2 min does not destroy the antibiotics in full, or it is taken down to some intermediate product, no less dangerous for the human health.

961

Table 1. Tested samples of raw cow milk for the presence of antibiotics and sulphonamides residues

Number of the tested milk samples	Number/percent positive samples for penicillin	Number/percent positive samples for tetracycline	Number/percent positive samples for sulphonamides	Number/percent positive samples total
From cows with clinical mastitis 317	129 57.8%	94 42.2%	23 7.2%	246 77%
From cows with subclinical mastitis 607	271 63.8%	154 36.2%	61 10.4%	486 80%
From healthy cows 139	0 0	0 0	0 0	0 0
Mixed milk from different dairy farms 832	382 62.4%	231 37.6%	89 10.6%	702 87.4%
Total: 1895	782 62.1%	479 37.9%	173 9.1%	1436 75.6%

(ii) The milk, containing residues of antibiotics and sulphonamides has also changed technological qualities, inhibiting the activity of the lactic acid yeast, used for the production of the traditional Bulgarian products – Bulgarian youghurt and Bulgarian white cheese. It is observed a delay of the run of the fermentation, which leads to production of non-qualitative and in most cases not good for consumption milk products (bitter taste, a specific smell of medicine, not good acidity or pulpy consistence).

The economic losses from the waste production are significant. They can not be determined for the particular private farmer. We have tried to count the losses from waste milk (in tones) obtained from the 8 dairy farms of the region of Plovdiv. The results are presented in Fig. 1, which shows the distribution of the positive samples for residues of antibiotics and sulphonamides by years and the losses of waste milk, related to them.

It is obvious that the higher number of positive samples in 1998 is in relation to the higher quantity of waste milk – 1145.4 t. It is observed a tendency for decreasing the economical losses, respectively: 212 positive samples in 1999 – 630.6 t waste milk; 97 positive samples in 2000 – 285 t losses of cow milk. In our opinion, it is of great significance (importance) the increasing professional competence and knowledge of the management staff of the single production units (dairy farms), but it is different for the private farmers.

Having in mind that the number of the animals decreased significantly during the last years, and from there the milk production, the determined economical losses from waste milk as a result of the presence of residues of antibiotics and sulphonamides – total of 12 366 t can be considered as significant.

962

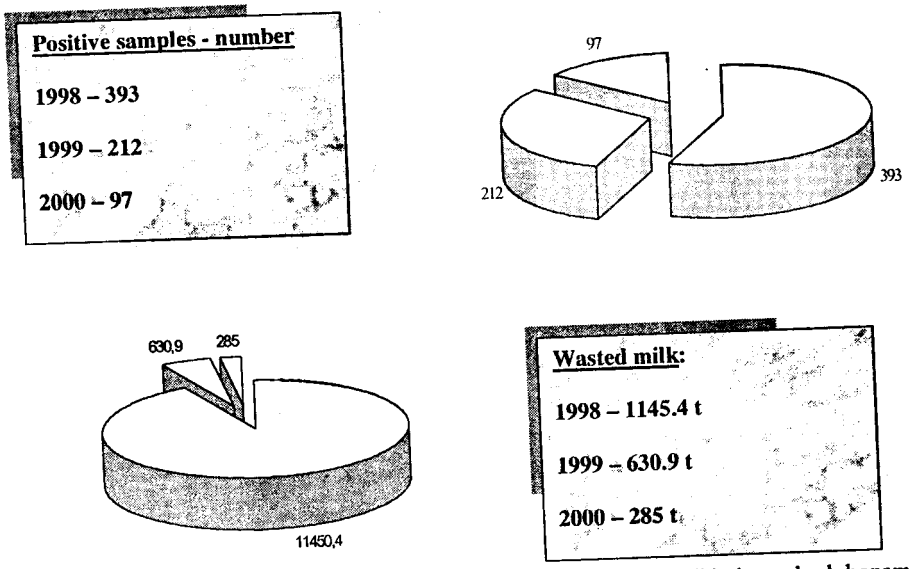

Fig. 1. Percentage of the positive samples for the presence of residues of antibiotics and sulphonamides and the losses of wasted milk

Figure 2 presents the distribution of the positive samples of raw cow milk for the presence of residues of antibiotics from the penicillin and tetracycline series, according to the inhibitor zone extent. It is obvious that the number of positive samples with extent of the zone up to 20 mm is higher for penicillin – 165 positive samples, for tetracycline – 97 samples, comparing to samples with diameter of the inhibitor zone in the boundaries between 40 – 50 mm, respectively 52 and 21 samples.

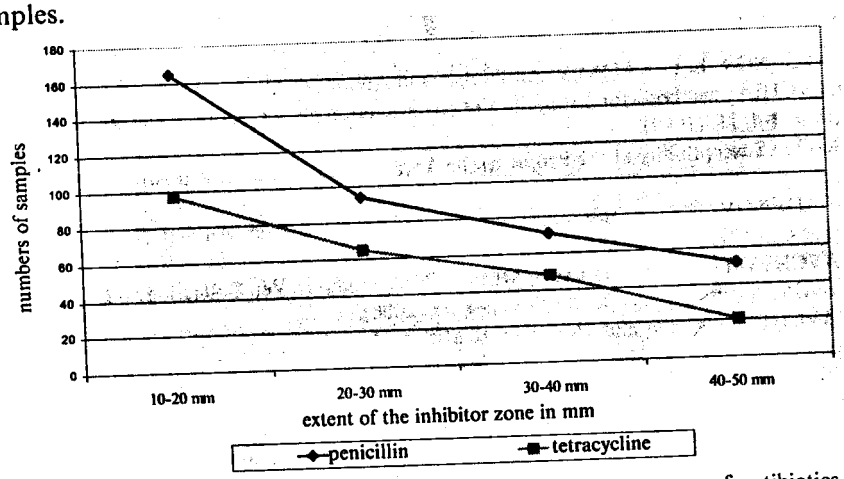

Fig. 2. Distribution of the positive samples of cow milk for the presence of antibiotics from the penicillium and tetracycline series, according to the extent of the inhibitor zone

963

This could be accepted as a fact, which shows that the bigger part of the private farmers consider the requirements to respect the quarantine time limits by milk delivering, when the dairy animals are treated with antibiotics with prophylactic and medicative activity, but not in full and not always, which is confirmed by the quantity of the waste milk, because of the above-mentioned reason.

CONCLUSIONS

According to the obtained results, the following conclusions can be made. From the tested total number of 1895 samples of raw cow milk, 1436 samples are positive for the presence of residues of antibiotics and sulphonamides Of these, 702 samples (87.4%) are from the included in the experimented 8 dairy farms at the territory of the region of Plovdiv and 732 samples are from private farmers, from which 486 (80.0%) – from cows with subclinical mastitis and 246 (77.0%) – from cows with clinical mastitis.

It was found that the number of the positive samples of cow milk for the presence of residues of antibiotics from the penicillin and tetracycline series with a size of the inhibitor zone until 20 mm is higher, respectively 165 and 97 samples, comparing with these with a size of the zone with the boundaries between 40-50 mm – 52 samples for penicillin and 21 – for tetracycline.

It has been shown that in most cases the quarantine time limits are considered by the private farmers during the milk delivery, when the dairy animals are treated with antibiotics with prophylactic and medicative activity, but not in full and not always, which is confirmed by the quantity of the waste milk – 12 366 t for the period of research.

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964