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RESEARCH ARTICLE

Human health hazards associated with tetracycline drugs residues in food

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Abstract

This study aimed to investigate health risk from tetracycline residues in food, as one of the most widespread veterinary antibiotic. Health risk assessment of veterinary drugs residues in food in particular within the World Trade Organization, the Eurasian Economic Community and the Eurasian Economic Community customs union is one of the priority areas in the field of consumer health safety.

According to results of gut flora alterations modeling for children it was founded that tetracycline residues concentration in food more than 10 μg/kg increases risk of digestive system diseases to 0.000461 (up to 4% of cases), risk of dermatitis to 0.000725 (up to 0.9% of cases), risk of alimentary allergy to 0.000149 (up to 0.1% of cases), risk of diseses of the blood to 0.001372 (up to 8% of cases).

Health risk assessment on tetracycline in food showed that tetracycline residues at 10 μg/kg (allowable residue level for Customs Union members) led to no health risk increase including most sensitive population

Introduction

The obligatory application of health risk assessment methodologies is firstly focused on consumers’ health and product safety. These methodologies are constantly developed within the activity of the World Trade Organization, the Eurasian Economic Community and the Customs Union (CAC/GL 30-1999, CAC/GL 62-2007).

In most countries and international organizations the risk assessment concept is the main mechanism for developing policies, strategies and priority actions aimed at maximizing economically justified reducing of the negative health impact (Zaitseva N.V. et al.,2013).

According to WHO, the number of antibiotics used in agriculture to promote growth and prevent infections in healthy animals and birds, is two times more today than the amount of these drugs in medicine. This inescapably leads to the accumulation of veterinary drugs residues in products of animal origin.

The majority of hygienic standards in the Russian Federation and the Customs Union for antibiotics in food is harmonized with the international standards of the European Union (EU) and the Codex Alimentarius Commission (CAC). Differences occur only for tetracyclines in all kinds of standardized products and bacitracin in meat and eggs.

The necessity to apply risk criteria, when developing hygienic standards, is determined by the high probability of getting these drugs residues in animal products in connection with the extremely large-scale application in agriculture in comparison with other antibiotics. Thus, according to domestic and foreign sources generally in the veterinary and animal agriculture, more than half of consumed antibiotics are tetracyclines, and veterinary monitoring of the products, imported to the Russian market, shows that among the residues of antimicrobials almost 78% is chlortetracycline.

Tetracycline group - bacteriostatic antibiotics - are among the most common drugs used for the treatment and prevention of poultry and beef cattle diseases. Along with the increase of meat food production efficiency, use of tetracycline may lead to the formation of health risk due to residues in meat, milk, eggs and other products. Regulated residual concentrations in foods in the United States, the European Union and the Russian Federation are quite different (Zaitseva N.V. et al.,2012). The main health hazard, associated with exposure of tetracycline drugs residues from food, is an imbalance of intestinal microflora (Van den Bogaard A.E. et al.,1999,2000; Lisbeth Elvira de Vries et al.,2009; Yvonne Agersø et al.,2002; Nowrouzian F. et al.,2003; Saarela M. et al.,2007).

In Russian and international medical practice a number of diseases, associated with an imbalance of intestinal microflora, is identified. For the child population with microflora imbalance in the intestine, the following disoders may be obderved: irritable bowel syndrome with diarrhea (K58.0); constipation (K59.0); functional bowel disorder, unspecified (K59.9); duodenitis (K29.8, K29.9); food allergies (T78.0, T78.1, T78.4); iron deficiency anemia (D50); other atopic dermatitis (L20.8), common variable immunodeficiency, unspecified (D83.9) (Kamalova A.A.,2011; Gorodkova E.N.,2007).

Material and Methods

Health risk assessment, associated with the presence of tetracycline residues in foods, included four steps in accordance with the Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240, WHO 2009 (WHO,2009):

– *Hazard Identification*. Establishment of the specific risk factors in accordance with the principal exposure scenarios, possible health effects; identification of susceptible population;

– *Dose-Response Assessment*. Determination and evaluation of benchmark levels for chemicals exhibiting a threshold for toxic effects and establishment of parameters of "exposure - effect (response)" for the non-threshold chemical contaminants;

– *Exposure Assessment*. Identification and evaluation of quantitative and qualitative characteristics, frequency, duration and route of exposure, using a scenario approach, taking into account the levels of product consumption (maximum, recommended, actual);

– *Risk Characterization*. Description of risk as the probability of certain effects with their quantitative characteristics, as well as evaluation of the acceptability of risk level and its classification.

When calculating the intermediate values of minimum inhibitory concentrations, the model proposed in the paper by A.Fazil (1996) and based on the Poisson beta-distribution (1), has been used:

$$p_i = (1-(1+\alpha C)^{-\beta}) \cdot 100\%, \qquad (1)$$

where p_i – percentage of inhibited bacteria of *i*-type, *C* – tetracycline concentration in the intestine, α, β - model parameters.

Animal products daily consumption levels of child population aged 3-7 in the Russian Federation are adopted in accordance with Moscow Guidelines "Catering in preschool educational institutions" (2007) and are presented in Table 1.

Result and Discussion

Table 1 – Standards of daily consumption rates of animal origin products for the child population in the Russian Federation

Food products	Level of daily consumption, kg
Meat (muscle)	0.074
Liver	0.007
Eggs	0.048
Milk	0.558

At the step of *Hazard Identification* it was found that tetracycline is one of the most common drugs, used for the treatment and prevention of poultry and meat cattle diseases. Along with the increase of production efficiency of meat food products, use of tetracycline may lead to the public health risk due to residues in meat, milk, eggs and other products.

Standards of residues concentrations in foods in the United States, the European Union and the Russian Federation differ significantly (Table 2).

Table 2 - Tetracycline MRLs for different types of animal products (μg/kg)

Product	WHO 1990	FAO/WHO 1998	USA	Russia
Milk	100	100	300	10
Muscle	100	200	2000	10
Fat	10	-	10	10
Eggs	200	400	200	10
Liver	300	600	300	10
Kidney	600	1200	600	10

Tetracycline has a range of negative effects on human health:
- imbalance of intestinal microflora;
- resistant flora growth;
- suppressing the growth of sensitive bacteria;
- decrease of function of the obligate anaerobic flora (Dominguez E. et al.,2002).

At the step of *Dose-Response Assessment* simulation of facultative microflora growth against the obligate flora inhibition, principally, Bifidobacterium, was conducted.

As the basis of the dose-response assessment, the well-known microbiological studies, as well as the data provided in the WHO Additives Series: 41. Tetracycline, have been used (FAO/WHO,1998). Due to reported data, the minimum concentrations, at which tetracycline inhibits 50 and 90% of bacteria - Mic_{50}, $Mic_{90,}$ have been established. Table 3 shows normal microflora composition and data on the minimum inhibitory concentrations $Mic_{50,}$ Mic_{90} for tetracycline.

Table 3 - Values of Mic_{50} and Mic_{90} of tetracycline against human intestinal microflora

Species of bacteria	Share in the normal intestinal microflora,%	$Mic_{50,}$ μg/ml	$Mic_{90,}$ μg/ml
Bifidobacterium spp.	85	16	32
Bacteroides spp.	4,99	1	32
Clostridium spp.	0,01	0,062	32
Fusobacterium spp.	2	0,125	–
Lactobacillus	4	2	2
Peptostreptococcus	2	2	32
E. Coli (intestinal bacterium)	1	32	64
Optional flora	1	–	–

Results of parameters' assessment for each type of bacteria inhibition in the intestinal microflora are reproduced in Table 4.

Table 4 - Parameters of tetracycline concentration effect models on the bacteria inhibition in the intestinal microflora

Species of bacteria	α	β
E. Coli (intestinal bacterium)	0,00024	148,4
Bifidobacterium spp.	0,00048	148,4
Bacteroides spp.	3,09	0,5
Clostridium spp.	0,01	4,56
Fusobacterium spp.	0,68	14,27
Lactobacillus	0,0078	148,4
Peptostreptococcus	0,89	0,68

Figure 1 presents the dependence models of the inhibition percent and tetracycline concentration for various bacteria.

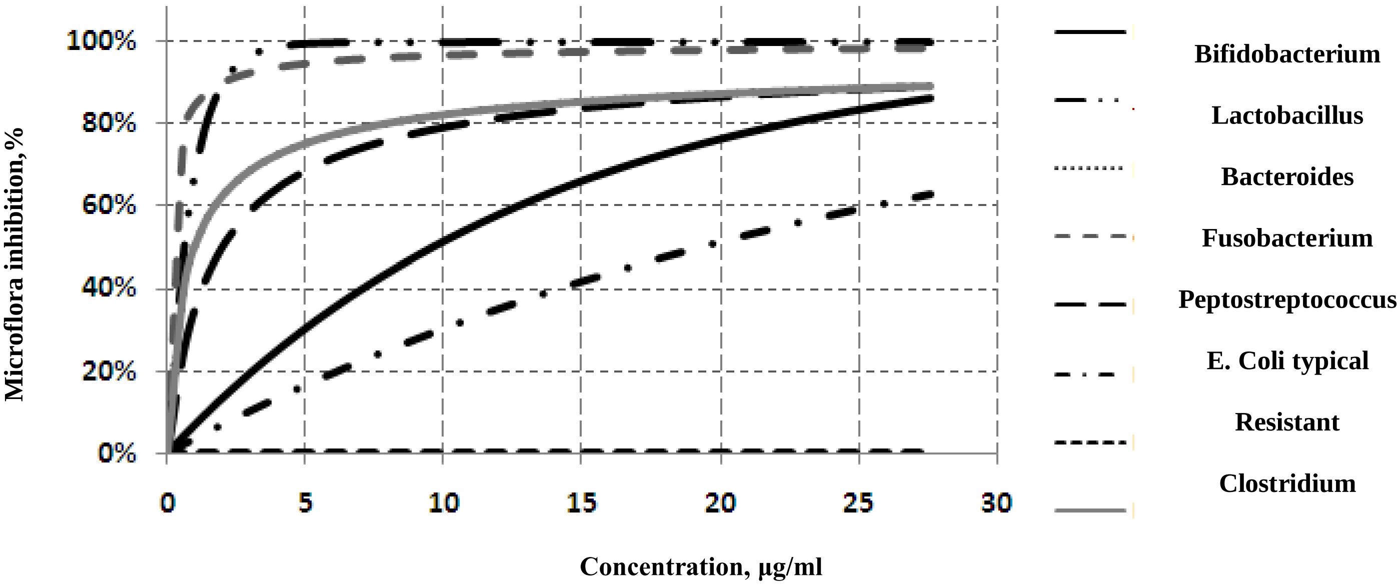

Figure 1 - Correlation model of the inhibition percent and tetracycline concentration for various bacteria

Figure 1 shows that there is variability in the sensitivity of different bacteria in the intestines to tetracycline. Hence, there is a reason to believe that an increase in the content of tetracycline in the intestine leads to a change in the relationship between different types of bacteria. In this case, based on the studies (Corpet D.E.,1993,2000; Shuhaimi M. et al.,1999; Levy J.,2000; Perrin-Guyomard A. et al.,2001), which describe the growth of facultative microflora at the oppression of obligate species of microorganisms, it is possible to perform modeling of changes in the composition of the microflora under the tetracycline exposure on the basis of balance ratios.

When modeling the influence of tetracycline on the microflora state in the intestine microbial balance equation was used in the following form:

$$\sum_i n_i = const = N \, , \tag{2}$$

where - n_i -the number of *i*-type bacteria, N - total number of microorganisms.

Since certain bacteria inhibition leads to the growth of other bacteria, microflora balance is changed. In other words, based on (2) we can make a ratio of bacteria overall quantity equality in the form of:

$$\sum_i \frac{n_i}{N} \cdot 100\% = \sum_i \lambda_i \cdot 100\% = 100\% \, , \tag{3}$$

where $\lambda_i = \dfrac{n_i}{N}$ - relative quantity of the intestinal microflora of *i*-type.

On the basis of the balance equation (3) based on the model of tetracycline effect on the inhibition of various kinds of bacteria (1) the relative composition of the intestinal microflora was calculated according to the concentration of tetracycline by the relation:

$$\lambda_i(C) = \frac{\lambda_i(0)(1 - p_i(C))}{\sum_i \lambda_i(0)(1 - p_i(C))} \tag{4}$$

The calculation results are presented in Figure 2.

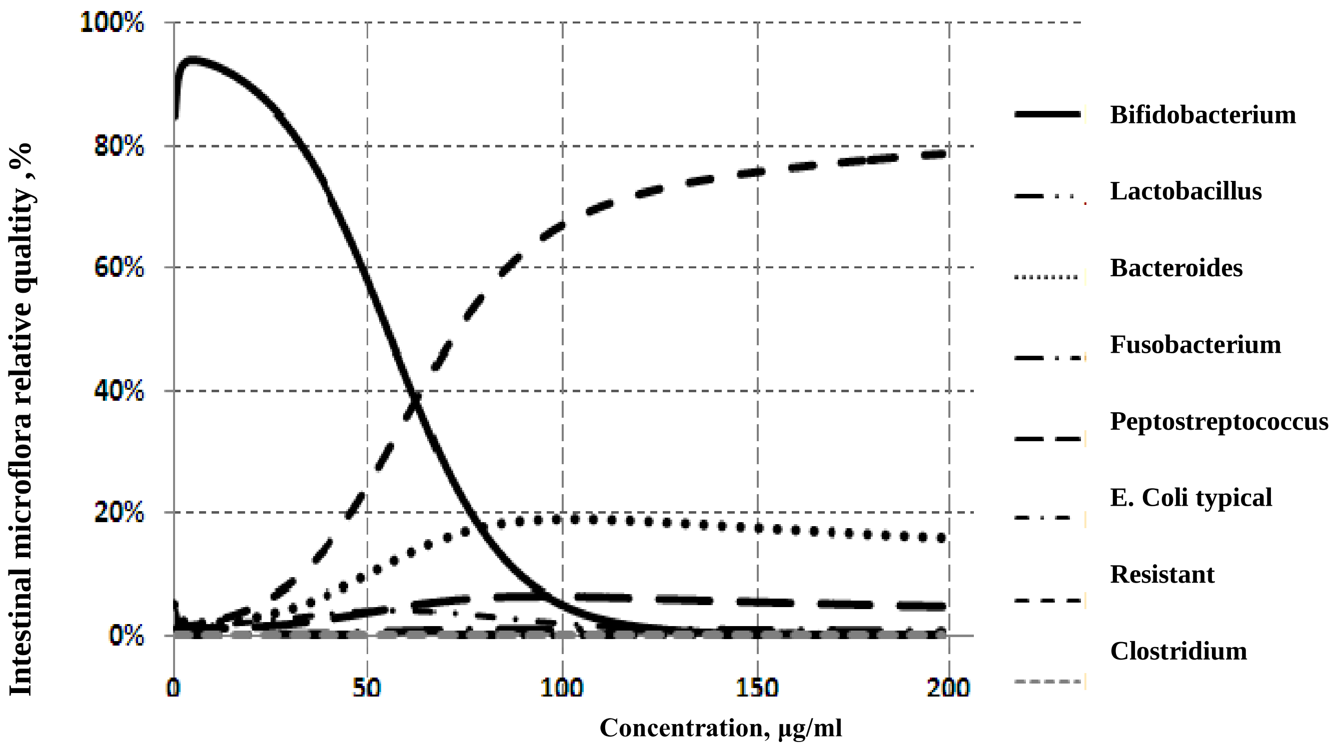

Figure 2 – The dependence of the intestinal microflora relative quantity (%) on the tetracycline concentration

Figure 2 shows the patterns of change in the microflora composition under the conditions of growth inhibition of certain bacteria and their replacement by others, resistant to tetracycline. So, when tetracycline concentration increases, the relative quantity of Bifidobacterium falls, reaching 1%. At this, a facultative, tetracycline resistant flora is developing and which at the concentration increasing can reach up to 79% of the entire bacterial flora population.

As a most susceptible population children aged 1-11 years with tetracycline sensitive intestinal microflora, constituting up to 70% of the total child population, were selected (Kamalova A.A.,2011).

At the step of exposure evaluation 4 exposure scenarios have been developed. They take into account data on the average daily consumption of animal origin foods by the child population of the Russian Federation and the values of MRLs tetracycline used in Russia (scenario 1), tetracycline maximum residue levels (MRL) recommended by WHO (1990) (scenario 2), FAO/ WHO (1998) (scenario 3) and by the United States (scenario 4).

The obtained values of the maximum daily intake for children population of tetracycline under the conditions of the studied exposure scenarios, considering the average body weight (20 kg) are shown in Table. 5.

Table 5 - Values of the tetracycline maximum daily intake according to the studied exposure scenarios

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Tetracycline maximum daily intake, µg/kg	3,2	89,4	160,3	360,95

With regard to the average mass of the children intestinal contents (50g) (Kamyshnikova V.S.,2007) tetracycline concentration in the gastrointestinal tract was calculated for the conditions of four exposure variants (Table 6).

Table 6 - Tetracycline concentrations in the gastro-intestinal tract of children for the studied exposure scenarios

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Tetracycline concentration in the gastro-intestinal tract, µg/g	0,069	1,79	3,2	7,2

Thus, the tetracycline concentration in the gastro-intestinal tract of children population for the four studied exposure scenarios ranged from 0.069 µg/g (Scenario 1), up to 7.2 µg/g (Scenario 4).

At the step of *Risk Characterization* it was found that the risk of intestinal microflora imbalance in children, associated with tetracycline residues in food, is absent only at concentrations not exceeding 10 µg/kg (Scenario 1) (Table 7). So, such a concentration of tetracycline does not impose risk of diseases associated with intestinal microflora imbalance.

Table 7 - Intestinal microflora balance in children under different scenarios of tetracycline residues exposure, % of the total number of bacteria

Species of bacteria	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Bifidobacterium spp.	89,58	78,99	71,37	53,44
Bacteroides spp.	2,74	1,17	0,91	0,62
Clostridium spp.	0,006	0,002	0,002	0,002
Fusobacterium spp.	0,64	0,11	0,07	0,04
Lactobacillus	2,79	0,38	0,08	0,00
Peptostreptococcus	0,96	0,52	0,40	0,26
E. Coli (intestinal bacterium)	0,99	0,93	0,88	0,76
Facultative bacteria	2,29	17,9	26,28	44,87

Taking into account the frequency of various diseases associated with the intestinal microflora imbalance in children and child morbidity in the model region of the Russian Federation the individual risk of digestive diseases (K58.0, K59.0, K59.9, K29.8, K29.9) will be from 0.000081 to 0.001238. In addition, intestinal microflora imbalance can cause additional risk of anemia, dermatitis, allergy and immunodeficiency states in children (Table 8).

Table 8 - Individual risk of additional morbidity for various degrees of microflora balance violations

Group (code within ICD10)	1 degree	2 degree	3 degree	Total
IBS with diarrhea (K58.0)	0.000016	0.000033	0.000032	0.000081
Constipation (K59.0)	0.000133	0.000032	0	0.000165
Functional bowel disorder, unspecified (K59.9)	0.000025	0.000094	0.000007	0.000126
Iron deficiency anemia (D50)	0.001012	0.000796	0.001372	0.00318
Other atopic dermatitis (L20.8)	0.000334	0.000206	0.000725	0.001265
Duodenitis (K29.8, K29.9)	0.000452	0.000325	0.000461	0.001238
Food allergy (T78.0, T78.1, T78.4)	0.000012	0.000030	0.000149	0.000191
Common variable immunodeficiency, unspecified (D83.9)	0	0.000010	0.000013	0.000023

In the course of the study possible uncertainties have been also evaluated, including the following ones:
- due to the missing or incomplete information;
- associated with the parameters used to evaluate exposure and risk calculation (uncertainty of parameters);
- due to the gaps in scientific theory required for prediction based on the causal relationships (model uncertainties).

The first category includes the insufficient information about the possible effects of tetracyclines in chronic exposure on the human health, the uncertainties associated with the transfer of data from animal experiments and in vitro on human, variability in the intestinal microflora because of its resilience.

The second category includes uncertainties associated with food consumption. For example, in the Russian Federation there is a tendency to increase the proportion of meat and dairy products in the population diet. There are no reliable forecasts of tetracycline content in foods. To the model uncertainty one should also include a limited number of these studies, under the results of which model parameters were calculated. The values of these parameters can be varied in the population, the inaccuracy in their definition can be determined using the generalized averaged data for large populations. Application of standard values of parameters increases the uncertainty of exposure and risk assessment, characterized by the degree of the sensitivity analysis of parameters. In addition, the uncertainty of such parameters as acceptable daily intakes is associated with the use of modifying factors or unreasonable refusal to use them.

The third category uncertainty is caused by the incomplete knowledge of the patterns forming microflora balance disorders and related health disorders.

In general, the uncertainty of study results should be characterized as high. Herewith, public health safety can be achieved in the nearest future through the application of uncertainty factors in establishing hygienic standards with their adjustment in the future based on the results of epidemiological studies.

Conclusion:

Thus, in children in case of intestinal microflora imbalance, particularly because of suppressing the growth of sensitive bacteria and resistant flora growth against the continued bactericidal activity related to the obligate anaerobic flora (Bifidobacterium, Bacteroides, Clostridium, Fusobacterium, Peptostreptococcus, Lactobacillus), due to residual tetracycline concentrations in food more than 10 mg/kg, the risk of digestive system diseases increases to the level of 0.000461, dermatitis to 0.000725, food allergies to 0.000149, blood diseases to 0.001372. Increased risk of child morbidity in the Russian Federation with the digestive system diseases can be up to 4% of cases, diseases of the blood - up to 8% of cases, skin diseases - to 0.9% of cases, allergic diseases - to 0.1% of cases.

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