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CHLORAMPHENICOL-INDUCED ALTERATIONS IN THE LIVER AND SMALL INTESTINE EPITHELIUM IN PIGS*

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Abstract

An effect of the exposure to chloramphenicol (CAP) at doses used therapeutically was studied in pigs at the age of slaughter. Pigs were treated with CAP intramuscularly (20 mg/kg b.w. two times every 24 hours). Histomorphometrical and immunohistochemical analyses of small intestine and liver were done. CAP increased the thickness of myenteron and submucosa, and the length of villi; decreased the depth of crypts in the duodenum and jejunum. CAP influenced the Auerbach plexus. A decrease in cell proliferation, an increase in the number of apoptotic cells and T lymphocytes in the CAP-treated pigs were observed. CAP induces hepatotoxicity, neurotoxicity and disturbed intestinal epithelium. It can be concluded that short exposure of pigs to CAP at doses used therapeutically results in disturbed digestion and absorption process in the intestine.

Key words: chloramphenicol, food-producing animals, intestinal epithelium, liver, pigs

Chloramphenicol (CAP) is an antibiotic originally derived from the bacterium *Streptomyces venezuelae* (1947) found in soil. It has a broad spectrum against gram-

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positive and gram-negative bacteria, anaerobic bacteria, and rickettsias. Despite numerous side effects like bone marrow toxicity, lymphocytopenia, neutropenia, anorexia, vomiting, diarrhea, depression, peripheral neuritis and hepatitis, CAP is still used in parenteral treatment in humans, as well as in eye-drops (Friedman et al., 1998; Brady and Weil, 2002; Nielsen et al., 2013). An absorption of CAP in adult human beings is rapid and extensive after an oral dose (Yunis, 1973). CAP passes into breast milk, can cross the placenta and has a tendency towards accumulation in the liver and other tissues (Friedman et al., 1998; Shukla et al., 2013).

In United Europe, CAP is placed on the list of pharmacologically active substances for which the MRPL (Minimum Required Performance Limit) level is determined and since 1994 the use in veterinary medicine is forbidden in all food-producing animals to prevent residues of CAP in foodstuffs of animal origin (Hanekamp and Calabrese, 2007; EC, 2010; Shukla et al., 2013). In muscle of pigs (27 kg) treated with CAP via feed twice daily at a dose of 25 mg/kg b.w. for 3 days, CAP was detected (40–270 μg/kg) in animals killed after 10 days. In liver and fat, CAP was detected in some animals even on day 21 (EFSA, 2014). The exposure of animals to CAP at doses formerly used therapeutically (typically 25–50 mg/kg b.w. per day) results in residues of CAP in meat (EFSA, 2014).

Since 2002 CAP also is banned in Ukraine, but despite the prohibition its residues are occasionally found in foodstuffs of animal origin such as milk and honey (Yanovych et al., 2013). The investigation of causes of CAP residues ingress into milk products does not always detect the non-authorized use of this antibiotic for the treatment of animals. For this reason, the causes of the presence of CAP in the tissues of animals are sought after. The source of CAP in organism of farm animals could be the feed contaminated by soil, because CAP is obtained from soil microorganism *Streptomyces venezuelae* (Yanovych et al., 2013). Also, plants occurring in straw or diet of farm animals may be the source of CAP ingress into animal organisms and foodstuffs of animal origin (Berendsen et al., 2010; Yanovych et al., 2013; Nordkvist et al., 2016). The presence of non-compliant residues is also a consequence of not respecting the principles of good farm practice or good veterinary practice. CAP is still used in non-food-producing animals. Thus, the most common cause of non compliance of concentration of CAP in animal tissues is the use of it in those species for which they are not intended and not respected grace period. Therefore, it seems that there is still a risk of exposure of human being to CAP in edible tissues or foodstuff of animal origin.

To our knowledge, there is no information in the literature concerning the alteration in intestinal epithelium or liver tissue after even short exposure to CAP. These findings and the lack of particular knowledge on the exposure to CAP give afresh a reason to the broad study of the influence of CAP on living organism. The aim of the present study was therefore to investigate if the short treatment at low therapeutic doses of CAP could cause any alteration in intestinal epithelium and liver in a pig model.

Material and methods

Ten castrated male pigs were used in the study. Used research material consisted of tissues and organs derived from 6 castrated porkers at the age of 6 months which were treated with CAP at the dose of 20 mg/kg body weight two times every 24 hours (intramuscularly) before slaughter. For comparison, analogous tissues and organs from 4 pigs which were not CAP-treated were used. The experiment was approved by The Local Ethics Committee in the State Scientific Research Control Institute of Veterinary Medicinal Products and Feed Additives (SCIVP), Lviv, Ukraine.

All the pigs were held under standard rearing conditions with constant access to fresh water. Pigs through the fattening were fed with commercial diet (twice a day) supplied in accordance with the stage of production cycle and age as recommended (Table 1).

After slaughter, two 10-mm long segments of the small intestine from the duodenum (5 cm distal to the pylorus) and 50% of the total intestinal length were taken from each animal. Fragments of liver (0.5 cm^3) were also obtained from each animal. All samples were subjected to histological procedure and staining as previously described (Kisielinski et al., 2002; Abu Eid and Landini, 2003; Suvara et al., 2013; Tomaszewska et al., 2014). The morphometric variables which were analyzed also were previously described (Abu Eid and Landini, 2003; Suvara et al., 2013; Tomaszewska et al., 2012). A total number of 5 measurements of each parameter per pig was performed (a total number of 30 and 20 measurement for CAP and control group, respectively).

Apoptotic cells were counted per square millimeter of tissue (in Hoechst eosin-stained sections); the criteria for recognizing apoptotic cells were those outlined as described previously (Lizard et al., 1995).

The following parameters of liver tissue were analyzed: per mm^2 – hepatocyte number, number of hepatocyte nuclei, number of double nucleated hepatocytes, total number of hepatocyte nuclei, other cells, total cells; intercellular space as an area (%); fractal dimension of intercellular space (D); average size of nuclei, perimeter of nuclei, Feret diameter and circularity (Śliwa et al., 2009; Tomaszewska et al., 2015 a, b, 2017). A total number of 5 measurements of each parameter per pig was performed (a total number of 30 and 20 measurements for CAP and control group, respectively).

Immunohistochemistry

Immunohistochemical staining was performed using monoclonal rabbit anti-pig antibodies against CD3 epsilon chain of T cells (Abcam, Cambridge, UK, dilution 1:200; to mark T lymphocytes in the small intestine wall); polyclonal rat anti-pig antibodies against Ki-67 (Abcam, Cambridge, UK, dilution 1:50; to indentify proliferating cells); rabbit polyclonal to 200 kD neurofilament heavy subunit neuronal marker (Abcam, Cambridge, UK, dilution 1:200; to localize Meissner and Auerbach plexuses). Immunohistochemical procedure is described previously (Tomaszewska et al., 2012).

Table 1. Diet composition for pigs in the study

Diet composition	Prestarter	Starter	Grower-finish
Ingredient			
wheat middlings (%)	53.00	41.50	15.00
barley middlings (%)	–	30.00	36.00
soybean meal (%)	–	–	10.00
rye middlings (%)	–	–	32.00
rape middlings (%)	–	–	4.00
Panto Ferkelgold F 2501 (%)	22.00	–	–
Panto Multivit-Premix M 512 (%)	–	3.50	–
Panto Multivit-Premix M 393 (%)	–	–	3.00
corn, grain (%)	25.00	10.00	–
Composition			
dry matter (%)	87.66	87.28	87.30
crude protein (%)	16.96	16.66	16.00
ME (MJ/kg)	14.01	13.00	12.63
crude fibre (%)	2.72	3.56	4.03
crude fat (%)	1.98	1.98	1.75
ash (%)	4.60	5.86	5.65
starch (%)	47.60	45.34	43.60
Ca (%)	0.64	0.82	0.79
P (%)	0.48	0.48	0.48
available P (%)	0.08	0.14	0.15
Na (%)	0.17	0.25	0.21
Mg (%)	0.20	0.20	0.19
carbohydrates (%)	4.37	3.49	3.54
Lys (%)	1.17	1.00	0.93
Met (%)	0.55	0.33	0.30
Met + Cys (%)	0.79	0.65	0.63
Trp (%)	0.18	0.19	0.19
Thr (%)	0.74	0.63	0.57
vitamin A (IE)	15.840	14.000	12.000
vitamin D (IE)	1.760	1.750	1.200
vitamin E (mg)	99.00	140	75.00
vitamin C (mg)	33.00	–	–
vitamin K (mg)	2.20	1.40	0.90
vitamin B_1 (mg)	2.20	1.40	0.90
vitamin B_2 (mg)	6.60	4.24	2.70
vitamin B_6 (mg)	4.40	4.20	2.70
vitamin B_{12} (µg)	33.00	35.00	–
nicotinic acid (mg)	33.00	–	–

[1]The protein-vitamin-mineral concentrate (1 kg): crude protein 38.0%, ash 14.5%, L-lysine 3.8%, crude fibre 3.75%, crude fat 3.5%, Ca 2.7%, P 1.0%, Na 0.7%, vitamin A 72,000 IU, vitamin D_3 8,000 IU, vitamin E 450 mg, Cu 600 mg, endo-1,4-β-xylanase 1,200 FXU, 3-phytase 2,000 FTU, Ca $(H_2PO_4)_2$, lactic acid, formic acid, aromas.

[2]The vitamin and mineral premix (1 kg): Ca 21.0%, P 3.0%, Na 6.0%, L-lysine 7.0%, threonine 1.0%, Mg 2.0%, methionine 2.0%, vitamin A 400,000 IE, vitamin D_3 50,000 IE, vitamin E 4,000 mg, Cu 5,000 mg, 3-phytase 16,650 FTU, salinomycin-Na 1,000 mg.

[3]The vitamin and mineral premix (1 kg): Ca 22.5%, P 3.5%, Na 5.5%, L-lysine 6.5%, Mg 2.0%, methionine 1.0%, vitamin A 400,000 IE, vitamin D_3 50,000 IE, vitamin E 2,500 mg, Cu 1,000 mg, salinomycin-Na 1,000 mg.

Microscopic images of the immunohistochemistry reactions were subjected to further analysis and the variables analyzed included: regarding Ki-67 – the number of proliferating cells/0.01 mm^2 of the gland surface and the proliferating cell index as the number of proliferating cells in relation to all the gland cells (%); regarding neurofilament detection – the cross section area of the nerve ganglion, sphericity, perimeter, and mean diameter, and regarding T cells: the total number of T cells per mm^2 of tissue. A total number of 5 measurements of each parameter per pig was performed (a total number of 30 and 20 measurements for CAP and control group, respectively).

Statistical analysis

All results are expressed as means ± SD (standard deviation). Differences between means were tested with the Student-T test. Normal distribution of data was examined using the W. Shapiro-Wilk test and equality of variance was tested by the Brown-Forsythe test. When there was no normal distribution and/or unequal variance of data, we had to use the Mann-Whitney U test to test the differences between the means. A p-value less than 0.05 was considered statistically significant. All statistical analyses were carried out by means of Statistica 12 software (StatSoft, Inc., Tulsa, OK, USA).

Results

At slaughter, CAP-treated pigs weighed 99.4±3.8 kg and did not differ from the non-treated animals (95.0±4.7 kg, P>0.05).

Table 2. Effect of chloramphenicol treatment on the histomorphometry of duodenum in pigs

Parameter	C[1]	CAP[2]
Myenteron thickness (µm):		
longitudinal lamina	403±63	714±49*
transversal lamina	503±35	681±103*
Submucosa thickness (µm)	94±28	128±17*
Mucosa thickness (µm)	862±50	680±80*
Enterocyte number/100 µm of villus	20.9±6.7	16.2±1.5*
Villi epithelium thickness (µm)	38.3±13.4	38.0±4.5
Villi length (µm)	566±145	478±130
Villi thickness (µm)	37.3±17.4	28.5±25.8
Total villi number/mm	6.1±2.0	6.3±0.5
Crypts depth (µm)	286±49	198±30*
Crypts width (µm)	46.5±7.9	107.8±14.9*
Active crypt number/mm	5.4±1.0	7.4±0.7*
Inactive crypt number/mm	2.9±1.4	1.2±0.2
Total crypt number/mm	8.4±0.9	7.4±0.7*
Small intestine absorptive surface (μm^2)	3.1±0.9	5.8±1.3*
Ki number/0.01 mm^2 of the gland surface	40.6±10.5	28.2±3.1*
Ki index	41.8±6.5	32.5±2.4*

Data given are Mean ± SD, * P < 0.05.
[1]C – control group, no chloramphenicol.
[2]CAP – animals treated with chloramphenicol.

The histomorphometric analysis of the duodenum and jejunum and nerve plexuses is presented in Tables 2, 3, and 4, respectively. CAP administration resulted in an increase in the thickness of the myenteron (longitudinal and transversal in both parts of intestine about 73% and 45%, respectively) and submucosa (about 41%; P<0.001). Mucosa thickness decreased about 21% in duodenum (P<0.001), while increased about 60% in jejunum (P<0.001). The length of villi increased in the CAP-treated pigs in jejunum about 67% (P<0.001) and decreased about 15% in duodenum (P=0.03). The depth of crypts was lower in both the duodenum and jejunum (about 42%; P<0.001), although their width and the number of active crypts rose in the duodenum (131% and 37%, respectively; P<0.001). Absorptive surface increased in duodenum by 87% (P<0.001) and in jejunum by 30% (P<0.001). What is more, administration of CAP influenced the size of nerve plexuses and resulted in an increase in the Auerbach plexus diameter in the duodenum (44%; P<0.001) and an increase in the perimeter, diameter, and area of the Auerbach plexus in the jejunum (135%, 85%, 230%, respectively; P<0.001).

Table 3. Effect of chloramphenicol treatment on the histomorphometry of jejunum in pigs

Parameter	C[1]	CAP[2]
Myenteron thickness (μm):		
longitudinal lamina	253±41	432±77*
transversal lamina	436±30	669±33*
Submucosa thickness (μm)	129±38	189±41*
Mucosa thickness (μm)	480±75	770±80*
Enterocyte number/100 μm of villus	15.3±1.9	16.3±2.3
Villi epithelium thickness (μm)	24.8±3.9	24.4±2.8
Villi length (μm)	419±142	701±140*
Villi thickness (μm)	112±12	116±9.7
Total villi number/mm	7.5±1.2	6.7±0.9
Crypts depth (μm)	226.2±35.2	124.1±18.5*
Crypts width (μm)	59.3±16.8	61.6±13.1
Active crypt number/mm	7.3±1.4	7.1±0.9
Inactive crypt number/mm	2.1±1.1	3.2±2.1
Total crypt number/mm	9.2±1.3	8.7±1.6
Small intestine absorptive surface (μm^2)	7.8±2.1	10.2±2.0*
Ki number/0.01 mm^2 of the gland surface	71.0±15.9	54.5±2.4*
Ki index	49.3±6.9	37.7±1.6*

Data given are Mean ± SD, * P<0.05.
[1]C – control group, no chloramphenicol.
[2]CAP – animals treated with chloramphenicol.

The number of T cells in the duodenum did not differ significantly between the control (1460 ± 442 cells per mm^2 of tissue) and CAP-treated animals (1162 ± 435 cells per mm^2 of tissue). The analysis showed a significantly higher number of T lymphocytes in the jejunum in the CAP group (2836 ± 627 cells per mm^2 of tissue) compared with the control group (1148 ± 353 cells per mm^2 of tissue; P<0.001) (Figure 1).

Table 4. Effect of chloramphenicol treatment on the histomorphometry of nerve plexuses in duodenum and jejunum of pigs

Parameter	C[1]	CAP[2]
	Duodenum	
Auerbach plexus		
area (μm²)	19512±14443	29439±17106
perimeter (μm)	922±563	967±360
mean diameter (μm)	112±24	161±30*
sphericity	0.77±0.18	0.86±0.13
Meissner plexus		
area (μm²)	3938±4137	3763±3844
perimeter (μm)	572±584	384±271
mean diameter (μm)	49±20	48±18
sphericity	0.04±0.02	0.06±0.04
	Jejunum	
Auerbach plexus		
area (μm²)	2184±2209	7202±3693*
perimeter (μm)	181±110	426±137*
mean diameter (μm)	44.5±23.8	82.4±21.6*
sphericity	0.35±0.28	0.22±0.12
Meissner plexus		
area (μm²)	2402±1942	2039±1675
perimeter (μm)	299±131	311±214
mean diameter (μm)	42.7±19.5	35.6±13.3
sphericity	0.10±0.13	0.03±0.02

Data given are Mean ± SD, * $P<0.05$.
[1]C – control group, no chloramphenicol.
[2]CAP – animals treated with chloramphenicol.

The Ki index was decreased in the CAP group in the duodenum and in the jejunum (about 23%; $P=0.004$ for duodenum and $P<0.001$ for jejunum). Moreover, the number of proliferating cells also decreased in the CAP-treated pigs ($P<0.001$) (Tables 2 and 3; Figure 1).

After administration of CAP, an increase in the number of apoptotic cells was observed ($P<0.001$). There were 151% more apoptotic cells in the duodenum and 19% more apoptotic cells in the jejunum in comparison to the control group.

The histomorphometric analysis of the liver is presented in Table 5. It revealed a significant decrease in the numbers of hepatocytes by 24%, hepatocyte nuclei by 30%, and an increase of other cells by 70% in the group of pigs treated with CAP. The degree of the diversity of the cell outline increased after CAP by 7% ($P<0.01$).

Further examination revealed 13% less apoptotic cells in the liver of pigs treated with CAP in contrast to the control group ($P<0.001$).

The histological examination of the control liver tissue showed normal architectural hepatocytes with more or less centrally located mainly one nucleus.

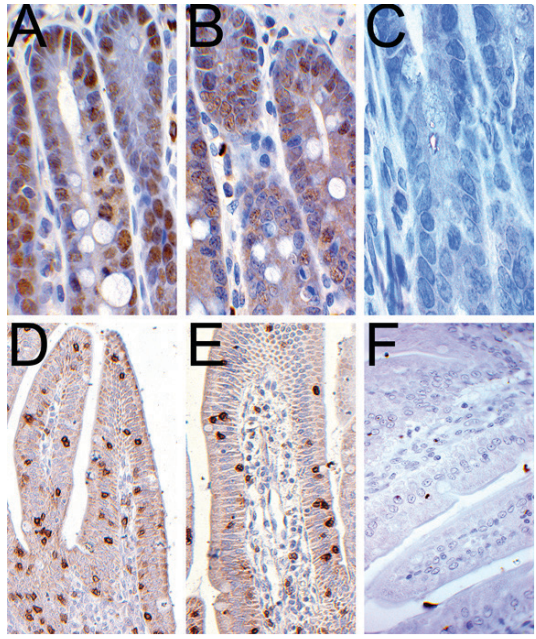

Figure 1. Upper row: Representative pictures of the immunohistochemical reactions for Ki-67 carried out on formaldehyde-fixed sections from the jejunum of pigs from the control group (A) and from the CAP group (B). C – control section for antibody
Lower row: Representative pictures of the immunohistochemical reactions for CD3 carried out on formaldehyde-fixed sections from the jejunum of pigs from the control group (C) and from the CAP group (D). F – control section for antibody

Table 5. Effect of chloramphenicol on the histomorphometrical parameters of liver in pigs

Parameter	C[1]	CAP[2]
Hepatocyte		
hepatocyte number/mm^2	2034±297	1548±165*
hepatocyte nuclei number/mm^2	1758±363	1230±153*
double nucleated hepatocyte number/mm^2	337±131	320±109
total hepatocyte nuclei number/mm^2	2434±263	2152±238
other cell/mm^2	547±212	932±222*
total cell number/mm^2	2590±339	2460±498
intercellular space (%)	10.1±4.6	12.3±3.3
fractal dimension	1.54±0.07	1.65±0.07*
Nuclei of hepatocyte		
nuclei average size (µm)	51.7±12.3	43.6±4.8*
perimeter (µm)	20.8±5.2	20.9±4.9
feret (µm)	7.4±1.7	7.5±1.7
circularity	0.75±0.09	0.75±0.08

Data given are Mean ± SD, * P<0.05.
[1]C – control group, no chloramphenicol.
[2]CAP – animals treated with chloramphenicol.

Microscopic assessment of the liver structure in the CAP-treated pigs showed no marked differences in the distribution of portal triads and terminal hepatic venules in the tissue compared with the control group. However, slight fatty degeneration, ballooning, and vacuolization in the livers were observed. No fibrosis was observed in the liver.

Discussion

Modern intensive farming methods and the associated high density of animals cause still rising demand for veterinary medicines, including antibiotics (EMA, 2012). These substances are widely used in human or veterinary medicine for therapeutic and prophylactic purposes or to protect the public health by limiting the spread of zoonoses (Greger, 2007; EFSA and ECDC, 2015 a, b). On the other hand, improper use or overuse of antibiotics in agriculture as well as human or veterinary medicine led to the emergence and spread on a very large scale of antibiotic-resistant microorganisms with more efficient mechanisms of resistance (Phillips et al., 2004; Hammerum and Heuer, 2009). The overuse of antibiotics in livestock led to their presence in food products (edible tissues) obtained from food-producing animals (Shea, 2003; Khanna et al., 2007). It should be noted that among these antibiotics is CAP which is still used as a growth promoter or bacteriostatic agent in some countries (Shalaby et al., 2006; Shukla et al., 2013).

However, our analysis showed that there was no impact of CAP on body weight in pigs at slaughter age. Still, it cannot be unambiguously excluded that the use of CAP promotes animal body weight gain. Sanchez-Martínez et al. (2008) have shown that fish supplemented with antibiotic exhibit a significant increase in weight gain which suggested a growth promoting action despite negative effects on the immune system of fish (Guardiola et al., 2012). Sanchez-Martínez et al. (2008) have also shown significant increase in weight gain and decrease in food conversion ratio, which could be beneficial for the producer, but it is linked with the presence of residual antibiotics in fish tissue and fish products (Samanidou and Ewaggelopoulou, 2007). It should be emphasized that other structural analogs of CAP with more activity against bacteria are also used as growth promoter (Cannon et al., 1990). It seems that the effect of antibiotic growth promoter depends upon the kind of animals and type of antibiotic used.

The current results showed that short CAP treatment increased wall of small intestine in pigs. The use of CAP or other antibiotics may lead to proliferation of pathogenic bacteria re-homing and releasing their toxins. Among those activated bacterias that invade the host leading to complications could be *Proteus* or *Pseudomonas* (Dhama et al., 2014).

In our study the activity of bacteria in gastrointestinal tract was not investigated, but after CAP treatment an increase of the number of active crypts was observed, probably in response to the change in the balance of intestinal microflora and their composition. In crypts there are specialized epithelial cell populations such as Paneth

cells which secreted antimicrobial peptides. The number of T cells in the intestine of the CAP-treated pigs also increased.

To our knowledge, there are numerous studies with CAP analysis and monitoring in food of animal origin in different countries suggesting a high risk of illegal use of CAP in farms (Shen et al., 2009; Mehdizadeh et al., 2010; Tajik et al., 2010). For this reason, it is difficult to discuss our other results because there is a lack of any study describing histomorphometric alteration in intestinal epithelium. Our study showed that CAP decreased mitosis in the intestinal crypts and slowed down proliferation. CAP treated pigs also showed a tendency to a decrease in the number of enterocytes. This is an important element in the contact digestion. Furthermore, CAP showed negative effect on nervous system in small intestine. These changes might influence the processes of proliferation, differentiation, and regeneration of the intestinal epithelium.

Biotransformation of CAP takes place mainly in the liver besides kidneys, where CAP reaches the highest residue levels. It correlates with the present results of the histomorphometric analysis. These changes can indicate considerable damage of liver tissue. The influence of CAP on the liver resulted in an increased number of other cells than hepatocytes. This change could be caused by the increased number of phagocytic cells indicating an inflammatory process. Our results are consistent with other studies showing liver toxicity after CAP treatment (Gaikowski et al., 2012; Ahmadizadeh et al., 2013).

In general, the problem of the influence of pharmacologic treatment of farm animals on the nutritional value of meat and its products, and its safety for human health is still open for discussion (Grela et al., 2013).

Conclusion

It can be concluded that short exposure of pigs to CAP at doses used therapeutically results in disturbed digestion and absorption process in the intestine. Our results showed the negative impact of CAP-treatment on histological structure of the intestine, liver and innervation of small intestine. CAP induces hepatotoxicity, neurotoxicity and disturbed intestinal epithelium.

Conflict of interest

The authors declare that they have no conflict of interests regarding the publication of this article.

References

Abu Eid R., Landini G. (2003). Quantification of the global and local complexity of the epithelial-connective tissue interface of normal, dysplastic, and neoplastic oral mucosae using digital imaging. Pathol. Res. Pract., 199: 475–482.

Ahmadizadeh M., Esmailpoor M., Goodarzi Z. (2013). Effect of phenobarbital on chloramphenicol-induced toxicity in rat liver and small intestine. Iran J. Basic Med. Sci., 16: 1282–1285.

Berendsen B., Stolker L., de Jong J., Nielen M., Tserendorj E., Sodnomdarjaa R., Cannavan A., Elliott C. (2010). Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Anal. Bioanal. Chem., 397: 1955–1963.

Brady N.C., Weil R.R. (2002). The nature and properties of soils. Upper Saddle River, USA, Prentice Hall, 13th ed., 960 pp.

Cannon M., Harford S., Davies J.A. (1990). A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. J. Antimicrob. Chemother., 26: 307–317.

Dhama K., Tiwari R., Khan R.U., Chakraborty S., Gopi M., Karthik K., Saminathan M., Desingu P., Sunkara L. (2014). Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: the trends and advances – a review. Int. J. Pharm., 10: 129–159.

EC (2010). Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Off J EU, L15, 20.1.2010: 1–72.

EFSA (2014). EFSA Panel on Contaminants in the Food Chain: Scientific Opinion on Chloramphenicol in food and feed. EFSA J., 12: 3907.

EFSA and ECDC (2015 a). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J., 13: 162.

EFSA and ECDC (2015 b). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J., 13: 4329.

EMA (2012). Sales of veterinary antimicrobial agents in 19 EU/EEA countries in 2010. EMA/88728/2012, pp. 74.

Friedman Y., Weisman Y., Avidar Y., Bogin E. (1998). The toxic effects of monensin and chloramphenicol on laying turkey breeder hens. Avian. Pathol., 27: 205–208.

Gaikowski M.P., Wolf J.C., Schleis S.M., Tuomari D., Endris R.G. (2012). Safety of florenicol administered in feed to tilapia (*Oreochromis* sp.). Toxicol. Pathol., 41: 639–652.

Greger M. (2007). The human/animal interface: emergence and resurgence of zoonotic infectious diseases. Crit. Rev. Microbiol., 33: 243–299.

Grela E.R., Kowalczuk-Vasilev E., Klebaniuk R. (2013). Performance, pork quality and fatty acid composition of entire males, surgically castrated or immunocastrated males, and female pigs reared under organic system. Pol. J. Vet. Sci., 16: 107–114.

Guardiola F.A., Cerezuela R., Meseguer J., Esteban M.A. (2012). Modulation of the immune parameters and expression of genes of gilthead seabream (*Sparus aurata* L.) by dietary administration of oxytetracycline. Aquacult., 334–337: 51–57.

Hammerum A.M., Heuer O.E. (2009). Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. Clin. Infec. Dis., 48: 916–921.

Hanekamp J.C., Calabrese E.J. (2007). Chloramphenicol, Europeans legislation and hormesis – commentary. Dose Response, 5: 91–93.

Khanna T., Friendship R., Dewey C., Weese J.S. (2007). Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet. Microbiol., 128: 298–303.

Kisielinski K., Willis S., Prescher A., Klosterhalfen B., Schumpelick V. (2002). A simple new method to calculate small intestine absorptive surface in the rat. Clin. Exp. Med., 2: 131–135.

Lizard G., Fournel S., Genestier L., Dhedin N., Chaput C., Flacher M. Mutin M., Panaye G., Revillard J.P. (1995). Kinetics of plasma membrane and mitochondrial alterations in cells undergoing apoptosis. Cytometry, 21: 275–283.

Mehdizadeh S., Kazerani H.R., Jamshidi A. (2010). Screening of chloramphenicol residues in broiler chickens slaughtered in an industrial poultry abattoir in Mashhad. Iran. J. Vet. Sci. Technol., 2: 25–32.

Nielsen I.R., Seim A., Bentzen N. (2013). Chloramphenicol eye drops in the treatment of conditions indicative of maxillary sinusitis. Tidsskr. Nor. Laegeforen., 133: 2146–2148.

Nordkvist E., Zuidema T., Herbes R.G., Berendsen B.J.A. (2016). Occurrence of chloramphenicol in cereal straw in north-western Europe. Food Addit. Contam. Part A: Chem. Anal. Control. Expo. Risk. Assess., 33: 798–803.

Phillips I., Casewell M., Cox T., De Groot B., Friis C., Jones R., Nightingale C., Preston R., Waddell J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother., 53: 28–52.

Samanidou V.F., Evaggelopoulou E.N. (2007). Analytical strategies to determine antibiotic residues in fish. J. Sep. Sci., 30: 2549–2569.

Sanchez-Martínez J.G., Pérez-Castañeda R., Rábago-Castro J.L., Aguire--Guzmán G., Vázquez-Sauceda M.L. (2008). Preliminary study on the effects on growth, condition, and feeding indexes in channel catfish, *Ictalurus punctatus*, after the prophylactic use of potassium permanganate and oxytetracycline. J. World Aquaculture Soc., 39: 664–670.

Shalaby A.M., Khattab Y.A., Rahman A.M. (2006). Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). J. Venom. Anim. Toxins. incl. Trop. Dis., 12: 172–201.

Shea K.M. (2003). Antibiotic resistance: what is the impact of agricultural uses of antibiotics on children's health? Pediatrics, 112: 253–258.

Shen J., Xia X., Jiang H., Li C., Li J., Li X., Ding S. (2009). Determination of chloramphenicol, thiamphenicol, florfenicol, and florfenicol amine in poultry and porcine muscle and liver by gas chromatography-negative chemical ionization mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 877: 1523–1529.

Shukla P., Bansode F.W., Singh R.K. (2013). Chloramphenicol toxicity: a review. J. Med. Med. Sci., 2: 1313–1316.

Suvara S.K., Layton C., Bancroft J.D. (2013). Bancroft's theory and practice of histological techniques. Edinburgh, UK, Churchill Livingstone, 7th ed., 654 pp.

Śliwa E., Dobrowolski P., Tatara M.R., Piersiak T., Siwicki A., Rokita E., Pierzynowski S.G. (2009). Alpha-ketoglutarate protects the liver of piglets exposed during prenatal life to chronic excess of dexamethasone from metabolic and structural changes. J. Anim. Physiol. Anim. Nutr., 93: 192–202.

Tajik H., Malekinejad H., Razavi-Rouhani S.M., Pajouhi M.R., Mahmoudi R., Haghnazari A. (2010). Chloramphenicol residues in chicken liver, kidney and muscle: A comparison among the antibacterial residues monitoring methods of Four Plate Test, ELISA and HPLC. Food Chem. Toxicol., 48: 2464–2468.

Tomaszewska E., Dobrowolski P., Puzio I. (2012). Postnatal administration of 2-oxoglutaric acid improves the intestinal barrier affected by the prenatal action of dexamethasone in pigs. Nutrition, 28: 190–196.

Tomaszewska E., Dobrowolski P., Puzio I., Prost L., Kurlak P., Sawczuk P., Badzian B., Hulas-Stasiak M., Kostro K. (2014). Acrylamide-induced prenatal programming intestine structure in guinea pig. J. Physiol. Pharmacol., 65: 107–115.

Tomaszewska E., Winiarska-Mieczan A., Dobrowolski P. (2015 a). Hematological and serum biochemical parameters of blood in adolescent rats and histomorphological changes in the jejunal epithelium and liver after chronic exposure to cadmium and lead in the case of supplementation with green tea vs black, red or white tea. Exp. Toxicol. Pathol., 67: 331–339.

Tomaszewska E., Winiarska-Mieczan A., Dobrowolski P. (2015 b). The lack of protective effects of tea supplementation on liver and jejunal epithelium in adult rats exposed to cadmium and lead. Environ. Toxicol. Pharmacol., 40: 708–714.

Tomaszewska E., Dobrowolski P., Kwiecień M. (2017). Alterations in intestinal and liver histomorphology, and basal hematological and biochemical parameters in relation to different sources of dietary copper in adult rats. Ann. Anim. Sci., 17: 477–490.

Yanovych D., Rydchuk M., Korobova O., Zasadna Z. (2013). Natural ways of antibiotics' ingress into the products of animal origin. In: 6th International Symposium on Recent Advances in Food Analysis, Pulkrabová J., Tomaniová M., Nielen M., Hajšlová J. (eds). Institute of Chemical Technology, Prague, Czech Republic, p. 449.

Yunis A.A. (1973). Chloramphenicol toxicity. In: Blood disorders due to drugs and other agents, Girdwood R.H. (ed.), Excerpta Medica, Amsterdam, Netherlands, pp. 107–126.

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