HISTOMORPHOLOGICAL CHANGES IN ORGANS USING AN EXPERIMENTAL MODEL OF BALB/C MICE, INFECTED WITH DIFFERENT *E. COLI* STRAINS

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Abstract. The aim of this work was to determine an effect of endotoxin of *E. coli* strain ATCC 35218 serotype O6:H31:K-, strain RRW-1 serotype O8:H24:K-, and strain K-12 MG165 serotype O12:H48:K- on histomorphological changes in the organs using an experimental model of BALB/c mice. The first group (n=3) was control group and 0.1 ml 0.85 % NaCl solution was injected into the peritoneum. The second group of mice (n=12) was divided into the 4 subgroups (n=3) and 0.1 ml of strain *E. coli* RRW-1 serotype O8:H24:K- according to the dilutions 1:1; 1:10; 1:100 and 1:1000 were injected into the peritoneum. The third group of mice (n=12) was divided into the 4 subgroups (n=3) and 0.1 ml of strain *E. coli* ATCC 35218 serotype O6:H31:K- according to the dilutions 1:1; 1:10; 1:100 and 1:1000 were injected into the peritoneum. The fourth group of mice (n=12) was divided into the 4 subgroups (n=3) and 0.1 ml of strain *E. coli* K-12 MG165 serotype O12:H48:K- according to the dilutions 1:1; 1:10; 1:100 and 1:1000 were injected into the peritoneum. All mice of the third group were dead within 20 hours after injection of strain *E. coli* ATCC 35218 serotype O6:H31:K- dilution 1:1. All strains and serotypes of *E. coli* caused histomorphological changes in the mice organs. The most severe changes in the organs were caused by ATCC 35218 serotype O6:H31:K- dilutions 1:1, 1:10, similar changes were caused by *E. coli* RRW-1 serotype O8:H24:K-, dilutions 1:1 and 1:10. Dilution 1:1 of K-12 MG165 serotype O12:H48:K- caused mild lesions in some organs. Pathogenic *E. coli* strains caused blood circulatory disturbances: hyperaemia, thrombosis, haemorrhages; hepatosis and nephrosis with immune response reaction, hyperplasia of lymphoid organs (spleen, lymph nodes).

Keywords: mouse, *E. coli*, strains, organs, pathology

Introduction. *Escherichia coli* strains are commonly found in the gut microflora of warm-blooded animals. Most *E. coli* are harmless and actually are an important part of the healthy human and animal intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness intra - or extra-intestinal tract, and include septicaemia, urinary tract infections (UTIs), meningitis and pneumonia (Weinstein et al. 1997, Peterson et al. 2005). The types of *E. coli* that cause diarrhea can be transmitted through contaminated water, food or by contact with animals or persons (Golenbock et al. 1991, Russo et al. 2003, Bearman et al. 2005, Russel, 2006). Blood infections that influence morbidity and mortality are increasing worldwide (Bearman et al. 2005). One of the most important diseases of *E. coli,* are gram-negative rods from the family *Enterobacteriaceae* (Starlander et al. 2014). There is currently seen an increasing broad-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* bacteria prevalence not only in hospitals but also in society (Goulenok et al. 2013).

The **aim** of this work was to determine an effect of endotoxin of *E. coli* strain ATCC 35218 serotype O6:H31:K-, strain RRW-1 serotype O8:H24:K-, and strain K-12 MG165 serotype O12:H48:K- on histomorphological changes in the organs using an experimental model of BALB/c mice.

Materials and methods

Animals and samples. BALB/c mice (n=39) of 10 weeks old were used in the study. The animals were purchased from the Animal Facility of Veterinary Academy, Lithuanian University of Health Sciences (Kaunas, Lithuania). The experiments were performed in compliance with the relevant laws of the local Animal Ethical Committee (No 92-16 (21-07-2014). The animals were housed individually in standard polycarbonate cages (Techniplast, Italy), according to the Directive 2010/63 EU with free access to food and acclimatized for one week before the study. They were housed under conditions of constant temperature (22 ± 2°C), humidity (55±5 percent), and the light/dark cycle (12 h/12 h). A commercial pellet diet was provided ad libitum. The commercial standard diet for rodents contained crude protein 19.91 percent, fat 12.05 percent, and crude fibers 2.79 percent and 7.72 percent cellulose in 1 kg of feed. The first group (n=3) was control group and 0.1 ml 0.85 % NaCl solution was injected into the peritoneum. The second group of mice (n=12) was divided into the 4 subgroups (n=3) and 0.1 ml of strain *E. coli* RRW-1 serotype O8:H24:K- according to the dilutions 1:1; 1:10; 1:100 and 1:1000 were injected into the peritoneum. The third group of mice (n=12) was divided into the 4 subgroups (n=3) and 0.1 ml of strain *E. coli* ATCC 35218 serotype O6:H31:K- according to the dilutions 1:1; 1:10; 1:100 and 1:1000 were injected into the peritoneum. The fourth group of mice (n=12) was divided into the 4 subgroups (n=3) and 0.1 ml of strain *E. coli* K-12 MG1655 serotype O12:H48:K- according to the dilutions 1:1; 1:10; 1:100 and 1:1000 were injected into the peritoneum. Strain of *E. coli* our named RRW-1 serotype O8:H24:K- was obtained from a patient who died of

sepsis. Using PCR analysis it was identified that serotype RRW-1 is O8:H24:K- is very toxigenic, producing ESBL, do not producing exotoxins, and very resistant to wide range of antibiotics. The primary suspension contained 10 Mc Farland (3000 CFU (x106/ml). *E. coli* strain (our named) RRW-1 serotype O8:H24:K- was obtained from tissues of a child who died from this infection. *E. coli* strain ATCC 35218 was as a Quality Control Isolate and is recommended by NCCLS (National Committee for Clinical Laboratory Standards, 1997) as the quality control organism for the β-lactam–β-lactamase inhibitor agents. However, current guidelines recommend the testing of *E. coli* ATCC 35218 with Mueller-Hinton (MH) medium. Strain MG1655 *E. coli* K-12 serotype O12:H48:K- was sequenced by the Blattner laboratory because it approximates wild-type E. coli and "has been maintained as a laboratory strain with minimal genetic manipulation, having only been cured of the temperate bacteriophage lambda and F plasmid by means of ultraviolet light and acridine orange, respectively." (Blattner et al. 1997).

Health condition of animals was observed 24 hours per day for two weeks. After two weeks all mice were killed by an overdose of carbon dioxide in chamber.

Histomorphological examination. Samples of kidney, liver, lungs, heart, thymus, lymph nodes, and intestines for histomorphological examination were taken during the autopsy. The samples were immediately fixed in 10% buffered formalin. The paraffin blocks were made using „Shandon Pathcentre“ and „TES 99 Medite Medizintechnik“ equipment. Serial 4-µm sections were prepared with a “Sakura Accu-Cut SRM“ microtome from each sample and served for routine H&E staining.

Statistical analysis. For percentage estimates, Wilson (Score) 95 % confidence intervals (CI 95 %) were calculated. Statistical characteristics of the samples were calculated using statistical software SPSS (Version 15, SPSS Inc., Chicago, IL). Results were considered statistically significant if $P<0.05$.

Results

All mice of the third group were dead in period of 20 hours after injection of strain *E. coli* ATCC 35218 serotype O6:H31:K- dilution 1:1. Clinically mice show tachycardia and tachypnea. All mice of 2, 4 and 3 (except where the dilution was 1:1) groups were euthanized after 2 weeks.

Histomorphological findings. All strains and serotypes of *E. coli* caused histomorphological changes in mice organs ($\chi^2=15.18$; $P<0.001$). Most severe changes in organs were caused by ATCC 35218 serotype O6:H31:K- dilutions 1:1, (95% CI 31.4-56.7) and 1:10 (95% CI 20.3-44.0) ($P<0.001$). Similar changes were caused by *E. coli* RRW-1 serotype O8:H24:K-, dilutions 1:1 (95% CI 23.6-57.6) and 1:10 (95% CI: 12.7-43.4) ($P<0.01$). Only dilution 1:1 of K-12 MG165 serotype O12:H48:K- produced more lesions in some organs (Fig. 1). Pearson's Chi-squared test $\chi^2 = 3.7813$.

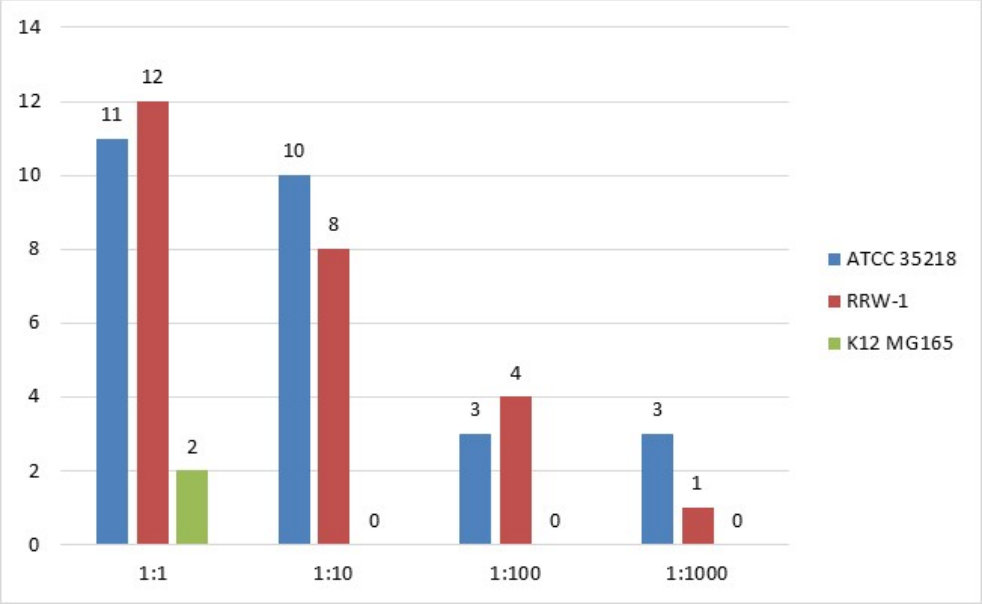

Fig. 1. **Incidence of main pathologies in mice with *E. coli* different serotypes**

E. coli ATCC 35218 serotype O6:H31:K- dilution 1:1 caused circulatory disturbances (43.6%) almost in all organs. Grossly and microscopically we identified hyperaemia, thrombosis of blood vessels, haemorrhage, oedema, emphysema and leucocytes infiltration in lungs (Fig. 2); hyperaemia, haemorrhages in kidney, liver, immunological response in lymphoid organs (lymph nodes, spleen) (Fig. 3). There were mild changes in the small intestines – mild infiltration of leucocytes in some areas and degeneration of epithelial cells.

In case of *E. coli* ATCC 35218 serotype O6:H31:K- dilution 1:10 there were outspread reaction of lymphoid

tissue, blood circulatory disturbances, and lesions of the liver and kidney. There were hyperplasia of lymphoid tissue in the spleen - lymphoid follicles were much enlarged. The marked disorders were determined in the liver and kidney such as degeneration and inflammatory reaction. Perivasculitis, hyperaemia, degeneration of hepatocytes were found in the liver and there were a lot inflammatory focuses which consist of lymphocytes, heterophils, eosinophils, infiltration by lymphocytes nearby biliary ducts (Fig. 4). Hyperaemia, epithelial degeneration, perivasculitis, degeneration of blood vessels wall (fibrinoid) with perivascular oedema of kidneys were determined (Fig. 5). Hemosiderosis and thrombosis of some vessels were found in lungs (Fig. 6). A wall of intestines was infiltrated by some leucocytes, with swelling of enterocytes. The same serotype (O6:H31: K-) dilution 1:100 caused severe swelling and lipidosis of the liver (Fig. 7). No severe changes were identified in other organs: hyperaemia and moderate degeneration of kidney, moderate hyperplasia of lymphoid follicles in spleen and lymph nodes, mild leucocyte infiltration in lungs. Dilution 1:1000 showed mild changes in parenchymal organs. Moderate hyperplasia of follicles was found in the spleen and swelling of parenchymal cells in the liver and kidney.

In case of *E. coli* RRW-1 serotype O8:H24:K-, dilution 1:1 active thymus with proliferation of lymphocytes in follicles was found (40.4%). Spleen follicles were of different sizes, from normal to large with proliferation of lymphocytes. In the liver there were swellings, lipidosis or necrosis of hepatocytes and focuses of leucocytes accumulation; in perivascular tissue and in triads - moderate infiltration by lymphocytes and plasmocytes (Fig. 8). Similar changes were found in the kidneys - hyperaemia, perivasculitis, degeneration of tubular epithelium (Fig. 9). The heart vessels were infiltrated with leucocytes. In the lungs (there were identified haemorrhages, hyperaemia, mild infiltration with leucocytes and bronchiolitis. The small intestines were infiltrated with few leucocytes. RRW-1 serotype O8:H24:K-, dilution 1:10 caused infiltration by leucocytes in the intestinal mucosa, swelling of hepatocytes, perivascular infiltration by lymphocytes and plasmocytes in liver. In kidney there were degeneration and mild necrosis of some tubular epithelial cells, in some glomerulus were found only few erythrocytes (Fig. 10). In samples of the lungs there were alveolar emphysema, hyperaemia, oedema and hyperplasia of bronchial lymphoid follicles (Fig. 11). In spleen was mild hyperplasia of lymphoid follicles. Dilution 1:100 showed changes in the liver: swelling or lipidosis, or necrosis of hepatocytes, hyperaemia, and perivasculitis (from mild to moderate). In the lungs there were alveolar emphysema, hyperaemia, oedema. A mild immune response was in the lungs, kidney, intestines and lymphoid organs. Dilution 1:1000 cause leucocytes infiltration in the intestinal mucosa, hyperplasia of epithelial cells in crypts; hyperaemia of kidney and degeneration of hepatocytes in the liver. In the spleen there were hemosiderosis and mild accumulation of macrophages. In the lungs - mild oedema, peribronchial infiltration of lymphocytes and proliferation of II type alveolar cells.

K-12 MG165 serotype O12:H48:K- dilution 1:1 caused mild infiltration by leucocytes in intestinal mucosa, swelling of hepatocytes (mild perivascular infiltration by lymphocytes and plasmocytes in stroma of the liver (Fig.12), haemorrhages and swelling of tubular epithelial cells in kidney and mild immune response in the lungs. In the spleen - proliferation of lymphocytes, plasmocytes and hemosiderosis (Fig. 13). Other dilutions - 1:10, 1:100, 1:1000 of K-12 MG165 serotype O12:H48:K- did not cause changes in organs.

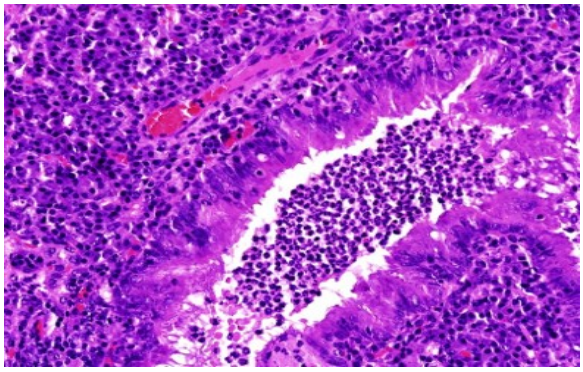

Fig. 2. **Severe lung inflammation: hyperaemia, oedema and inflammatory cells infiltration in lung and in the lumen of bronchioli. HE, x200**

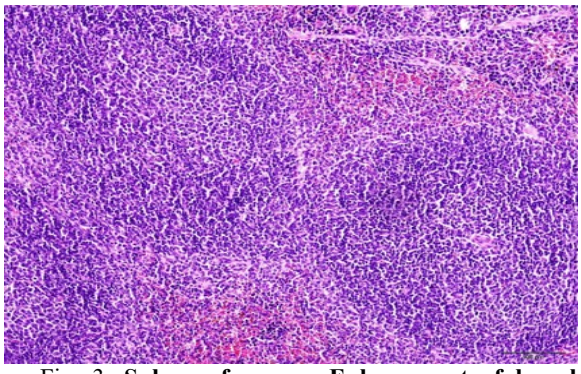

Fig. 3. **Spleen of mouse. Enlargement of lymph follicles. HE, x100**

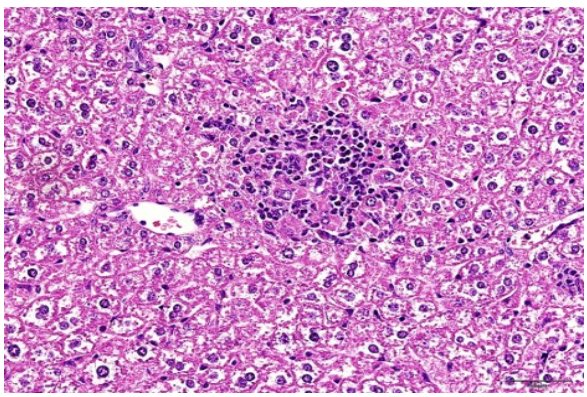

Fig. 4. **Lymphocytes infiltrates in mouse liver nearby biliary ducts. Lipidosis and swelling of hepatocytes. HE, x200**

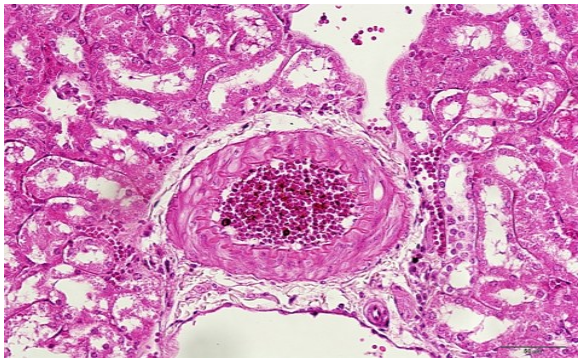

Fig. 5. **Degeneration of blood vessels wall (fibrinoid), perivascular oedema in mouse kidney. HE, x200**

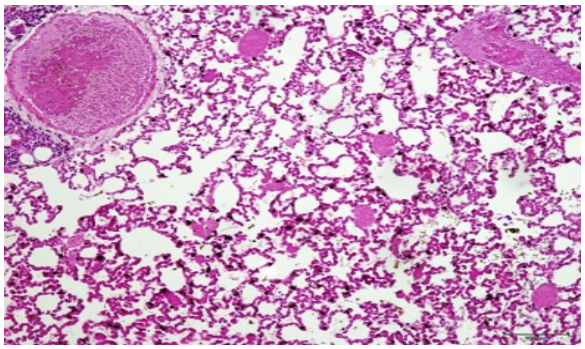

Fig. 6. **Thrombosis of lung blood vessels. HE, x100**

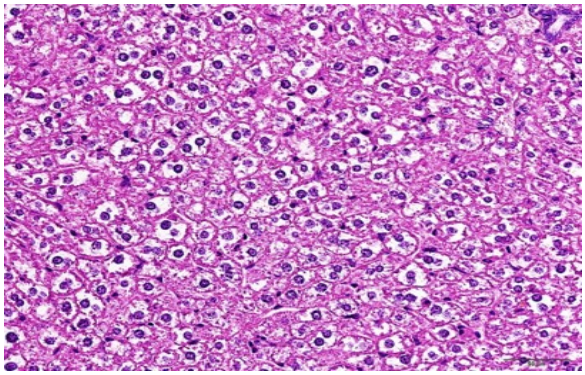

Fig. 7. **Severe swelling and lipidosis of hepatocytes. HE, x200**

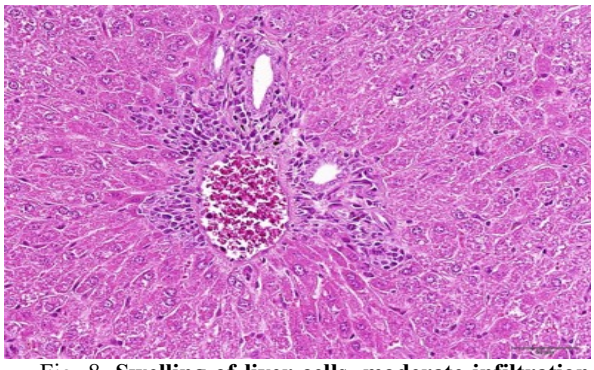

Fig. 8. **Swelling of liver cells, moderate infiltration of perivascular areas and triad by lymphocytes and plasmocytes. HE, x200**

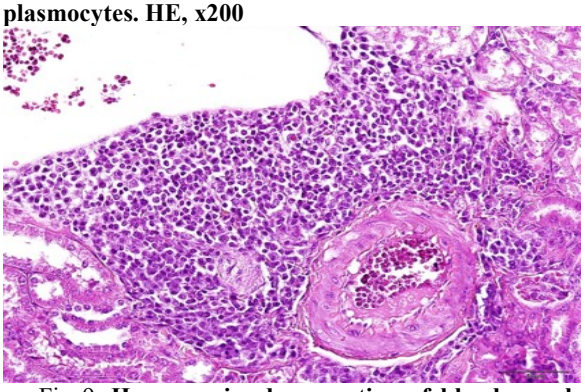

Fig 9. **Hyperaemia, degeneration of blood vessels, large perivascular lymphocytes-plasmocytes infiltration in mouse kidney. HE, x200**

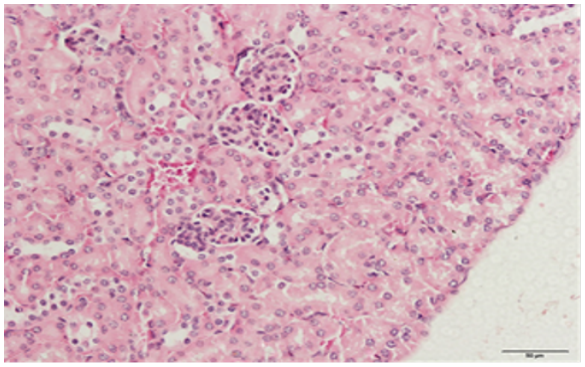

Fig. 10. **Degeneration and mild necrosis of some tubular epithelial cells in kidney. HE, x200**

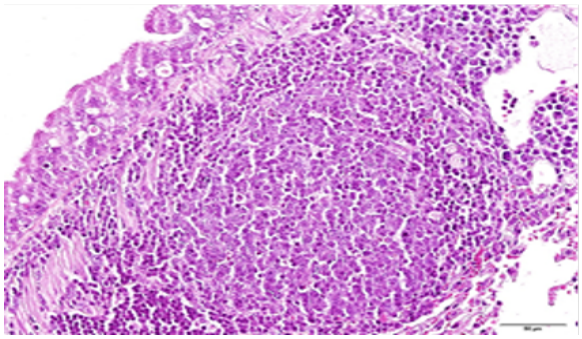

Fig. 11. **Hyperplasia of bronchus associated lymphoid tissue (BALT). HE, x200**

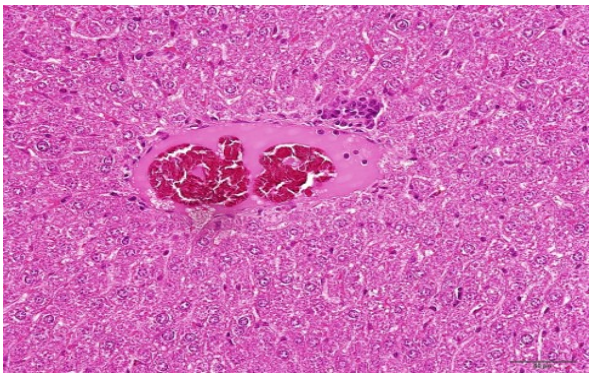

Fig. 12. **Hyperaemia and swelling of cells in liver.**

HE, x200

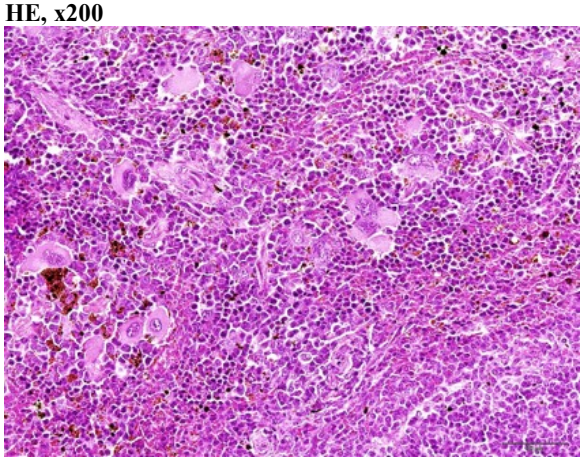

Fig. 13. **Red pulp hyperplasia in mouse spleen (proliferation of plasmocytes and lymphocytes). Extramedullary haematopoiesis also visible. HE, x200**

Discussion

From the dawn of modern biology, the intestinal bacterium *Escherichia coli* have been the most intensively studied organism. Many basic molecular processes, best understood in *E. coli*, are universal throughout the natural world. *E. coli* is the main cause of hospital infections, especially bloodstream and urinary tract infections. According Martin et al. (2003), Peterson et al. (2005) despite improves care, the hospital mortality rate from severe sepsis and septic shock has not improved significantly. Gram-negative bacterial lipopolysaccharide (LPS) is regarded as the strongest sepsis and septic shock pathogenetic mediator (Ronco et al. 2010). It is believed that the lipid A component of LPS is toxigenic (Fu et al. 2008). Among the various strains of bacteria is observed not only O-antigen but also variability of lipid A from which gives depends the respond of organism (Rietschel et al. 1994, Borzęcka et al. 2013).

We analysed *E. coli* strains RRW-1, K-12 MG165, ATCC 35218 lysates impact to BALB/c mice and compared with the control group. In our study death of mouse (n=3) cause only ATCC ESBL 35218 serotype O6:H31:K- dilution 1:1. Injections of small doses of endotoxin results in death in most mammals. Time of death varies on the dose of the endotoxin, species of animal, its susceptibility to endotoxin, route of administration. LPS endotoxin is responsible for the clinical syndrome of Gram-negative septicemia or septic shock (Golenbock et al. 1991; Fu et al. 2008).

E. coli ATCC ESBL 35218 serotype O6:H31:K- dilution 1:10 caused severe changes in organs, such as: hyperplasia of lymphoid tissue, blood circulatory disturbances and blood vessels changes such as hyperaemia, haemorrhages, fibrinoid necrosis and thrombosis with perivasculitis. In parenchymatous organs (liver kidney) caused degeneration or necrosis. Cytokines and secondary mediators participate in systemic vasodilatation and cause hypotension and hypoxia, hart failure and dysfunction of other organs, endothelial injury with disseminated intravascular coagulation (DIC) formation, and increase of capillary permeability. Tissue hypoxia and bacterial toxins cause degenerations, systemic leukocyte adhesion and diffuse alveolar capillary damage. Less changes were found when dilution was 1:100 or 1:1000. According to present knowledge LPS, through its lipid A component, interacts with various host cell types including mononuclear cells, endothelial and smooth muscle cells, polymorphonuclear granulocytes, and thrombocytes, among which macrophages/monocytes are of particular importance. LPS responsible for toxic manifestations of severe Gram-negative infections and generalized inflammation (Rieschel et al., 1993). LPS are extremely strong stimulators of innate or natural immunity in alive organisms (Galanos & Freudenberg, 1992; Galanos et al., 1993; Alexsander & Rietsckel, 2001).

E. coli RRW-1 serotype O8:H24:K-, dilution 1:1 was more aggressive than the other dilution and causes a stimulation of lymphoid organs, degenerative and inflammatory reactions in parenchymal organs. LPS induced activation of macrophages (phagocytosis and cytotoxicity) results in the production of bioactive lipids, reactive oxygen species, and peptide mediators such as tumour necrosis factor a (TNF), interleukin 1 (IL-I), IL-6, IL-8, and IL-b. An activation of the complement cascade C3a and C5a causes histamine release leading to vasodilatation and, as result of inflammation, effect neutrophil chemotaxis and accumulation. The secondary, hormone-like proteins are endowed with potent bioactivities and inducing many of the typical endotoxin effects by acting independently, in sequence, synergistically or antagonistically (Vogel at al. 1990, Haveman et al. 1999, Borzecka et al. 2013).

K-12 MG165 serotype O12:H48:K- only dilution 1:1 caused mild changes in all organs. Tourret et al., (2011) determined that synergy of pathogenic and non pathogenic *E. coli* had several consequences. In their experiment the mixed infection killed more mice and more rapidly than its components were separately.

Many studies have highlighted the increasing prevalence of b-lactam-resistant strains, particularly extended-spectrum b-lactamase (ESBL)-producing enterobacteriaceae (ESBL-PE) in the hospital and community. RRW-1 is O8:H24:K- produce ESBL and resistant for many antibiotics. Antibiotic resistance is a major public health concern worldwide. Most ESBL-PE are resistant to other antimicrobial therapy such as fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole (Peterson et al. 2005, Starlander et al. 2014, Goulenok et al. 2013).

Conclusions

Pathological morphological changes caused all dilutions of strains *E. coli* RRW-1 serotype O8:H24:K- and ATCC 35218 serotype O6:H31:K-. More severe changes in the mice organs are caused *E. coli* ATCC 35218 O6:H31:K- and *E. coli* RRW-1 serotype O8:H24:K-, dilutions 1:1, 1:10. K-12 MG165 serotype O12:H48:K- dilution 1:1 causes mild changes in the mice organs. The main pathological morphological changes on all 2, 3 groups were degeneration or necrosis, immune

response in the liver, kidney and intestines; hyperplasia of lymphocytes in immune organs, vascular disturbances: hyperaemia, disorders of endothelium, thrombosis and perivasculitis.

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