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RELEVANCE OF FOOD BACTERIAL ZOONOSES IN THE DNIPROPETROVSK REGION (UKRAINE) DURING 2020

Martynenko H. A.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: anna29.10.76@i.ua

Summary. When the world is facing an unprecedented COVID-19 pandemic, the importance of animal disease surveillance has become apparent. A significant proportion of new and existing human diseases are zoonoses. Microbiological researches remain relevant today. They guarantee the quality of food raw materials and products, as well as identify sources of infection. The aim of our work was to study the spread of foodborne bacterial zoonoses in the Dnipropetrovsk region during 2020. The study used the methods provided by DSTU ISO 6887-2:2005, DSTU ISO 4833:2006, DSTU ISO 4832:2015, DSTU EN 12824:2004, DSTU ISO 11290-1:2003, DSTU 7444:2013, DSTU ISO 6888-2:2003, DSTU ISO 7937:2006. As a result of the study of the epidemic situation regarding outbreaks of acute intestinal infectious diseases and food poisoning in 2020 in Ukraine, 52 cases were investigated, including cases in which children have been affected (43.8%). In the etiological structure, the largest share of outbreaks caused by salmonella (34.6%) and outbreaks of viral etiology (25%) was observed. The registration of outbreaks of human salmonellosis in 82% of regions in Ukraine proves that geographical conditionality, climatic conditions, species structure of farm animals and the level of socio-economic status of the population are important factors in the development of the disease. The presence of positive for animal salmonellosis localities also demonstrates the constant spread of this zoonosis in Ukraine, which causes contamination by these bacteria of food products of animal origin and environmental objects. The dominance of the Dnipropetrovsk Region in 2020 in the number of outbreaks of acute intestinal infectious diseases contributed to a detailed investigation of bacteriological studies of raw materials of animal origin and food products, which revealed 2.56% of non-compliant samples, where the highest percentage of violations of safety criteria was poultry products. Thus, high-risk products included semi-finished products and culinary products from meat, in particular poultry; minced meat and mechanically rolled meat, as well as ready-to-eat meat products — sausages, namely poultry, and eggs. Poultry products of seven types (n = 3,226) were analyzed for the presence of sanitary-indicative microflora (coliform bacteria, mesophilic aerobic and facultative-anaerobic microorganisms), opportunistic microflora (coagulase-positive staphylococci, *Proteus*, sulfite-reducing clostridia), and pathogenic microflora (bacteria of the genera *Salmonella, Listeria).* The percentage of samples that did not meet the requirements of regulatory documentation by indicators was: mesophilic aerobic and facultative anaerobic microorganisms up to 10.92%; coliform bacteria — up to 3.90%. Pathogenic contaminants of poultry products of domestic Ukrainian production were: *Salmonella* Enteritidis (up to 4.20%), *Listeria monocytogenes* — 12.50%. The paper shows problematic in 2020 nosological forms in the structure of pathogens of food bacterial zoonoses and identifies their potential sources. Microbiological studies have shown that the contaminants of poultry products of domestic Ukrainian production in the Dnipropetrovsk Region were sanitary-indicative (coliform bacteria, mesophilic aerobic and facultative-anaerobic microorganisms), opportunistic pathogens (*Staphylococcus aureus*, sulfite-reducing clostridia), and pathogenic microflora (bacteria of the genera *Salmonella, Listeria).* The author has monitored the geographical serotypic predisposition of *Salmonella*, which is due to the prevalence of O-group D in the Dnipropetrovsk Region, and the possibility of their passage through the food chain. It was found that minced meat and meat of mechanical deboning from poultry is one of the factors of transmission of a potentially pathogenic for humans and animals species of *Listeria — L. monocytogenes* in the region

Keywords: poultry products, *Salmonella* Enteritidis, *Listeria monocytogenes*, meat of mechanical deboning

Introduction. At a time when the world is facing an unprecedented pandemic COVID-19, the importance of animal disease surveillance has become apparent. Zoonoses have been widespread since the Bible (Will, 1994). Thus, in Ukraine every year more than 50 nosological forms of infectious diseases are registered among the population (Markovych and Grynevych, 2013).

According to scientists, at least 60% of known human infectious diseases can be transmitted from animals, and 75% of new or emerging human diseases are derived from animals (CDC, 2021). Quite a significant part of the most dangerous zoonotic diseases account for emergent food zoonoses (Grynevych, Markovych and Markovych, 2012) Sources of pathogens of most food poisoning in humans: salmonella, *Escherichia* coli, yersinia, listeria, campylobacter, are farm animals and animal products (Trykhlib, 2018). Among all the acute intestinal infections that occur due to eating poor-quality food, salmonellosis occupies the leading place.

To date, due to the widespread of this disease, it is just a matter of reducing the incidence and containment of pathogen spread among the main sources of this infection (Zarytsky, Hlushkevych and Bubalo, 2016). In recent years, *Salmonella* Enteritidis occupies the leading place in the etiology of salmonellosis.

The main source of alimentary infection is poultry products (Afshari et al., 2018). In addition, currently food products produced by catering enterprises of individual ownership can be added to the above

categories of products. Microbiological monitoring serves as an information base of modern epidemiology and allows to influence the effectiveness of epidemiological surveillance (Druzhaeva, 2014).

In the late twentieth century, listeriosis re-emerged as a foodborne infection, leading to a significant number of outbreaks associated with human consumption of pasteurized milk, cheese, ice cream, meat, vegetables, and other food products (Mengesha et al., 2009).

Listeria is widespread in nature, causing diseases with polymorphism of clinical manifestations and high mortality. Listeriosis also causes significant economic damage to agriculture by morbidity and high animal deaths. The work of recent decades shows that *L. monocytogenes* undergoes adaptive changes.

This occurs mainly under the influence of anthropogenic factors (use of preservatives, uncontrolled use of antibiotics and disinfectants, etc.), which change the biological properties of bacteria, most often it is the emergence of resistance to certain antibacterial drugs, the ability to form biofilms, and the appearance of low virulent mutants (Vovk, 2009).

According to the Public Health Center of the Ministry of Health of Ukraine, from 2007 to 2017, 22 cases of listeriosis have been registered in Ukraine, both in adults and children (PHCU, 2021). Of particular concern is listeriosis in pregnant women, which leads to severe pathology of the fetus and newborns and has mortality rates of 10–12% or more (Tartakovski, 2000).

The lack of an effective system of sanitary and epidemiological surveillance for listeriosis and the unsatisfactory quality of laboratory diagnostics have led to a kind of vacuum between the real role of listeria in human infectious pathology and practical research in clinical microbiology. Not all cases of human listeriosis are detected and registered.

However, we can shortly predict an increase in listeriosis due to the high adaptive properties of listeria, the ability to reproduce in an abiotic environment, increasing the percentage of people with immunodeficiency, the predominance of the food route of infection (Tartakovski, Maleev and Ermolaeva, 2002). Now a new stage in the study of this disease has begun, associated with the widespread introduction into modern medical practice of up to date laboratory methods of specific diagnosis (Yushchuk and Vengerov, 2021).

Thus, microbiological research remains relevant today, because they guarantee the quality of food raw materials and products, as well as reveal sources of infection.

The **aim of our work** was to study the spread of foodborne bacterial zoonoses in the Dnipropetrovsk region during 2020.

Materials and methods. The material for the study was data from annual reports and data from the State Service of Ukraine on Food Safety and Consumer Protection. The compliance of microbiological indicators with the requirements of normative documents in seven types of poultry products in the Dnipropetrovsk Region was determined. A total of 3,226 samples were studied in real-time (Table 1), of which 19.5% were export products, 78.6% — domestic Ukrainian production, 1.27% — state control, and 0.62% — imports.

Table 1 — List of samples of studied poultry food products in the Dnipropetrovsk Region during 2020 (n = 3,226)

Type of poultry products	Total number, samples
Semi-finished and culinary products from poultry meat	1,048
Sausages from poultry	615
Poultry meat	761
Eggs	284
Minced meat and mechanically deboned poultry meat (MDPM)	119
Egg products	344
Poultry by-products	55

Preparation of the tested samples was performed in accordance to DSTU ISO 6887-2: 2005 (DSSU, 2005a).

The methods provided by DSTU ISO 4833:2006 (DSSU, 2008), DSTU ISO 4832:2015 (SE 'UkrNDNC', 2018), DSTU EN 12824:2004 (DSSU, 2005d), DSTU ISO 11290-1:2003 (DSSU, 2005c), DSTU 7444:2013 (MEDTU, 2014), DSTU ISO 6888-2:2003 (DSSU, 2005b), DSTU ISO 7937:2006 (DSSU, 2009) were used in the work.

Results. Analysis of the epidemic situation in Ukraine regarding outbreaks of acute intestinal infectious diseases and food poisoning in 2020, according to the annual report of the State Service of Ukraine on Food Safety and Consumer Protection, shows an investigation of 52 cases with 646 victims, including 283 children (43.8%). The decrease in the number of outbreaks and casualties by almost four times compared to 2019, when 204 outbreaks were recorded, was due to anti-epidemic measures to prevent the spread of acute respiratory disease COVID-19 in Ukraine. Outbreaks were registered in almost all regions of Ukraine, except for Zhytomyr, Luhansk, Khmelnytsky, and Chernihiv regions.

Most outbreaks (n = 6) were registered in the Dnipropetrovsk Region. In the etiological structure, the largest share of outbreaks caused by salmonella was observed — 18 (34.6%) against 74 (36.3%) in 2019 and outbreaks of viral etiology (rota-, adeno-, noroviruses) — 13 (25%) against 42 (20.6%) in 2019. Thus, the monitoring of foodborne infections in Ukraine during 2019–2020 shows that 35.4% of outbreaks were caused by salmonella, which is quite dangerous due to the ease of transmission of the pathogen. The structure of salmonella allows them to experience environmental conditions and continue their life cycle in water and soil (WHO, 2018).

Thus, the registration of outbreaks of human salmonella infection in 82% of regions of Ukraine proves that important factors in the development of the disease are geographical conditionality, climatic conditions, species structure of farm animals and the level of socio-economic status of the population.

To assess the prevalence of salmonellosis in Ukraine, statistical data were analyzed (Fig. 1).

Thus, the annual presence of positive on salmonellosis settlements demonstrates the constant spread of this zoonosis in Ukraine, which causes contamination by these bacteria of food products of animal origin and the environment, and proves not only veterinary but also medical, environmental, and social problems of this infection.

Since the Dnipropetrovsk Region was dominated by the number of acute intestinal infectious diseases outbreaks, a detailed study of quantitative data in this region was conducted. Thus, the analysis of the results of bacteriological studies of raw materials of animal origin and food products in the Dnipropetrovsk Region shows that during 2020, 136 samples with discrepancies were detected, which was 2.56% of the total number of samples (n = 5,320).

Thus, according to the results of microbiological studies, poultry products had the highest percentage of violations of safety criteria (Fig. 2).

It has been established that the number of high-risk products includes semi-finished products and culinary products made of meat, in particular poultry; minced meat and MDPM, as well as ready-to-eat meat products (those that have undergone heat treatment) — sausages, including poultry, and eggs (Table 2).

The results of samples culturing showed that MA&OAMO exceeded the allowable norms in 1.14% of samples from semi-finished and culinary products made of poultry meat; in 0.90% — in sausages; in 10.92% — in minced meat and MDM from poultry.

Coliform bacteria were found in samples from semi-finished products — 3.90%; in sausages — 2.13%.

Bacteria of the genus Salmonella serological group D were isolated in 4.20% of samples of minced meat and MDM from poultry and 0.47% of samples of eggs, which proves the transmission of the pathogen by food.

The presence of staphylococci in semi-finished products is not normalized, but studies have shown that they are present in 12.82% of samples from semi-finished and culinary products from poultry meat.

Clostridia were found in semi-finished products — in 0.48% of samples.

L. monocytogenes were isolated in 12.5% of minced meat and MDM from poultry, which indicates the presence of listeriosis in the regions where the poultry was raised.

Thus, the main source (85.7%) of salmonellosis infection were poultry products of domestic production (Table 3). Sporadic outbreaks of salmonellosis can be supported by the constant supply of salmonella-contaminated meat products that have not been detected during processing, which is a risk factor for zoonosis.

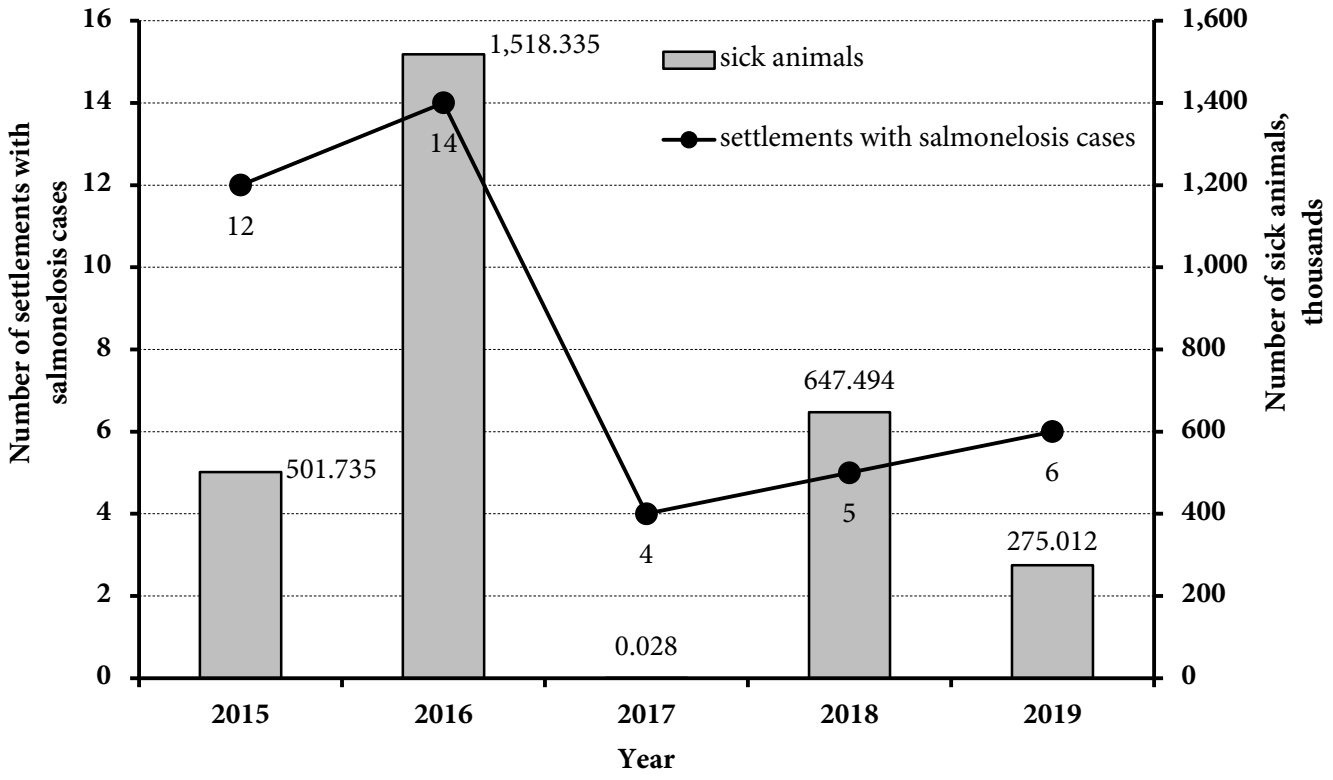
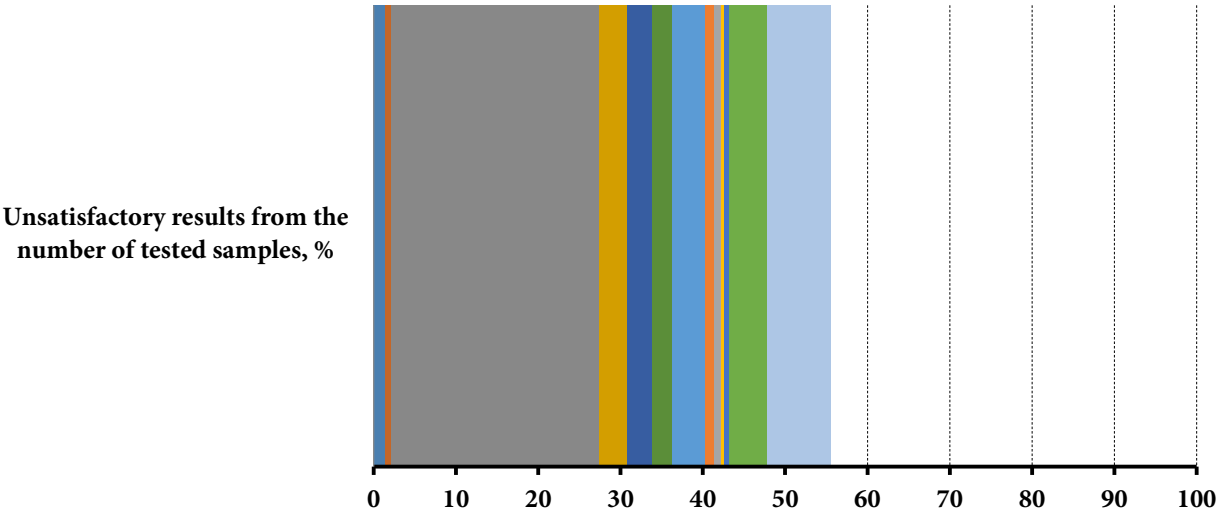


Figure 1. Statistical data on animal salmonellosis in 2015–2019 in Ukraine



	Unsatisfactory results from the number of tested samples, %
pork meat	1.41
beef meat	0.8
poultry minced meat and MDPM	25.21
minced meat and MDM of other types of animal meat	3.4
semi-finished products and culinary products from meat, including poultry	3.05
sausages, including poultry	2.51
semi-finished products and culinary products without meat	3.89
milk and dairy products	1.14
cheese	0.85
eggs	0.35
fresh fisg	0.57
salted, smoked, dried fish, herring	4.68
caviar, mollusks, crustaceans and other seafood	7.69

Figure 2. Standardized histogram of positive results of microbiological studies of food and food raw materials of animal origin in the Dnipropetrovsk Region in 2020

Table 2 — Violation of microbiological indicators in poultry products in the Dnipropetrovsk Region in 2020

Type of poultry products	Groups of dangerous factors, absolute number of samples/percentage of violations					
	MA&OAMO	Bacteria of the *E. coli* group	*Salmonella* spp.	*S. aureus*	Sulfite-reducing clostridia	*L. mono-cytogenes*
Semi-finished and culinary products from poultry meat	961/11 1.14	410/16 3.90	-	39/5 12.82	-	-
Sausages from poultry meat	332/3 0.90	516/11 2.13	-	-	413/2 0.48	-
Minced poultry meat and MDPM	119/13 10.92	-	119/5 4.20	-		96/12 12.50
Chicken eggs	-	-	212/1 0.47	-	-	-

Table 3 — Serological identification of salmonella, which were isolated during microbiological studies of raw materials, food products in the Dnipropetrovsk Region in 2020 (n = 7)

Serological group	Serological variant	Products and their origin		
		Minced meat and MDM from poultry, frozen (domestic), %	Beef meat (domestic), %	Chicken eggs (domestic), %
D	*Salmonella* Enteritidis 0:9, phase (H:g/H:m)	71.4	14.3	14.3

Discussion. Microbiological studies have shown that poultry products in 0.9–10.9% of cases were characterized by high content of QMA&OAMO. The largest number of microorganism-contaminated samples (10.9%) was observed in minced meat and MDM, compared with other types of products, as also reported by other sources (Danylenko et al., 2017). It is known that the nature of microbial contamination is influenced by the physicochemical properties of products. Thus, mechanical processing (making minced meat, etc.) increases the probability of contamination and promotes the homogeneous spread of microorganisms throughout the product (Kovalenko and Zamaziy, 2021).

The analysis of other sanitary-indicative criteria proved the increased number of bacteria of the *E. coli* group in 2.1–3.9% of the studied samples. The presence of an increased amount of *E. coli* bacteria in the finished product indicates unsatisfactory sanitary conditions of processing and storage of the product.

The number of spore-forming sulfite-reducing rods (genus *Clostridium*) is limited because they can multiply intensively in food in the presence of anaerobic conditions and at a concentration of 10^6 or higher in 1 ml/g to cause food poisoning. Their presence in poultry sausages at the level of 0.48% indicates the non-compliance of certain samples with regulatory documentation.

Isolation of *S. aureus* is dangerous due to its ability to produce enterotoxin and cause food poisoning. Increased amounts of coagulase-positive staphylococci in finished products usually indicate secondary contamination of the product: through contact with contaminated equipment, staff hands, or by airborne droplets.

Detection of pathogens *Salmonella* spp. and *L. monocytogenes* in minced meat and MDPM proves that these poultry products are a potential source of human infection. Thus, Zaytseva and Digo (2017) also report the isolation of listeria at the level of 15–80% from poultry meat. Therefore, human infection with listeria in the region is possible through food products at any stage of their receiving, processing, and storage, without the participation of animals in the cycle of transmission. The urgency of the problem of listeriosis is confirmed by reports in 2019. Thus, in Spain, 150 people were injured, and one died; in the Netherlands and Belgium, three people died and one stillborn child as a result of eating meat in which the causative agent of listeriosis was found (European Pravda, 2019a, 2019b).

Regarding the detection of bacteria of the genus *Salmonella*, according to the official data of the RASFF system (Rapid Alert System for Food and Feed) in the EU in the period from 2010 to 2015 in raw materials of animal and plant origin contamination of poultry and poultry meat products dominated (Smajhel and Shadrova, 2018).

As salmonella during mass reproduction in products can cause not only toxicoinfections but also infectious diseases, Commission Regulation (EU) No. 142/2011 requires the absence of the pathogen in 25 g of the product. Council Directive 94/65/EC inclused minced meat to the list of products for which Salmonella control is regulated, which is explained by the scattering of *Salmonella* from the smallest lymph nodes over the entire area and more intensive reproduction of bacteria on a large surface of minced meat (Sevalniev et al., 2020).

The study of the structure of the flagellar H-antigen proved the circulation in the Dnipropetrovsk Region of the serological variant *Salmonella* Enteritidis, which according to the WHO is one of the most important serotypes transmitted from animals to humans in most regions of the world (WHO, 2018). Regarding Ukraine, the analysis of salmonellosis incidence and its etiological structure also shows that the predominant pathogen was *Salmonella* Enteritidis (Zarytsky, Hlushkevych and Bubalo, 2016). Detection of egg samples containing salmonella indicates a risk of eating raw or undercooked eggs, which can cause up to 45% of all known cases of human salmonellosis (EFSA and ECDC, 2021; Whitworth, 2019).

In addition, according to RASFF (Smajhel and Shadrova, 2018), *Salmonella* Enteritidis is the serotype most commonly found in poultry meat. The detection of salmonella in beef indicates the importance of controlling food safety. Thus, 13 cases of disease and one death were recorded in the United States in 2019 due to the use of infected ground beef (CDC, 2019).

Studies of the structure of the somatic O-antigen of *Salmonella* revealed exclusively serological group D in four different types of products, which indicates a geographical serotypic predisposition, caused by the dominance of group D pathogens in the Dnipropetrovsk Region (Martynenko and Rula, 2020). Thus, according to statistical reports of the Sanitary and Epidemiological Service of Ukraine, the dominant serogroup of the pathogen among sick people and carriers remains O-group D (MHU, 2016).

Close attention to poultry products imported to the territory of Ukraine is attracted by the detection of non-compliance of DSTU EN 12824:2004 (DSSU, 2005d) in 2019 (Martynenko and Rula, 2020) and 2020 (MDSFCSIFR, 2020). This situation may be due to the fact that EU legislation does not establish microbiological criteria for the content of bacteria of the genus *Salmonella* in raw materials. Thus, today the number of countries that have international veterinary certificates for the import of minced meat and/or MDPM into the customs territory of Ukraine has increased to seven countries (UK, Denmark, Poland, Hungary, Czech Republic, Lithuania, and Latvia). Thus, imported products are a risk factor, according to a report by EFSA and ECDC (2021), which showed a stable prevalence since 2015 of serovars of salmonella-positive flocks among breeding chickens, laying hens, broilers and turkeys for fattening.

Thus, the analysis of information on animal and poultry diseases from the territories-importers of agricultural products allows to formulate epizootological problems in a timely manner and to ensure the welfare of animals and the health of the population of the importing region.

Infected products were the source of pathogens of food bacterial zoonoses in the Dnipropetrovsk Region in 2020: minced meat and MDM from poultry; chicken eggs and beef, the probability of which is also reported by other authors (Malysh, 2020).

Conclusions. The paper shows problematic in 2020 nosological forms in the structure of pathogens of food bacterial zoonoses and identifies their potential sources.

Microbiological studies have established that the contaminants of poultry products of domestic Ukrainian production in the Dnipropetrovsk Region were sanitary-indicative (Bacteria of the *E. coli* group, MA&OAMO), opportunistic (*S. aureus*, sulfite-reducing clostridia) and pathogenic microflora (bacteria of the genera *Salmonella, Listeria*).

The author observed the geographical serotypic predisposition of *Salmonella*, which is caused by the prevalence of O-group D in the Dnipropetrovsk Region, and the possibility of their passage through the food chain.

It was found that minced meat and MDM from poultry are among the factors in the transmission of a potentially pathogenic for humans and animals species of *Listeria — L. monocytogenes* in the region.

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