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Incidence and risk factors of salmonellosis in Ukraine

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Abstract: The article, based on the reports of the Ministry of Health of Ukraine, presents the materials of the epidemiological surveillance of salmonellosis in 2011–2018. To assess the influence of factors on the epidemic process of salmonellosis, the demographic situation, income and living conditions of the population were studied; average monthly air temperature, relative humidity, precipitation; the quantitative and qualitative composition of the microbiocenosis of patients with signs of acute intestinal infection.
It was found that in Ukraine the incidence of salmonellosis is high. Outbreaks of salmonellosis are recorded. S. enteritidis is most often isolated from the clinical material of patients, carriers and human objects ($p < 0.05$). The risk groups for salmonellosis are children ($p < 0.05$), as well as the rural population ($p < 0.05$). The low level of sanitary and epidemiological control at the stages of production, transportation and sale of food products, water supply contributes to the spread of salmonellosis. Natural factors have a regulating effect on the intensity of the epidemic salmonella process: a strong direct relationship is established between the incidence and air temperature and precipitation ($p < 0.05$). Salmonella enters into a competitive or synergistic relationship with other microorganisms in the intestinal biotope.
Thus, the intensity of the epidemic process of salmonellosis can be influenced not only by external (natural and social), but also by internal factors.

Key words: salmonellosis, epidemic situation, epidemiological surveillance, correlation, factors.

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Introduction

According to the data of WHO salmonella is one of four main reasons of diarrhoeal diseases in the world [1]. The processes of globalization contribute to salmonellosis transmission. In the USA up to 1.2 mln cases of salmonellosis incidence are registered every year [2]. In the European countries salmonellosis ratio in the structure of food zoonosis is 30.2% [3]. About half of the cases of salmonellosis are caused by salmonella *enteritidis*, *typhimurium*, *newport* and *javiana* [4]. Hens and pigs are considered to be a major reservoirs of salmonellosis [5].

In Ukraine the problem of salmonellosis is to be examined. It is necessary to determine territories of risk, groups of risk, and dominant factors of transmission and to develop preventive measures in accordance with available epidemical situation.

Objective of the paper is to study the incidence of salmonellosis in various regions of Ukraine, determine factors contributing to its transmission.

Material and Methods

The article according to the data of reports of the Ministry of Health of Ukraine represents materials of epidemiological surveillance over salmonellosis in 2011–2018.

In order to assess the influence of factors on the epidemic process of salmonellosis, demographic situation, income and living conditions of population were studied according to the data of the Main Administration of Statistics in Sumy oblast; spread of diseases of the digestive system — research and information centre; average monthly air temperature, relative humidity, precipitation — according to the data of the centre of hydrometeorology; quantitative and qualitative composition of microbiocenosis — according to the research results of 93 faecal extracts of the patients showing signs of acute intestinal infection. While examining intestinal microflora, the following was determined: pathogenic *Enterobacteria*, *Lactobacillus* and *Bifidobacteria*, *Escherichia coli*, *enterococcus*, *staphylococci*, *Proteus*, yeasts of the genus *Candida*, opportunistic enterobacteria, non-fermenting microorganisms. During identification of the isolated microorganisms Bergey's classification was observed [6]. Enzyme-linked immunoassay and the method of polymerase chain reaction were applied in the virus identification studies.

In order to determine nature of quantitative interaction between salmonella and other microbiocenosis members, the Jaccard similarity coefficient (g) was calculated according to the formula: $g = c/(a+в-c) \times 100\%$, where, a — number of sampling with a species; в — number of sampling with в species, c — number of sampling with both microorganism species [7].

Depending on value of the Jaccard coefficient, nature of interactions between microorganisms was determined: antagonistic, synergistic or mutualistic. If g <30%

— interactions between the species are antagonistic; at g 30 to 70% — bacteria are able to coexist, synergism is observed; at g >70% — mutualistic interactions.

Epidemiological and statistical research methods are applied. Software Microsoft Office Excel 2010, Statstica 6 is used.

Results

In Ukraine according to the notification rate salmonellosis belongs to the infections of frequent occurrence. In 2011–2018 the incidence varied within 17.3–22.1 per 100 thousand people and there was no downtrend of the incidence (Rate of incidence average: $R_{inc.}^{aver.}$ = –0.78%).

The incidence of salmonellosis was substantially different in various regions of Ukraine. The most unfavourable situation was in one of densely populated, industrially developed oblasts — Kharkiv oblast, where median salmonellosis incidence in 2011–2018 was 53.3 per 100 thousand people. The incidence of salmonellosis was also higher than average one in Ukraine (Fig. 1).

Fig. 1. Regions of Ukraine with the highest and lowest incidence of salmonellosis.

Salmonellosis outbreaks were registered throughout Ukraine. The proportion of salmonellosis patients involved in the outbreak incidence was 8.8%.

Most outbreaks of salmonellosis occurred in public catering establishments (72.5% of cases), at home, during various events: weddings, funeral repasts, etc. (15.9%). The outbreaks of salmonellosis were most often reported in May–September ($p < 0.05$). 92.6% of cases were connected with alimentary route of transmission.

Eating food consisting of eggs and meat of questionable quality was mentioned by the affected as the major reasons of salmonellosis. While investigating the outbreaks of salmonellosis, employees of the State Sanitary and Epidemiological Service established that most public catering establishments didn't have documents certifying origin, quality and safety of food, ready-made meals technology, storage and transportation conditions were violated, periodic laboratory control of finished products was not conducted, terms of periodic medical examinations were not adhered to.

The range of salmonella serovars most commonly isolated from the clinical material correlated to the scenes of the serovars isolated from the persons examined for preventive purposes, objects of human activity environment, including food (Table 1).

Table 1. Frequency of salmonella serovar isolation from the samples isolated from people and objects of the environment (%).

	Patients		Carriers		Objects of human activity environment, including food	
1	*S. enteritidis*	75.8	*S. enteritidis*	62.7	*S. enteritidis*	70.7
2	*S. typhimurium*	15.4	*S. typhimurium*	15.2	*S. typhimurium*	15.3
3	*S. blegdam*	3.5	*S. blegdam*	3.5	*S. infantis*	2.3
4	*S. infantis*	2.2	*S. infantis*	3.0	*S. virchow*	1.8
5	*S. muenchen*	0.7	*S. muenchen*	1.6	*S. szentes*	1.8
6	*S. virchow*	0.4	*S. give*	1.1	*S. give*	1.8
7	*S. derby*	0.3	*S. java*	0.8	*S. muenchen*	0.9
8	*S. java*	0.3	*S. rissen*	0.7	*S. blegdam*	0.7
9	*S. tshiongwe*	0.3	*S. virchow*	0.7	*S. coeln*	0.7
10	*S. give*	0.3	*S. agona, derby*	0.6	*S. derby*	0.7

In the samples taken at the objects of human activity environment in 57.6% of cases salmonella was isolated from food, in 26.3% — in washes taken in public catering establishments and food production companies (Fig. 2).

Regarding food products, salmonella was most commonly isolated from the samples of ready-cooked foods, eggs, fresh meat (Table 2).

The risk of infection depends on the conditions to which the agent and susceptible organism are subjected. As exemplified by Sumy oblast, in order to determine the factors, which could indirectly influence the epidemic process of salmonellosis, social and natural conditions of the region have been studied.

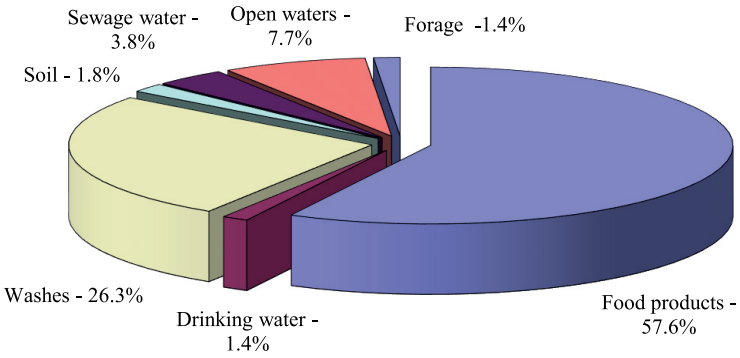

Fig. 2. The proportion of salmonella isolated from the objects of human activity environment.

Table 2. Frequency of salmonella serovar isolation from the food samples (%).

	Meat	Poultry	Eggs	Dairy products	Fish	Vegetables	Confectionery	Ready-cooked foods	Other food
S. typhimurium	2.7	1.2	2.7	—	1.2	—	1.6	0.8	2.3
S. enteritidis	12.1	3.1	18.8	2.0	2.3	1.2	6.3	13.7	19.9
S. infantis	1.2	0.8	—	—	—	—	—	—	—
S. virchow	—	2.3	—	—	—	—	—	0.4	—
S. give	—	0.4	—	—	—	—	—	1.2	—

Sumy oblast is situated in the north-eastern part of Ukraine. It occupies an area of 23.8 thousand km^2, its population is 1094.3 thousand inhabitants. 66.7% of the population lives in the city. It has moderately continental climate. The demographic situation in Sumy oblast during the period under study (2011–2018) is characterized by gradual decline in total population from 1161.5 thousand people to 1094.3, density of the population living per 1 km^2 from 48.8 to 45.9 persons per 1 km^2. In the age structure of the population children share (aged from 0 to 17 years) was insignificant and varied from 15.2 to 15.7%.

The incidence of salmonellosis in Sumy oblast ranged 16.7–21.7 per 100 thousand people (Fig. 3).

Children's incidence varied from 35.9 to 45.9 per 100 thousand children, had a moderate upward trend ($R_{inc.}^{aver.}$ = 2.2%). Children's median incidence exceeded adult's median incidence by 3.2 times.

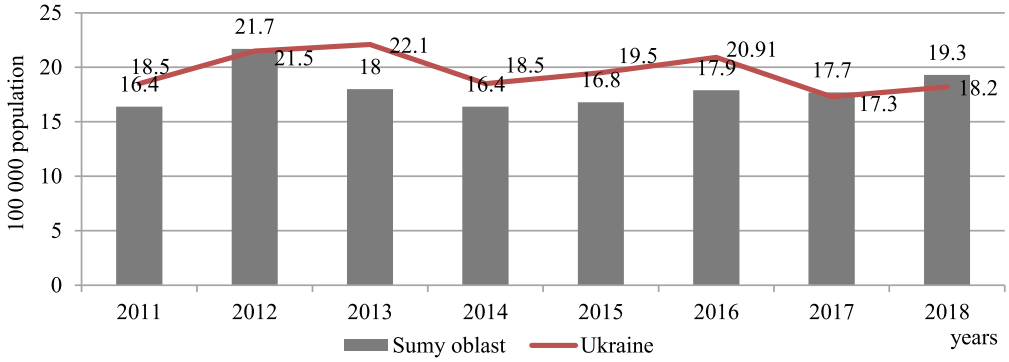

Fig. 3. The incidence of salmonellosis in Ukraine and Sumy oblast.

The highest incidence of salmonellosis is registered in the group of children aged 1 to 4 years old ($p < 0.05$). Median incidence was 58.3 per 100 thousand of cohesive and exceeded the one of children aged up to 1 year old by 2.6 times, 5–9 years — by 1.6 times, 10–14 years — by 2.1 times, 15–17 years — by 1.6 times.

The children's incidence of salmonellosis, which belonged to "non-organized" category, in other words those, who didn't go to children's pre-school institutions and schools, was the highest ($p < 0.05$). Median incidence of "non-organized" children exceeded the one of children from children's pre-school institutions by 2.5 times and pupils — by 7.9 times.

The epidemic process of salmonellosis in cities and villages is characterized by various incidence rates, presence of both synchronous and asynchronous rises in its dynamics. Generally, median salmonellosis incidence of city inhabitants was 18.8 per 100 thousand people and exceeded of village inhabitants by 1.3 times. At the same time 2011 to 2018 period has a pronounced upward trend in salmonellosis incidence of village inhabitants ($R_{inc.}^{aver.} = 12.1\%$), while the salmonellosis incidence of city inhabitants declined ($R_{inc.}^{aver.} = -4.2\%$). In 2016–2018 the incidence of village inhabitants exceeded the one of city inhabitants.

In monthly incidence the peak of salmonellosis case registration fell upon June (15.6%) and July (14.5%).

It has been established that there is no dependence between the dynamics of salmonellosis incidence and demographic indicators (population, dwelling density, natural and migratory movements), as correlation rates (r) were $r = -0.006$, $r = -0.002$, $r = 0.203$ and $r = -0.340$, $p > 0.05$, respectively.

Consuming various food containing salmonella can be the risk factor, which can influence the incidence of salmonellosis. While studying presence of correlation regressive connections between consuming food and the incidence of salmonellosis,

we have established a direct correlation connection of average force between consuming meat and meat products and the incidence of salmonellosis (r = 0.526).

In Sumy oblast frequency of the water samples detected not corresponding to regulatory sanitary-microbiological rates from the village water pipelines ranged from 4.0 to 13.3%. According to the results of the correlation-regression analysis, it has been found that in Sumy oblast there is a strong direct correlation between the incidence of salmonellosis of village inhabitants and the frequency of detection of "non-standard" samples of drinking water taken from rural water systems (r = 0.806).

In order to discover natural factors able to determine activation of the epidemic process of salmonellosis, air temperature, humidity and precipitation in Sumy oblast were studied. It was found that the coldest months of the year in the region were January and February (average long-term air temperature was (-5.3°C)), the warmest — July (average long-term indicator — (+21.2°C)). The level of water vapour content in the air (relative humidity) was the lowest in May (average long-term rate was 64.7%), the highest — in November and December (87.1 and 87.2% respectively). Most precipitation fell in July (average long-term level — 76.2 mm).

Statistically confirmed ($p < 0.05$) direct correlation relationships (r = 0.834 and r = 0.692, respectively) were discovered between the dynamics of the incidence of salmonellosis and average monthly air temperature and precipitation.

To define internal impact factors on the epidemic process of salmonellosis, faeces of the people admitted to Sumy Oblast Clinical Infectious Hospital with signs of acute intestinal infection were studied. It has been established that in (59.3 ± 5.6)% of cases one agent was isolated from stools, in (40.7 ± 5.6)% — virus associations, opportunistic pathogens, salmonella, yeasts of the genus *Candida.*

The share of salmonella was the highest among the isolated agents and amounted to 14.5% (where 81.8% — *S. enteritidis).* The percentage of *K. pneumonia* was 9.2%, *S. aureus* — 6.7%, diarrheal *E. coli* — 6.6%, Norovirus — 5.3%, *P. aeruginosae* and rotavirus — 2.6% each. *Proteus, Citrobacter*, *Enterobacter* were isolated in individual cases.

It was found that Norovirus in associations with *K. pneumonia*, *S. aureus*, *S. typhimurium*, *K. pneumonia* and *P. aeruginosae* was isolated in 21.1% of cases, rotavirus with *S. aureus*, diarrheal *E. coli*, *S. enteritidis*, *C. albicans* — in 5.3%, opportunistic pathogens in association with other opportunistic pathogens (*K. pneumonia* and *S. aureus*, *K. pneumonia* and *P. aeruginosae*) — in 3.9%, *S. aureus* from *S. typhimurium* and diarrheal *E. coli* — in 2.6%. In 12.9% of the examined patients with acute intestinal infections, *C. albicans* was isolated in number exceeding 10^4 in associations with pathogenic germs (*S. typhimurium*) and opportunistic pathogens (*S. aureus*, *K. pneumonia*, *P. aeruginosae*, *E. cloacae*, *Citrobacter*, *M. morganii*).

The results of the conducted studies have shown that stable associations of microorganisms are formed between Norovirus and *K. pneumonia* (36.4%), Norovirus and *S. typhimurium* (40.0%), Norovirus and *P. aeruginosae* (40.0%).

However, most germs in the intestine of patients with acute intestinal infections entered into competitive interactions among themselves and as a consequence, these microbial communities are unstable, short-life and able to exist for a short time. The Jaccard coefficient for *K. pneumonia* and *S. aureus*, *K. pneumonia* and *P. aeruginosae*, *K. pneumonia* and *C. albicans* was 9.5; 9.1 and 16.7% respectively. *S. aureus* strains in associations were in antagonistic interactions with Norovirus (18.2%), *C. albicans* (14.3%), *S. typhimurium* and diarrheal *E. coli* (7,7%). Similar interactions were established between *C. albicans* and *S. typhimurium* (7.1%), *C. albicans* and *P. aeruginosae* (5.3%), *C. albicans* and *E. cloacae* (20.0%).

Discussion

High incidence of salmonellosis in Ukraine indicates that the situation with this disease is drastically different from the global one. Salmonellosis is widespread and is an urgent veterinary and biomedical problem for most countries [8, 9]. The peculiarity of the salmonellosis epidemic situation in Ukraine is that the frequency of case registration differs significantly in various regions. The above mentioned regions are located in different parts of Ukraine and differ in physical and geographical characteristics. Kharkiv oblast is one of the most densely populated, with a strong industrial and scientific potential, located in the northeast of Ukraine in two natural areas — forest-steppe and steppe. The urban population is 80.6%. The population density is 86 thousand people per 1 km^2. Kherson oblast is situated in low basin of the Dnieper River, washed by the Black and Azov Seas. Agricultural sector prevails in the economy. The proportion of urban population is less than in Kharkiv oblast and amounts to 61.2%. The population density is the least in Ukraine and amounts to 36.1 thousand people per 1 km^2.

The epidemic salmonellosis situation can be significantly complicated by outbreaks, which are indicators of the epidemic conditions, mediated sign of low quality preventive and antiepidemic measures. In Ukraine salmonellosis outbreaks were most commonly registered in tourist, scientific and industrial regions with developed network of public catering establishments.

Most salmonellosis outbreaks were connected with public catering establishments, children's pre-school institutions as well as with attending commemorative events arranged at home. Food both in the world and in Ukraine is the prevailing factor of salmonellosis transmission [10].

Poultry farming and animal breeding are the leading sectors of agriculture of Ukraine. Solving of a wide range of social and economic problems, namely satisfaction of the population demand for high quality food, is connected with their further development. Intensification of the methods of animal breeding and fattening, non-obligatoriness of preventive vaccination contributed to the fact that in recent years

there was no downtrend of the incidence of salmonellosis. Researchers suggest that *S. enteritidis* is isolated from the samples of chicken meat in 81.3% of cases, in eggs — in 88.3% [11].

Salmonella is comparatively highly resistant to the effect of various environmental factors. However, while boiling, they are immediately destroyed and at temperature below 5°C the growth of salmonella is stopped. Fresh eggs and meat, even if they are contaminated with salmonella, can't be the reason of human food poisoning. At public catering and trade enterprises, where the storage precautions for meat and eggs are often violated, such an intensive infection of food products is a real fact. Salmonella reinoculation occurs most often in the processing shops of public catering establishments and stores [12].

Researchers state that the problem of low quality of meat products and semi-finished products especially is related to cooperative and private low-capacity shops [13]. They are often housed in adjusted premises, insufficiently equipped, insufficiently provided with containers, vehicles, warehouses and refrigeration units. Employees in most cases have poor professional training. Many entrepreneurs process such meat into various sausage or smoked products or sell it in the form of semi-finished meat products, despite the fact that forced-slaughter meat, as limitedly suitable, is allowed to be used only for production of cooked sausages and canned food.

The concept of globalization of the epidemic process of salmonellosis is confirmed by the fact that in addition to *S. enteritidis* and *S. typhimurium* outbreaks of salmonellosis in Ukraine were caused by S. *muenchen*, *S. blegdam*, *S. glostrup*, which are "imported", exotic for Ukraine.

Almost 60% of all samples isolated from the human activity environment and containing salmonella at the same time is accounted for by food. The above mentioned gives us the opportunity to think that in Ukraine the main way of salmonellosis agent transmission is food. Meat and eggs are basic transmission factors.

The compulsory condition for a comprehensive assessment of the epidemic situation of salmonellosis is to identify the reasons, which formed it. Given the variety of the salmonellosis incidence curves in the regions of Ukraine, we can assume that they are a reflection of impact on the epidemic situation of different socio-economic and natural conditions.

While studying the manifestations of the epidemic salmonellosis process in Ukraine as exemplified by Sumy oblast, it was found that the incidence of salmonellosis in Sumy oblast did not differ significantly from its average value across Ukraine ($p > 0.05$). The risk group was children, moreover aged 1 to 4 years and those belonging to the group of "non-organized", rural inhabitants. The discovered fact of increasing the incidence of salmonellosis in rural areas still needs to be studied. One of the reasons for the above mentioned, in our opinion, is internal migration of the population. Often rural people

work in the city, have numerous contacts in transport, at work, use public catering establishments.

In order to determine the factors that may activate the epidemic process of salmonellosis, correlation-regression relationships between the dynamics of incidence and demographic indicators of Sumy oblast were studied. It has been established that in the conditions of Sumy oblast there is no dependence between the incidence of salmonellosis and demographic indicators (population, population density per 1 km^2, natural and migratory population movements).

It is well known that poor quality of food, its imbalance, psycho-emotional overload is the risk factors for the appearance of diseases of the digestive system [14]. Some researchers have concluded that increase in the incidence of digestive diseases of non-infectious nature occurs after a sharp rise in the incidence of infectious diseases of the digestive system [15]. Despite the fact that among those admitted to in Sumy Oblast Infectious Diseases Hospital with a diagnosis of salmonellosis, one in three had chronic diseases of the digestive system in anamnesis, a correlation dependence between the prevalence of diseases of the digestive system in Sumy oblast and salmonellosis couldn't be established in the conducted study ($p > 0.05$).

The established correlation relationship between the incidence of salmonellosis and consumption of meat and meat products on the one hand indirectly points to low quality of raw meat consumed by the population of Sumy oblast and on the other hand proves that without taking veterinary measures the situation with salmonellosis will only get worse.

Researches indicate great significance of drinking water in the epidemiology of acute intestinal infections, as most large outbreaks of acute intestinal infections are connected exactly with drinking water [16, 17]. The established correlation relationship between the incidence of salmonellosis in rural areas and the frequency of detection of "non-standard" according to sanitary and microbiological indicators of drinking water samples, proves that in addition to salmonella-contaminated meat drinking water in rural areas is also the risk factor of salmonellosis development ($p < 0.05$).

It is well known that natural factors affect all components of the parasitic system: parasite population and its biological host, as well as the mechanism of transmission. In contrast to demographic indicators, we have found the influence of natural factors on the dynamics of salmonellosis incidence.

In the host organism and external environment, microorganisms form various biocenoses, which are constantly under the influence of biotic and abiotic factors. These relationships can be both integrative and competitive that absolutely affects manifestations of the epidemic process.

We found out that not one but several agents are discovered in faeces of almost half of the percentage of patients with acute intestinal infections. Salmonella is isolated in associations with Norovirus, rotavirus, opportunistic pathogens, *C. albicans*. The

Jaccard coefficient is an informative indicator of environmental relation of various microorganism species that determines the nature of microorganism relationships in associations by presence of conjugated bonds.

According to the results of the conducted study, it was found that there are synergistic relationships *in vivo* between Norovirus and *S. typhimurium*, as the Jaccard coefficient was 40.0%. At the same time, *S. typhimurium* entered into antagonistic relationships with *S. aureus* and *C. albicans* (the Jaccard coefficient was 7.7 and 7.1% respectively).

Thus, taking into consideration the above mentioned, it can be concluded that the nature of relationships between microorganisms in the intestinal biotope is another factor influencing the development of the epidemic process of salmonellosis.

Conclusions

Salmonellosis in Ukraine continues to remain an unresolved problem of modern times. Low level of sanitary and epidemiological control at the stages of production, transportation and sale of food, water supply contributes to the spread of salmonellosis. Natural factors have a regulatory impact on the intensity of epidemic salmonellosis process ($p < 0.05$). Salmonella in the intestinal biotope enters into competitive or synergistic relationship with other microorganisms. Depending on the nature of this relationship, the effect can be stimulating or inhibitory.

Conflict of interest

None declared.

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