

Teresa Grzelak

Katedra i Zakład Fizjologii, Uniwersytet Medyczny im. Karola Marcinkowskiego, Poznań

Paulina Wojtyła-Buciora

Wydział Nauk Medycznych i Nauk o Zdrowiu, Uniwersytet Kaliski im. Prezydenta Stanisława Wojciechowskiego, Kalisz

Gabriela Burzawa, Joanna Ciepiela, Iwona Kaczmarczyk

Wojewódzka Stacja Sanitarno-Epidemiologiczna, Kraków

Wojciech Giermaziak

Główna Biblioteka Lekarska, Warszawa

Lucyna Kapka-Skrzypczak

Zakład Biologii Molekularnej i Badań Translacyjnych, Instytut Medycyny Wsi im. Witolda Chodźki w Lublinie, Lublin

Edyta Mądry

Katedra i Zakład Fizjologii, Uniwersytet Medyczny im. Karola Marcinkowskiego, Poznań

SALMONELLA BACTERIA IDENTIFIED IN HEALTHY PEOPLE FROM THE LESSER POLAND PROVINCE IN THE YEARS 2017–2022

BAKTERIE SALMONELLI STWIERDZONA U ZDROWYCH OSÓB Z WOJEWÓDZTWA MAŁOPOLSKIEGO W LATACH 2017-2022

Introduction

Salmonella bacteria belong to the Gram-negative Enterobacteriaceae family. They are motile, relatively anaerobic and lactose-negative colony-forming bacteria that ferment glucose and lack cytochrome oxidase. According to the taxonomic classification drawn up in 1992 by the International Salmonella Reference Center in Paris, the Salmonella genus includes two species: *Salmonella enterica* and *Salmonella bongori*. The first species includes six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. en-*

terica subsp. *Houtenae*, and *s. enterica* subsp. *Indica*. The *Salmonella bongori* species constitutes a homogeneous group of bacteria [3, 6]. Within the *Salmonella enterica* species, around 2,800 serological varieties (serotypes), so-called serovars, have been distinguished, the majority of which = belong to the subspecies *Salmonella enterica* subsp. *Enterica* that consists of over 1,500 serovars [17]. The serovars are classified according to the Kauffmann-White serological classification scheme based on the differentiation of protein flagellar antigens (H antigen), lipopolysaccharide somatic antigens (O antigen), and, in some cases, the presence of a capsular antigen (Vi antigen). Serovars are serologically identified by using polyvalent antisera. The most important serovars of the *Salmonella enterica* subsp. *enterica* are: *s. Choleraesuis*, *s. Enteritidis*, *s. Gallinarum*, *s. Paratyphi A*, *s. Paratyphi B*, *s. Paratyphi C*, *s. Typhi*, and *s. Typhimurium* [3, 17].

Infections from *Salmonella* bacteria such as typhoid, paratyphoid, and salmonellosis can pose a threat to both human health and life [1]. Typhoid is a disease caused by the most prominent serological variant of the *Salmonella* genus, *Salmonella Typhi*, and spreads only among humans who also serve the only reservoir/sink of this infection. Typhoid develops over a relatively long time, 10-14 days. The first phase of infection consists of high fever associated with bacteremia in the bloodstream along with insomnia, headaches, abdominal pain, and constipation rather than diarrhea. A typhoid rash may develop on the skin around the chest and abdomen. During later stages of the disease, acute diarrhea and perforation of the intestine occur and, more rarely, peritonitis. After a few or dozen days of a fever-free period, the disease may relapse, usually with a milder course. Living through the infection often leaves the host as a carrier of these bacilli in the intestine or bile ducts, which then poses a threat to the environment of people who have undergone it [12].

Other disease entities are paratyphoid, caused by *Salmonella Paratyphi A*, *B*, and *C*. The clinical symptoms are the same as in the case of typhoid fever, but have a milder course with symptoms of gastroenteritis. The only reservoir of paratyphoid bacilli are the sick, convalescents or carriers and, therefore, they constitute a source of infection. Paratyphoid is much more common than typhoid and its clinical course is similar to that of food poisoning [3].

Salmonellosis is a disease caused by various *Salmonella* bacilli of animal origin, such as *Salmonella Enteritidis*, *Salmonella Infantis*, or *Salmonella Typhimurium*. The reservoirs of these are farm animals and animal products, e.g., poultry, eggs, cattle, and pigs, as well as wild animals for which these bacilli are the primary pathogen. The infection can be transmitted to humans and spreads mainly through dishes prepared from contaminated products which have not been subjected to sufficient heat treatment. The infection starts suddenly, as gastroenteritis with symptoms of diarrhea, abdominal pain, and vomiting, which usually last 2-6 days. The disease, however, resolves spontaneously. The infected person is contagious during the illness and the period of excretion of *Salmonella* bacilli (which may also include the post-morbidity period) when short-term carriage of *Salmonella* bacilli usually occurs [6].

Since *Salmonella* bacilli are so widespread in the environment and can infect humans, those who study the *Salmonella* bacilli or engage in work with a high risk of exposure are thus required to get tested for its presence in their stool. These tests are undertaken as part of the offi-

cial SANEPID surveillance according to the provisions of the Act of 5 December 2008 on preventing and combating infections and infectious diseases in humans (Journal of Laws of 2019, item 1239, 1495) and are performed on healthy people for the purposes of issuing a medical certificate to satisfy SANEPID requirements [9, 21].

Study aims

- To determine the rate of persons testing positive for carrying *Salmonella* bacilli relative to the number of healthy people tested in the Lesser Poland Province during 2017–2022.
- To establish which are the most frequently identified serotypes of *Salmonella* in healthy people residing in the Lesser Poland Province in the years 2017–2022.

Methodology

The laboratory test material to determine the presence of *Salmonella* in healthy people was three stool samples collected by study subjects once a day for three consecutive days by means of sampling into test tubes suitable for transport in accordance with laboratory instructions. Each stool sample delivered to the laboratory was inoculated directly onto a solid MacConkey agar medium. This is a selective and differential medium, where the selective factors are bile salts and crystal violet that inhibit the growth of Gram-positive bacteria, whereas the differentiating factors are lactose and neutral red. The presence of this sugar allows for the differentiation between lactose-positive bacilli and those unable to metabolize lactose. Neutral red serves as an indicator by means of which colonies of lactose-degrading bacteria turn pink as a result of acidifying the environment caused by their proliferation. After incubating the samples in MacConkey's agar at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18–24 hours, further diagnostic procedures were performed on isolated colonies characteristic of the genus *Salmonella*, i.e., lactose-negative, colorless and transparent [11, 18].

The tested fecal samples were also inoculated onto a liquid selenite-phosphate medium (SF). This is a selective multiplication medium, where the presence of sodium selenite enhances the growth of *Salmonella* bacilli. Culturing feces on the SF medium increases bacterial detection by 50% and enables latex tests to be performed on samples for detecting and identifying *Salmonella* group B, C1, C2, D, E, and G O antigens. The absence of *Salmonella*, as determined by negative results of the latex test from direct culture in the three examined stool samples, thereby authorizes the issuing of a no-carrier *Salmonella* result [19]. If the latex test proves positive, then the sample in SF medium needs to be cultured in media with strong selective and differentiating properties, which would allow for typical colonies of *Salmonella* to be obtained, e.g., *Salmonella-Shigella* medium (SS) and Wilson-Blair bismuth-sulfite medium (W-B) [10, 11, 22].

When growing on solid media, colonies suspected of belonging to the *Salmonella* genus should be subjected to a short series of biochemical tests based on a differential profile of responses to a Biochemical I series (Tab. I).

Table I: Substrate media of the Biochemical I series applied for detecting the characteristics of *Salmonella* [designed in-house by the authors]

Substrate media of the Biochemical I series	Media application (feature tested for)	Reaction characteristic to the <i>Salmonella</i> genus
Peptone water with tryptophan	Ability to metabolize tryptophan	Negative
Christensen mediumwith urea	Ability to metabolize urea	Negative
Semi-solid agar media for testing microbial motility	Ability to move	Positive
Kligler Agar	Ability to ferment glucose	Positive
	Ability to ferment lactose	Negative
	Release of gas other than hydrogen sulfide	Negative
	Release of hydrogen sulfide	Positive
Media containing 10% lactose	Ability to ferment lactose	Negative

Strains suspected of belonging to the *Salmonella* genus should be confirmed by an additional Biochemical II series (Tab. II).

Table II. Substrate media of the Biochemical II series applied for detecting the characteristics of *Salmonella* [designed in-house by the authors]

Substrate media of the Biochemical II series	Media application (feature tested for)	Reaction characteristic to the <i>Salmonella</i> genus
Containing potassium cyanide	Ability to grow in the presence of potassium cyanide (KCN)	Negative
Falkow agar with lysine, ornithine, and arginine	Ability to decarboxylate lysine, ornithine, and arginine	Positive
Media for testing sugar and alcohol metabolism	Ability to metabolize sugars and alcohols	Should be checked in biochemical tables according to any given serovar.
Simmons citrate agar	Ability to utilize ammonium and citrate ions	Negative
Media containing phenylalanine	Ability for aerobic deamination of phenylalanine	Depends on the strain
Clark medium	Ability to metabolize glucose to form acetyl methyl carbinol,	Depends on the strain
Sodium malonate medium	Ability to metabolize malonic acid	Positive
Christensen's agar with sodium citrate	Ability to metabolize sodium citrate	Depends on the strain
Agar slant	Obtaining a pure culture for subsequent serological testing	Depends on the strain

Based on the so-obtained biochemical profiles, strains suspected of belonging to the *Salmonella* genus were serologically confirmed by slide agglutination using diagnostic sera. Serological diagnosis of a sample isolate consists in determining its antigenic structure. *Salmonella* serotyping was performed according to the White-Kauffmann-Le Minor scheme. The study endpoint was to determine the specific serotype of a given sample strain; i.e., the serovar [2, 18].

Those bacterial strains from the *Salmonella* genus that cannot be assigned to a species and which require further molecular or serological diagnostics should be sent to a reference center. Each case of salmonellosis should be registered with the local SANEPID station [19].

The study data and statistical analyses were obtained from studies undertaken by the provincial SANEPID station in Cracow (WSSE in Cracow) regarding carriers of *Salmonella* bacilli in healthy, educated subjects whose work involves a risk of transmitting an infection or infectious disease to other people. These studies were carried out in the years 2017–2022 by the Laboratory Department of Clinical Microbiology at the WSSE in Cracow, a holder of a Testing Laboratory Accreditation Certificate.

Results

Study subjects included 163,938 people tested for carrier status at the WSSE in Cracow in the Lesser Poland Province in the years 2017–2022. On average, there were 28,312 healthy people tested for *Salmonella* carriers in each of these years, out of whom an average of 72 *Salmonella* carriers were detected. An exception was 2020 when only 22,377 subjects were tested, (Fig. 1 and Fig. 2). This was most likely due the ongoing SARS-CoV-2 virus epidemic at that time.

In 2017, 27,416 people were *Salmonella* carrier tested (Fig. 1) of whom 79 people (0.29%) tested positive (Fig. 2). The *Salmonella* Enteritidis serotype accounted for approximately 65% of all the identified serotypes (52 cases), *Salmonella* Infantis – approximately 14% (14 cases), *Salmonella* Typhimurium – approximately 6% (5 cases), and *Salmonella* Ohio – approximately 4% (3 cases). The remaining single cases accounted for approximately 10% of all the identified serotypes (Table III).

There were 31,919 people carrier tested for *Salmonella* in 2019 (Fig. 1) out of whom 70 tested positive 0.22% (Fig. 2). The breakdown of all the serotypes identified was as follows: 76% (53 cases) corresponded to *Salmonella* Enteritidis, approximately 9% (6 cases) to *Salmonella* Infantis, approximately 7% (5 cases) to *Salmonella* Typhimurium, and approximately 4% (3 cases) to *Salmonella* Agona. The remaining single cases together accounted for about 4% of all the identified serotypes (Table III).

In 2020, 22,377 people were tested for the presence of *Salmonella* (Fig. 1), out of whom 79 tested positive (0.35%) (Fig. 2). The serotype breakdown of all the identified serotypes was the following: *Salmonella* Enteritidis was identified in approximately 81% (64 cases), *Salmonella* Infantis in approximately 4% (3 cases), *Salmonella* Typhimurium in approximately 5% (4 cases), and *Salmonella* Livingstone in approximately 2% (2 cases). The remaining single cases accounted for about 8% of all the identified serotypes (Table III).

There were 24,721 people were subjected to Salmonella carrier testing in 2021 (Fig. 1), where 74 tested positive (0.30%) (Fig. 2). The serotypes were broken down as follows: Salmonella Enteritidis at approximately 77% (57 cases), Salmonella Infantis at approximately 8% (6 cases), Salmonella Virchow at approximately 3% (2 cases), and Salmonella Livingstone – approximately 3% (2 cases). The remaining single cases together accounted for approximately 9% of all the identified serotypes (Table III).

In 2022, 29,956 people were tested for the presence of Salmonella (Fig. 1), out of whom 54 people tested positive (0.18%); (Fig. 2). The serotypic breakdown of all those identified was as follows: Salmonella Enteritidis serotype was found in approximately 72% (39 cases), Salmonella enterica subsp. Enterica, monophasic strain with the antigenic formula 1,4 [5],12 in approximately 7% (4 cases), Salmonella Mbandaka in approximately 4% (2 cases), and Salmonella Agona in approximately 4% (2 cases). The remaining single cases together accounted for about 13% of all the identified serotypes (Table III).

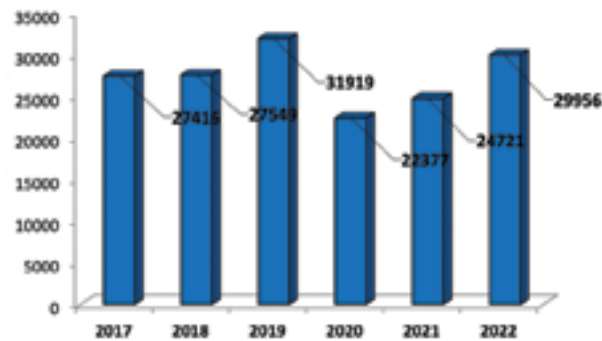


Figure 1: Number of Salmonella carrier tests performed on people from the Lesser Poland Province in the years 2017–2022

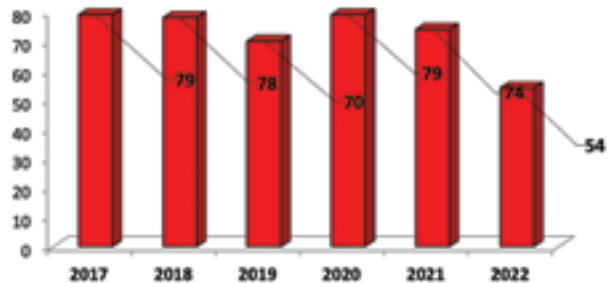


Figure 2. Number of people testing positive for Salmonella in the Lesser Poland Province in the years 2017–2022

Table III. *Salmonella* serotypes identified in healthy people from Lesser Poland in the years 2017–2022

Identified <i>Salmonella</i> serotype	Year					
	2017	2018	2019	2020	2021	2022
	Number of cases					
<i>S. Enteritidis</i>	52	44	53	64	57	39
<i>S. Typhimurium</i>	5	4	5	4	1	1
<i>S. Infantis</i>	11	16	6	3	6	1
<i>S. Indiana</i>	1	1	-	-	-	-
<i>S. Albany</i>	1	-	-	-	-	-
<i>S. Bareilly</i>	1	-	-	-	-	-
<i>S. Muenchen</i>	1	-	-	-	-	-
<i>S. Schleissheim</i>	1	-	-	-	-	-
<i>S. z gr. DO</i>	1	-	-	-	-	-
<i>S. Ohio</i>	3	-	-	-	-	-
<i>S. Agbeni</i>	1	-	-	-	-	-
<i>S. arizonae/diarizonae</i>	1	-	-	-	-	-
<i>S. Newport</i>	-	4	1	-	1	-
<i>D. Derby</i>	-	1	-	-	1	-
<i>S. Oranienburg</i>	-	1	-	-	-	-
<i>S. Brandenburg</i>	-	1	-	-	-	-
<i>S. Livingstone</i>	-	1	-	2	2	1
<i>S. Ordonez</i>	-	1	-	-	-	-
<i>S. Salamae</i>	-	1	-	-	-	-
<i>S. Itami</i>	-	1	-	-	-	-
<i>S. enterica subsp. enterica</i>	0	2	-	1	1	4
<i>S. Agona</i>	-	-	3	-	-	2
<i>S. Coeln</i>	-	-	1	1	1	-
<i>S. z gr CO</i>	-	-	1	1	-	-
<i>S. z gr BO</i>	-	-	-	1	-	-
<i>S. Strathcoma</i>	-	-	-	1	-	-
<i>S. Brunei</i>	-	-	-	1	-	-
<i>S. Virchow</i>	-	-	-	-	2	-
<i>S. Kottbus</i>	-	-	-	-	1	1
<i>S. Rissen</i>	-	-	-	-	1	-
<i>S. Paratyphi B</i>	-	-	-	-	-	1
<i>S. Mbandaka</i>	-	-	-	-	-	2
<i>S. Goldcoast</i>	-	-	-	-	-	1
<i>S. Thompson</i>	-	-	-	-	-	1

Discussion

Polish legislation stipulates the need for performing SANEPID testing/studies so as to prevent and fight infections and infectious diseases in humans, in accordance with the Act of 5 December 2008 (Journal of Laws of 2019, item 1239, 1495). SANEPID surveillance over carriers of the *Salmonella* bacilli is ongoing and aimed at preventing and reducing infections,

creating registers of this disease whenever detected in humans, and confirming the bacteriological diagnosis of the microbial agent actually causing the infection [21]. The primary objective of SANEPID examinations of healthy people is to detect asymptomatic carriers of *Salmonella* bacilli, who can infect food, water, or directly other people, which in turn may also lead to outbreaks of salmonellosis at national, European, or global level [16]. Furthermore, the diagnostic procedure carried out to identify *Salmonella* serotypes is vital, because it can indicate the origin and possible reservoir of the pathogen, as well as allow for an epidemiological investigation to be carried out in the event of any outbreaks [19].

Salmonellosis arising from reservoirs or infection sources of Typhi, Paratyphi A, Paratyphi B, and Paratyphi C serovars in sick or asymptomatic carriers account for a negligible percentage of all detected infections in Poland. At present, typhoid salmonellosis is observed sporadically, which is also confirmed by the above data obtained from the Lesser Poland Province. Only one case of Paratyphi B was identified in 2022. This outcome was achieved thanks to the improved hygienic situation observed in Poland post-World War II after many years of systematic typhoid vaccinations until 1960. In 1945, vaccination against typhoid was resumed [7]. These vaccinations are currently not considered mandatory, but recommended mainly for people travelling to at-risk regions of the world. An increased incidence of typhoid salmonellosis, due to endemic outbreaks of this disease, include South and Central America, Africa, Asia, India, and Pakistan, i.e., areas with a hot climate and a low standard of sanitary care/services. Indeed, the risk of importing various diseases, e.g., typhoid salmonellosis, to Europe and Poland has been constantly rising every year because of the greater number of trips made to countries with significantly different sanitary conditions. The potential risk of importing this disease from tropical countries can thus pose a serious epidemiological threat to those Polish people who do not travel abroad [13, 17]. As estimated by the World Health Organization (WHO), since 2019, there have been 9 million cases of typhoid registered in the world every year, of which 110,000 have resulted in death as a direct consequence of the disease. As of March 2023, the WHO pre-qualified two conjugate vaccines that could be administered as a single dose to children from 6 months of age in order to prevent typhoid in countries where this disease is endemic [7, 23].

Intestinal salmonellosis is caused by *Salmonella* bacilli other than *Salmonella* Typhi and *Salmonella* Paratyphi. The reservoir/sink of these bacilli is usually farm animals like poultry (including eggs), cattle, pigs, and wild animals. In Europe, the main source of salmonellosis in humans are foodstuffs [15]. In the years 2015–2019, the most significant source of contaminated food responsible for salmonellosis outbreaks in Europe were: eggs (33%), = pork (7%), and general meat products (6%). It is therefore important to identify the foodstuff that is linked to the contracting of intestinal salmonellosis in humans [14].

According to the Center for Disease Control and Prevention (CDC), there are over 2,000 different *Salmonella* serotypes of which *Salmonella* Enteritidis and *Salmonella* Typhimurium are responsible for over 70% of human cases of salmonellosis [4].

The tests performed in the Lesser Poland Province in 2017–2022 showed that the most frequently identified *Salmonella* serotypes were: *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Infantis, of which the first (*Salmonella* Enteritidis) accounted for 71.1% of all the identified *Salmonella* serotypes in healthy individuals. Infections with this serotype most often occur through the consumption of eggs, poultry meat, and pork [4], both in Poland and in Europe. Surveillance over flocks of laying hens is therefore important. *Salmonella* Enteritidis, however, can also infect the ovaries of laying hens, which causes the bacteria to enter the eggs, whereas other *Salmonella* serotypes remain in the feces, contaminating the surface of egg shells [20, 24]. There was an outbreak of an epidemic caused by *Salmonella* Enteritidis in the years 2016–2020 caused by eggs from a Polish poultry farm, which affected 18 countries of the European Union and has been the largest reported epidemic of this kind in Europe so far. Furthermore, in 2020–2021, an epidemic in Great Britain was caused by *Salmonella* Enteritidis from raw, breaded poultry products originating from two Polish meat suppliers [5].

Salmonella Infantis is one of the 10 most common serotypes that cause human salmonellosis in Europe and North America. In the years 2017–2022, *Salmonella* Infantis accounted for 9.9% of all the serotypes identified in healthy individuals in the Lesser Poland Province. In Europe, on the other hand, it is the most commonly reported *Salmonella* serotype of animal origin, where most of the strains are present in the poultry production chain. This serotype has also been isolated from various other sources, e.g., pigs [8].

The *Salmonella* Typhimurium bacilli accounted for 4.6% of all the serotypes identified in healthy people in Lesser Poland in 2017–2022. Outbreaks caused by *Salmonella* Typhimurium in Europe in 2015–2019 were mainly attributed to eggs and pork [14]. In 2019, *Salmonella* Typhimurium infections were identified in three EU countries, the UK, and Canada. Case-control studies indicate that Brazil nuts were the likely source of this infection. As of October 20, 2020, 123 cases of *s. Typhimurium* were reported, of which 104 were in the UK, 14 in France, 3 in Luxembourg and 1 in the Netherlands and Canada each [4].

Tests conducted in the Lesser Poland Province in 2017–2022 on healthy people demonstrated that single cases of *Salmonella* serotypes included the following: *s. Derby*, *s. Virchow*, *s. Agona*, *s. Newport*, *s. Mbandaka*, *s. Indiana*, *s. Oranienburg*, *s. Albany*, *s. Bareilly*, *s. Brandenburg*, *s. Goldcoast*, *s. Kottbus*, *s. Livingstone*, *s. Muenchen*, *s. Rissen*, *s. Schleissheim*, *s. Thompson*, *s. from Greek BO*, *s. from Gr. CO*, *s. from the group DO*, *s. Ohio*, *s. Agbeni*, *s. arizonae/diarizonae*, *s. Ordonez*, *s. salamae*, *s. Itami*, *s. enterica* subsp. *enterica*, monophasic strain with the antigenic formula 1,4 [12], 12:i:- and *s. Coeln*, *s. Strathcoma*, *s. Brunei*, which all accounted for a total of 14% of all the identified serotypes. Sources of infection for the above serotypes could have come from plants, animals, and products of animal origin. In 2015–2019, the most noteworthy sources of infection in Europe were eggs and egg products, followed by pork and its products, broiler meat, and beef and its derivatives. A much lower percentage of infections were observed from cereal products, including rice and seeds/legumes (nuts, almonds), herbs and spices, and tap water (including well/spring water) [14, 15].

The presented data demonstrated that a well-functioning system was in place for identifying *Salmonella* in healthy people in the Lesser Poland Province in the years 2017–2022. A large number of tests were performed and the number of detected carriers was low, which proves that detection, monitoring and SANEPID surveillance fully meets requirements. Most cases of typhoid salmonellosis are due to the continually intensifying tourist traffic around the world and the importing of *Salmonella* Typhi and Paratyphi bacilli to Poland from visited countries. It is therefore important to consult a specialist in tropical or travel medicine before visiting any country where the risk of contracting *Salmonella* is elevated. , . This should include preventive vaccination as recommended for international tourist traffic and compliance with the rules of tropical hygiene

The incidence of intestinal salmonellosis can be reduced by identifying its sources and the ways of its dissemination through the monitoring of animal infections and foodstuffs of animal origin, and examining feed by testing for the presence of these bacilli in order to identify them.

It is vital to observe the basic rules of hygiene, maintain a high level of sanitary and hygienic conditions in the places where catering services are rendered, and implement a quality control system in food production. Poland is covered by the Rapid Alert System for Food and Feed (RASFF) which notifies the European Commission whenever a food threat that poses a direct or indirect danger to human health is detected. It forms a part of official surveillance and monitoring by Polish SANEPID stations. Fast remedial action can thus be taken to eliminate the threat. Adopting preventative measures when travelling abroad and health education are important in preventing the incidence of the *Salmonella* genus bacteria cases. In order to effectively detect and identify infection factors, it is necessary to constantly develop our knowledge of clinical microbiology and improve the diagnostic methods employed.

Conclusions

- 1) The average rate of healthy people diagnosed with *Salmonella* bacilli living in the Lesser Poland Province in the years 2017–2022 was 0.25% . This was a constant for each year.
- 2) There were 35 different *Salmonella* serotypes identified in healthy people from the Lesser Poland Province in 2017–2022. The most frequent serotype detected was *Salmonella* Enteritidis, accounting for 71% of all the detected serotypes in *Salmonella* carriers. The second and third most commonly found serotypes were respectively *Salmonella* Infantis (9.9%) and *Salmonella* Typhimurium (4.6%). Other *Salmonella* serotypes were identified in individual cases and together accounted for 14.1% of all the detected serotypes. Only one case of *Salmonella* typhoid was identified, s. Paratyphi B, in 2022, during the 2017–2022 period.
- 3) The observed increase in food safety and a reduced number of cases in Poland are a result of the SANEPID supervision that includes microbiological food testing and testing healthy people to detect *Salmonella* carriers. It should be noted that typhoid strains may appear due to travelling without prior and proper preparation (i.e., vac-

nation, avoiding risky situations and observing tropical hygiene) to countries where Salmonella disease is endemic, as well as in the result of an influx of immigrants.

REFERENCES

1. Colin P. International Symposium on Salmonella and salmonellosis. Food Microbiol 2018; 71:1.
2. Dera-Tomaszewska B. *Epidemiologia i chorobotwórczość pałeczek Salmonella Enteritidis oraz serowarów Salmonella izolowanych w Polsce po raz pierwszy. Rozprawa habilitacyjna*. Gdański Uniwersytet Medyczny 2013; 102–106.
3. Dziubek Z. *Choroby zakaźne i pasożytnicze*. Wyd. IV. PZWL 2010, 140–150.
4. European Centre for Disease Prevention and Control, European Food Safety Authority. Multi-country outbreak of Salmonella Typhimurium and s. Anatum infections linked to Brazil nuts – 21 October 2020. EFSA Supporting Publications 2020. Available from: <https://www.ecdc.europa.eu/sites/default/files/documents/salmonella-typhimurium-rapid-outbreak-assessment-october-2020.pdf> (accessed: 07.10.2023).
5. European Centre for Disease Prevention and Control. Multi-country outbreak of Salmonella Enteritidis in chicken meat and chicken meat products. Available from: <https://www.ecdc.europa.eu/en/news-events/multi-country-outbreak-salmonella-enteritidis-chicken-meat-and-chicken-meat-products> (accessed: 11.10.2023).
6. Flisiak R. *Choroby zakaźne i pasożytnicze*. Tom II. *Dur brzuszny i inne salmonellozy*. Wyd. Czelej 2020, 48–56.
7. Gołofit-Szymczak M. Górny RL. *Szczepienia ochronne w Polsce* [Preventive vaccinations in Poland]. *Bezpieczeństwo Pracy: nauka i praktyka* 2021; 7: 18–21.
8. Gymoese P, Kiil K, Torpdahl M, Østerlund MT, et al. WGS based study of the population structure of Salmonella enterica serovar Infantis. BMC Genomics 2019; 20: 870.
9. Heczko PB, Wróblewska M, Pietrzyk A. *Mikrobiologia Lekarska*. PZWL, Warszawa, 2014; 141–147.
10. Kwiatek K. *Nowe normatywne wytyczne w zakresie wytwarzania i stosowania pożywek mikrobiologicznych* [New normative guidelines for production and use of microbiological culture media]. *Życie Weterynaryjne* 2013; 88(4): 315–318.
11. Mikołajczyk A, Stefaniuk E, Bosacka K, et al. *Właściwości i zastosowanie podłoży bakteriologicznych* [Properties and uses of bacteriological media]. *Post Mikrobiol* 2016; 55(3): 320–329.
12. Moser-van der Geest N, Schibli A, Huber LC. CME: Typhoid Fever – Clinical Manifestation, Diagnosis, Therapy and Prevention. *Praxis (Bern 1994)* 2019; 108(14): 937–943.
13. Paul M. *Dur brzuszny niedocenianym zagrożeniem zdrowotnym dla Polaków podróżujących do krajów strefy tropikalnej* [Typhoid fever as an underestimated health threat for Polish tourists travelling to tropical countries]. *Probl Hig Epidemiol* 2013; 94(4): 701–709.

14. Pinedo LC, Mughini-Gras L, Franz E, et al. *Sources and trends of human salmonellosis in Europe, 2015-2019: An analysis of outbreak data*. Int J Food Microbiol 2022; 379: 109850.
15. Pires SM, Vieira AR, Hald T, et al. *Source attribution of human salmonellosis: an overview of methods and estimates*. Foodborne Pathog Dis 2014; 11(9): 667–676.
16. Seweryn M. Funkcjonowanie Państwowej Inspekcji Sanitarnej. *Historia, rozwój, wyzwania*. Zdrowie Publiczne i Zarządzanie 2015; 13(2): 158–164.
17. Szewczyk EM. *Diagnostyka bakteriologiczna*. Wyd. Naukowe PWN, Warszawa, 2020; 554–555; 159–164.
18. Szewczyk EM. *Podłoża, próby i metody diagnostyczne. Schematy diagnostyczne*. In: Szewczek EM, eds. Diagnostyka mikrobiologiczna. PWN, Warszawa, 2013; 404–459.
19. Szych J. Rekomendacje laboratoryjnej diagnostyki zakażeń przewodu pokarmowego bakteriami rosnącymi w warunkach tlenowych oraz mikroaerofilnych. Rekomendacje Narodowego Instytutu Zdrowia Publicznego, Państwowego Zakładu Higieny i Krajowej Izby Diagnostów Laboratoryjnych [Recommendations for laboratory diagnosis of gastrointestinal infections caused by bacteria growing in aerobic and microaerophilic conditions. Recommendations of the National Institute of Public Health, the National Institute of Hygiene and the National Chamber of Laboratory Diagnosticians]. Warszawa, 2015. Available from: https://kidl.org.pl/get-file/166_to-wrzu- (accessed: 07.08.2023).
20. The Interagency Food Safety Analytics Collaboration (IFSAC), 2021. Foodborne illness source attribution estimates for 2019 for Salmonella, Escherichia coli O157, Listeria monocytogenes, and Campylobacter using multi-year outbreak surveillance data, United States. Available from: <https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2019-report-TriAgency-508.pdf> (accessed: 07.10.2023).
21. Ustawa z dnia 5 grudnia 2008 r. o zapobieganiu oraz zwalczaniu zakażeń i chorób zakaźnych u ludzi (Dz.U. 2008 nr 234 poz. 1570 z późn. zm.). Available from: <https://isap.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=wdu20082341570> (accessed: 07.10.2023).
22. Vandepitte J, Verhaegen J, Engbaek K, et al. *Najważniejsze podłoża i odczynniki (w). Podstawowe procedury laboratoryjne w bakteriologii klinicznej*, red. A. Przondo-Mordarska, PZWL, Warszawa, 2005; 157–170.
23. WHO. Immunizations, Vaccines and Biologicals. Available from: <https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/typhoid> (accessed: 14.10.2023).
24. Związek Przedsiębiorców i Pracodawców. Salmonella w stadach kur niosek towarowych. Występowanie, koszty oraz zapobieganie [Association of Entrepreneurs and Employers. Salmonella in flocks of laying hens. Occurrence, costs and prevention]. Available from: <https://zpp.net.pl/wp-content/uploads/2021/01/Raport-Salmonella.pdf> Salmonella (accessed: 07.08.2023).
25. Dera-Tomaszewska B. *Epidemiologia i chorobotwórczość pałeczek Salmonella Enteritidis oraz serowarów Salmonella izolowanych w Polsce po raz pierwszy. Rozprawa habilitacyjna*. Gdański Uniwersytet Medyczny 2013; 102–106.

26. Dziubek Z. *Choroby zakaźne i pasożytnicze*. Wyd. IV. PZWL 2010, 140–150.
27. European Centre for Disease Prevention and Control, European Food Safety Authority. Multi-country outbreak of Salmonella Typhimurium and s. Anatum infections linked to Brazil nuts – 21 October 2020. EFSA Supporting Publications 2020. Available from: <https://www.ecdc.europa.eu/sites/default/files/documents/salmonella-typhimurium-rapid-outbreak-assessment-october-2020.pdf> (accessed: 07.10.2023).
28. European Centre for Disease Prevention and Control. Multi-country outbreak of Salmonella Enteritidis in chicken meat and chicken meat products. Available from: <https://www.ecdc.europa.eu/en/news-events/multi-country-outbreak-salmonella-enteritidis-chicken-meat-and-chicken-meat-products> (accessed: 11.10.2023).
29. Flisiak R. *Choroby zakaźne i pasożytnicze*. Tom II. *Dur brzuszny i inne salmonellozy*. Wyd. Czelej 2020, 48–56.
30. Gołofit-Szymczak M, Górny RL. *Szczepienia ochronne w Polsce* [Preventive vaccinations in Poland]. *Bezpieczeństwo Pracy: nauka i praktyka* 2021; 7: 18–21.
31. Gymoese P, Kiil K, Torpdahl M, Østerlund MT, et al. WGS based study of the population structure of Salmonella enterica serovar Infantis. *BMC Genomics* 2019; 20: 870.
32. Heczko PB, Wróblewska M, Pietrzyk A. *Mikrobiologia Lekarska*. PZWL, Warszawa, 2014; 141–147.
33. Kwiatek K. *Nowe normatywne wytyczne w zakresie wytwarzania i stosowania pożywek mikrobiologicznych* [New normative guidelines for production and use of microbiological culture media]. *Życie Weterynaryjne* 2013; 88(4): 315–318.
34. Mikołajczyk A, Stefaniuk E, Bosacka K, et al. *Właściwości i zastosowanie podłoży bakteriologicznych* [Properties and uses of bacteriological media]. *Post Mikrobiol* 2016; 55(3): 320–329.
35. Moser-van der Geest N, Schibli A, Huber LC. CME: Typhoid Fever – Clinical Manifestation, Diagnosis, Therapy and Prevention. *Praxis (Bern 1994)* 2019; 108(14): 937–943.
36. Paul M. *Dur brzuszny niedocenianym zagrożeniem zdrowotnym dla Polaków podróżujących do krajów strefy tropikalnej* [Typhoid fever as an underestimated health threat for Polish tourists travelling to tropical countries]. *Probl Hig Epidemiol* 2013; 94(4): 701–709.
37. Pinedo LC, Mughini-Gras L, Franz E, et al. *Sources and trends of human salmonellosis in Europe, 2015-2019: An analysis of outbreak data*. *Int J Food Microbiol* 2022; 379: 109850.
38. Pires SM, Vieira AR, Hald T, et al. *Source attribution of human salmonellosis: an overview of methods and estimates*. *Foodborne Pathog Dis* 2014; 11(9): 667–676.
39. Seweryn M. *Funkcjonowanie Państwowej Inspekcji Sanitarnej. Historia, rozwój, wyzwania*. *Zdrowie Publiczne i Zarządzanie* 2015; 13(2): 158–164.
40. Szewczyk EM. *Diagnostyka bakteriologiczna*. Wyd. Naukowe PWN, Warszawa, 2020; 554–555; 159–164.
41. Szewczyk EM. *Podłoża, próby i metody diagnostyczne. Schematy diagnostyczne*. In: Szewczek EM, eds. *Diagnostyka mikrobiologiczna*. PWN, Warszawa, 2013; 404–459.
42. Szych J. Rekomendacje laboratoryjnej diagnostyki zakażeń przewodu pokarmowego bakteriami

- rosnącymi w warunkach tlenowych oraz mikroaerofilnych. Rekomendacje Narodowego Instytutu Zdrowia Publicznego, Państwowego Zakładu Higieny i Krajowej Izby Diagnostów Laboratoryjnych [Recommendations for laboratory diagnosis of gastrointestinal infections caused by bacteria growing in aerobic and microaerophilic conditions. Recommendations of the National Institute of Public Health, the National Institute of Hygiene and the National Chamber of Laboratory Diagnosticians]. Warszawa, 2015. Available from: https://kidl.org.pl/get-file/166_to-wrzu- (accessed: 07.08.2023).
43. The Interagency Food Safety Analytics Collaboration (IFSAC), 2021. Foodborne illness source attribution estimates for 2019 for Salmonella, Escherichia coli O157, Listeria monocytogenes, and Campylobacter using multi-year outbreak surveillance data, United States. Available from: <https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2019-report-TriAgency-508.pdf> (accessed: 07.10.2023).
44. Ustawa z dnia 5 grudnia 2008 r. o zapobieganiu oraz zwalczaniu zakażeń i chorób zakaźnych u ludzi (Dz.U. 2008 nr 234 poz. 1570 z późn. zm.). Available from: <https://isap.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=wdu20082341570> (accessed: 07.10.2023).
45. Vandepitte J, Verhaegen J, Engbaek K, et al. *Najważniejsze podłoża i odczynniki* (w). Podstawowe procedury laboratoryjne w bakteriologii klinicznej, red. A. Przondo-Mordarska, PZWL, Warszawa, 2005; 157-170.
46. WHO. Immunizations, Vaccines and Biologicals. Available from: <https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/typhoid> (accessed: 14.10.2023).
47. Związek Przedsiębiorców i Pracodawców. *Salmonella w stadach kur niosek towarowych*. Występowanie, koszty oraz zapobieganie [Association of Entrepreneurs and Employers. Salmonella in flocks of laying hens. Occurrence, costs and prevention]. Available from: <https://zpp.net.pl/wp-content/uploads/2021/01/Raport-Salmonella.pdf> Salmonella (accessed: 07.08.2023).

ABSTRACT

Introduction. Salmonella bacteria belong to the Gram-negative Enterobacteriaceae family. They are motile, relatively anaerobic, and lactose-negative colony-forming bacteria that ferment glucose and lack cytochrome oxidase. Infections from Salmonella bacteria such as typhoid, paratyphoid, and salmonellosis can pose a threat to both human health and life. Typhoid is a disease caused by the most prominent serological variant of the Salmonella genus, Salmonella Typhi, and it spreads only amongst humans who also serve as the only reservoir/sink of this infection.

Aim. To determine the rate of persons testing positive for carrying Salmonella bacilli relative to the number of healthy people tested in the Lesser Poland Province during 2017–2022. To establish the most frequently identified serotypes of Salmonella in healthy people residing in the Lesser Poland Province during 2017–2022.

Material and methods. The laboratory test material to determine Salmonella carriers among healthy people were three stool samples collected by study subjects once a day for three consecutive days by means of sampling into test tubes suitable for transport in accordance with laboratory instructions. Each stool sample delivered to the laboratory was inoculated directly onto a solid MacConkey agar medium. After incubating the samples in MacConkey's agar at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18–24 hours, further diagnostic procedures were performed on isolated colonies characteristic of the Salmonella genus, i.e., lactose-negative, colorless, and transparent.

Results. The average rate of healthy people diagnosed with Salmonella bacilli living in the Lesser Poland Province was 0.25% in 2017–2022. This was a constant for each year. There were 35 different Salmonella serotypes identified in healthy people from the Lesser Poland Province in the years 2017–2022. The most frequent serotype detected was Salmonella Enteritidis, accounting for 71 % of all the detected serotypes in Salmonella carriers. The second and third most commonly found serotypes were Salmonella Infantis (9.9%) and Salmonella Typhimurium (4.6%), respectively. Other Salmonella serotypes were identified in individual cases and together accounted for 14.1% of all the detected serotypes. Only one case of Salmonella typhoid was identified, s. Paratyphi B, in 2022, during the 2017–2022 period.

Conclusions: The observed increase in food safety and a reduced number of cases in Poland are a direct results of the Polish State Sanitary Inspection (SANEPID) supervision that includes microbiological food testing and testing healthy people to detect Salmonella carriers. It should be noted that typhoid strains may appear due to travelling without prior and proper preparation, (i.e. vaccination, avoiding risky situations and observing tropical hygiene) to countries where Salmonella disease is endemic, as well as in the result of an influx of immigrants.

KEYWORDS: Salmonella, serotype, typhoid, paratyphoid, salmonellosis.

STRESZCZENIE

Wprowadzenie. Bakterie *Salmonelli* należą do rodziny enterobakterii Gram-ujemnych. Są to ru-chliwe, stosunkowo anaerobowe i laktozo-ujemne bakterie tworzące kolonie, które fermentują glukozę i nie mają oksydazy cytochromowej. Zakażenia bakteriami *Salmonelli*, takie jak dur brzuszny, paraty-fus i salmonelloza, mogą stanowić zagrożenie zarówno dla zdrowia jak i życia ludzkiego. Dur brzuszny jest chorobą wywoływaną przez najważniejszy wariant serologiczny rodzaju *Salmonella*, pałeczki duru brzusznego, i rozprzestrzenia się tylko wśród ludzi, którzy są również jedynym rezerwuarem/gospoda-rzem docelowym tej infekcji.

Cel. Określenie odsetka osób z dodatnim wynikiem testu na nosicielstwo pałeczek *Salmonelli* w sto-sunku do liczby osób zdrowych przebadanych w województwie małopolskim w latach 2017-2022. Usta-lenie, które serotypy *Salmonelli* były najczęściej identyfikowane u osób zdrowych zamieszkających w woje-wództwie małopolskim w latach 2017-2022.

Materiały i metody. Laboratoryjnym materiałem badawczym do określenia nosicielstwa *Salmonelli* u zdrowych ludzi były trzy próbki kału pobierane przez badanych raz dziennie przez trzy kolejne dni za pomocą próbek nadających się do transportu zgodnie z instrukcjami laboratoryjnymi. Każda prób-ka kału dostarczona do laboratorium została zaszczipiona bezpośrednio na stałym podłożu agarowym MacConkeya. Po inkubacji próbek w agarze MacConkeya w temperaturze $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ przez 18-24 go-dziny, przeprowadzono dalsze procedury diagnostyczne na izolowanych koloniach charakterystycznych dla rodzaju *Salmonella*, tj. laktozo-ujemnych, bezbarwnych i przezroczystych.

Wyniki. W latach 2017-2022 średni odsetek osób zdrowych, u których zdiagnozowano pałeczki *Sal-monelli* mieszkających w województwie małopolskim, wynosił 0,25%. Była to stała dla każdego roku. W latach 2017-2022 u zdrowych osób z województwa małopolskiego zidentyfikowano 35 różnych se-rotypów *Salmonelli*. Najczęściej wykrywanym serotypem była *Salmonella* Enteritidis, stanowiąca 71% wszystkich wykrytych serotypów u nosicieli *Salmonelli*. Drugim i trzecim najczęściej występującym se-rotypem były odpowiednio *Salmonella* Infantis (9,9%) i *Salmonella* Typhimurium (4,6%). W pojedy-nych przypadkach zidentyfikowano inne serotypy *Salmonelli*, które łącznie stanowiły 14,1% wszystkich wykrytych serotypów. W latach 2017-2022 zidentyfikowano tylko jeden przypadek duru brzusznego *Salmonella*, s. Paratyphi B, w 2022 r.

Wnioski: Obserwowany wzrost bezpieczeństwa żywności i zmniejszenie liczby zachorowań w Polsce to zasługa nadzoru Sanepidu, który obejmuje badania mikrobiologiczne żywności oraz badania osób zdrowych w kierunku nosicielstwa *Salmonelli*. Należy zauważyć, że szczepy duru brzusznego mogą poja-wić się w wyniku podróży do krajów, w których *Salmonella* jest chorobą endemiczną, bez wcześniejszego i odpowiedniego przygotowania (tj. szczepień, unikania ryzykownych sytuacji i przestrzegania higieny tropikalnej), a także w wyniku napływu imigrantów.

SŁOWA KLUCZOWE: *Salmonella*, serotyp, dur brzuszny, paratyfus, salmonelloza.