

ORIGINAL ARTICLES

THE EPIDEMIOLOGICAL SIGNIFICANCE AND ETIOLOGICAL STRUCTURE OF *SALMONELLA SP.* AS A CAUSE OF FOOD- AND WATERBORNE INFECTIONS IN BULGARIA

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ABSTRACT

INTRODUCTION: *Salmonella* is a leading cause of gastroenteritis in humans and animals, causing typhoidal and non-typhoidal infections that can develop into widespread food- and waterborne outbreaks.

AIM: The incidence of salmonellosis among humans in our country has remained relatively high in recent years despite annual fluctuations. In this work, we aim to clarify the dynamics of the prevalence of salmonella-causing human infections in Bulgaria from 2017 to 2023.

MATERIALS AND METHODS: We used data from annual analyses of isolated *Salmonella* cultures from patients and contacts with NRL Enteric Infections, Pathogenic Cocci, and Diphtheria.

RESULTS AND DISCUSSION: During the study period, a total of 3208 human salmonellosis cases were laboratory confirmed by bacterial culture isolation, of which 1847 were from patients with enterocolitis syndrome and 1361 from infectious carriers. These cultures belonged to two species of *Salmonella enterica* and *S. bongori*, 4 subtypes – enterica, salame, arizonae and diarizonae, and 66 *Salmonella* serotypes. *Salmonella* Enteritidis plays the leading etiological role in the patients, occupying a relative share of 47%, followed by *Salmonella* Typhimurium – 32.8%, and 40.5% are monophasic. *Salmonella* Infantis ranked third with 4.85%. The following serotypes were isolated for the first time in our country: Brazzaville, Larochele, Ndolo, Kuoka, Jerusalem, Vejle, and the unique for the world, new serotype – *Salmonella* 11: z41: enz15. In carriers, in contrast to patients, other serotypes come to the fore Muenster, Bazenheid, Dublin, etc. The data obtained in the analysis again confirm that infants and young children are the group most affected by *Salmonella* infections. *Salmonella* outbreaks in the country have been declining sharply since 2020, in contrast to sporadic cases. In 2018, there was an outbreak of a rare serotype, London.

CONCLUSION: Nontyphoidal salmonellosis is a socioeconomic and public health problem that requires control of *Salmonella* infections in animals and humans. In the effort to contain them, it is necessary to know in detail and follow in dynamics the etiological structure of salmonellosis and to apply non-antibiotic-based approaches such as prophylaxis with probiotics, bacteriophages, and vaccines among farm animals.

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INTRODUCTION

Salmonella is a leading cause of gastroenteritis in humans and animals, causing typhoidal and non-typhoidal infections with a relatively low mortality rate and is included in the Biodefense and Emerging Infectious Diseases Priority Pathogens list by the National Institute of Allergy and Infectious Diseases (NIAID) (1). The pathogen covers more than 2600 characterised serovars, according to the White-Kauffmann-Le Minor scheme in the published supplement No. 48-2014 (2). Zoonosis is associated with the consumption of contaminated food products from poultry, beef, pork, fish, and non-animal foods such as fruits and vegetables. The disease is transmitted primarily through the faecal-oral route; however, it can also be contracted via less common modes of transmission, including domestic sources and, less frequently, sexual intercourse. (3,4). The World Health Organization (WHO) describes *Salmonella* as one of the four most important causes of diarrhoea worldwide and the second, after campylobacteriosis, most commonly reported gastrointestinal infection and an important cause of both sporadic cases and foodborne outbreaks in the European Union and the European Economic Area (5,6). Over 25% of all affected are paediatric patients, of whom over 80% are under 2 years of age; the second most affected age group are patients over 70 years (7,8). The severity of diseases caused by members of the genus *Salmonella* is dependent on the serotype of the bacteria and the immune status of the host. The infection is usually self-limiting, but in rare cases, immunocompromised patients, infants, and the elderly may develop invasive infections, bacteraemia, endovascular infections, and septic arthritis (9). A report from the European Food Safety Authority and the European Centre for Disease Prevention and Control's Zoonoses Monitoring and Surveillance reports 15.3 per 100 000 salmonellosis cases for 2022. The Centers for Disease Control and Prevention (CDC) estimates that approximately 1.35 million illnesses and 420 deaths are attributable to non-typhoidal *Salmonella* annually in the United States (10). The incidence of salmonellosis among humans in Bulgaria has remained relatively high in recent years, despite

annual fluctuations. In a 2006 study, Aseva et al. reported an increased incidence among the leading serotypes causing human salmonellosis in our country. The authors highlight Corvallis as a rare serotype in Europe, but in Bulgaria, it is the third most frequently reported causative agent of human infection after *S. Enteritidis* and *S. Typhimurium* since 1997 (11).

This report focuses on the epidemiological and etiological structure of salmonellosis in Bulgaria, drawing on laboratory-confirmed *Salmonella* isolates from across the country over a ten-year period, outlining in detail the etiological structure of salmonella enterocolitis as an important socioeconomic problem.

MATERIALS AND METHODS

To perform the retrospective epidemiological study, data from the annual analyses of isolated *Salmonella* cultures from patients and contacts of the NRL Enteric Diseases, as well as strains obtained for confirmation and serotyping sent by the network of microbiological laboratories operating in the country and performing primary diagnostics were used. Also, data on reported cases of salmonellosis (possible, probable, confirmed) from all 28 Regional Health Inspectorates (RHIs) in the country, weekly and monthly data from the National Centre for Communicable Diseases in Bulgaria, and annual analyses of communicable diseases in Bulgaria prepared by the Epidemiology Department of the NCIPD were used.

RESULTS

During the study period, a total of 3208 human salmonellosis cases were laboratory confirmed by bacterial culture isolation, of which 1847 (58%) isolates were from patients with diarrhoeal syndrome and 1361 (42%) were isolated from asymptomatic carriers. These bacterial cultures belonged to two types, *Salmonella* enterica and *S. Bongori*; 4 subtypes – *enterica*, *salame*, *arizonae*, and *diarizonae*, and 66 *Salmonella* serotypes were determined (Fig. 1, Table 1). The leading etiological role in patients is played by *Salmonella* Enteritidis, occupying a relative share of 47%, followed by *Salmonella* Typhimurium – 32.8%,

of which 40.5% – monophasic *Salmonella* 1,4,5,12:i:-, and 2.26% – *Salmonella* 1,4,5,12:-:1,2. *Salmonella* Infantis ranked third among the leading serotypes causing *Salmonella* in the country with 4.85% (Fig. 2). The following serotypes have been isolated in Bulgaria for the first time – Brazzaville, Larochelle, Ndolo, Kuoka, Jerusalem, Vejle, and the unique for the world, new serotype – *Salmonella* enterica subsp. enterica O:11 H: z41 H: enz15. Other serotypes are coming to the fore in the infectious hosts, in contrast to the diseased ones – Muenster, Bazenheid, Dublin, etc. The data obtained in the analysis again confirm that the group most affected by *Salmonella* in-

fections is the group of infants and young children (<5 years) (Fig. 3). Registered cases of salmonellosis predominate in the urban population. The sex differentiation between ill and asymptomatic *Salmonella* carriers is negligible in favour of the male sex – 54%. An outbreak of serotype London, a rare serotype in the country, was recorded in 2018, in contrast to regularly recorded outbreaks of serotype Enteritidis and Typhimurium. On average, 5 *Salmonella* outbreaks have been recorded per year.

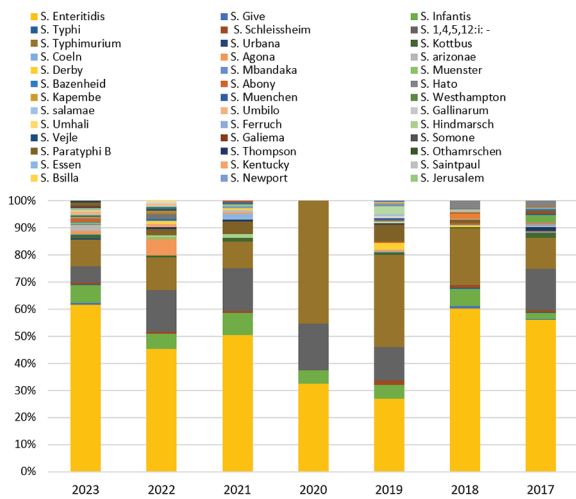


Fig. 1. Laboratory-confirmed *Salmonella* serotypes during the 2017–2023 study period.

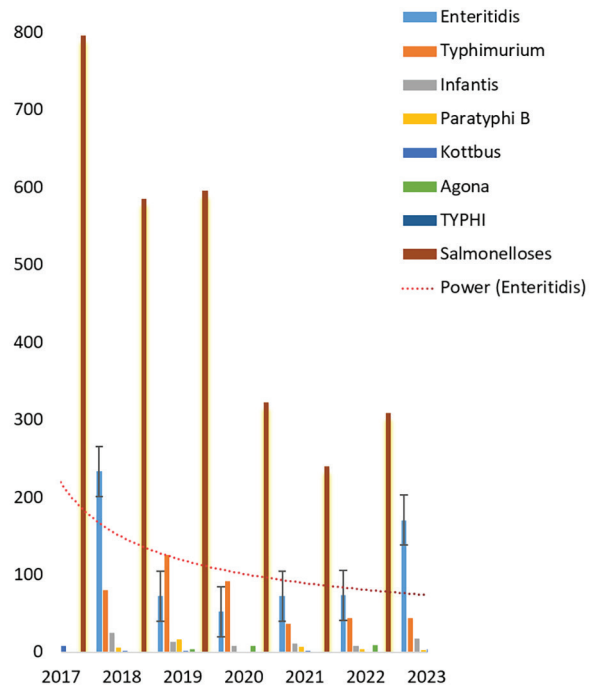


Fig. 2. Dynamics and distribution of *Salmonella* serotypes in Bulgaria for the 2017–2023 study period.

Table 1. Relationship of recorded infectious enterocolitis to reported possible/probable cases of salmonellosis and confirmed *Salmonella* serotypes (National Center for Communicable and Parasitic Diseases).

Year	Enterocolitis	Salmonellosis (Reported)	Salmonellosis (Isolates)
2017	14 101	796	457
2018	13 704	586	388
2019	13 121	596	271
2020	5 117	323	163
2021	2 953	240	145
2022	5 121	309	163
2023	7 137	358	278
Total	61 254	3 208	1 865

DISCUSSION

Acute infectious diarrhoeal disease caused by *Salmonella* remains a topical issue globally for developing and a different, but significant, problem for developed countries. The ability of *Salmonella* to adapt to the host environment and cause clinical symptoms in that particular host makes it variable in its degree of pathogenicity to both animals and humans. Representatives of the enterica subtype, such as *S. Enteritidis*, *S. Typhimurium*, *S. Newport*, and *S. Heidelberg*, can infect both humans and animals. Some serovars such as *S. Typhimurium*, *S. Abortusovis*, *S. Typhisuis*, *S. Gallinarum*, and *S. Pullorum* infect primarily sheep, swine, waterfowl, wildlife, and poultry, while *S. Dublin* and *S. Choleraesuis* infect cattle and swine. *S. Typhimurium*, in addition to being a leader among human salmonellosis, is known to shed from swine, cattle, horses, poultry, and rodents (12–14). In 2018 a US study showed that over 52% of *Salmonella* infections in poultry were caused by *S. Enteritidis*, making it one of the most prevalent *Salmonella* serotypes in the US (15–16). The data from our study do not differ from global data, namely that *S. Enteritidis* and *S. Typhimurium* are the leading causes of nontyphoidal *Salmonellosis* in the general population in Bulgaria. As in other EU countries, the number of isolates of monophasic *S. Typhimurium* (1,4,5,12: i: -) is increasing year by year at the expense of biphasic *S. Typhimurium* according to ECDC data (6,17).

But unlike the 1999–2004 period, when *S. Corvallis* reached statistically significant isolation rates in Bulgaria, in the current study period before and after the 2017–2023 epidemic, the *Infantis* serotype significantly displaced *Corvallis* and other common serotypes in the country and in the EU and EEA (17). Despite the high rate of salmonellosis due to *Infantis*, there was no confirmed outbreak; all 97 cases were sporadic in the country and unrelated to each other. In this regard *S. Enteritidis*, *S. Heidelberg*, and *S. Infantis* are associated with house flies, which are one of the vectors for *Salmonella* transmission, house flies can transmit serotypes (18). Chicken, pork, and beef are responsible for 34%, 25%, and 16% of salmonella outbreaks, respectively, and 10% of human salmonellosis is attributed to beef consumption. Likewise, pets can contaminate the environment and transmit infection to other animals kept for food by sporadically shedding bacteria in their faeces (18,19,20).

Pigs play a crucial role in the transmission of *Salmonella* to domesticated animals. Pets such as dogs fed raw food are more likely to contain *Salmonella* serovars such as *S. Typhimurium*, *S. Kentucky*, and *S. Heidelberg*. *Salmonella* is commonly found in a variety of wild mammals, such as wild boar. In humans, transmission usually occurs either through direct contact with contaminated faeces from infected animals or through consumption of contaminated meat from wild birds and other wild animals, such as deer or wild boar. Poultry and poultry products are considered to be at the highest risk of indirect or direct transmission of the pathogen from animals to humans and are considered to be the primary source of *Salmonella* infection in humans as *Salmonella* species are thought to be part of the normal gut or gall bladder microbiota of birds. Contamination of meat usually occurs as a result of improper handling of contaminated organs of these animals during carcass processing (20).

Our results regarding gender and age at *Salmonella* isolation from individuals with diarrhoeal syndrome are compliance with the literature. The group most vulnerable to these diseases remains that of paediatric patients (Fig. 3), where the severity of infection depends on host factors and *Salmonella* serotype. *Salmonella* usually causes self-limiting gastroenteritis manifested by diarrhoea, abdominal pain, nausea, vomiting, and fever, but can occasionally cause serious and potentially life-threatening infection in infants (21–23). An explanation for the high incidence of salmonellosis among young children may be required. On the one hand, there is the greater susceptibility to intestinal infections and the physiological characteristics of the child's organism, and on the other hand, there is the increased consumption of lyophilized and prepared foods, from which salmonellae are frequently isolated. *S. Muenster*, *Banzenheid*, and *Dublin* accounted for the largest relative proportion of isolated *Salmonella* from infectious vectors. Again, the finding that these three zoonoses were most commonly isolated from children in the 4–6 years age group is striking. This high prevalence in the paediatric population is probably due to the annual compulsory prophylactic testing for enteric pathogens of children before their admission into kindergartens.

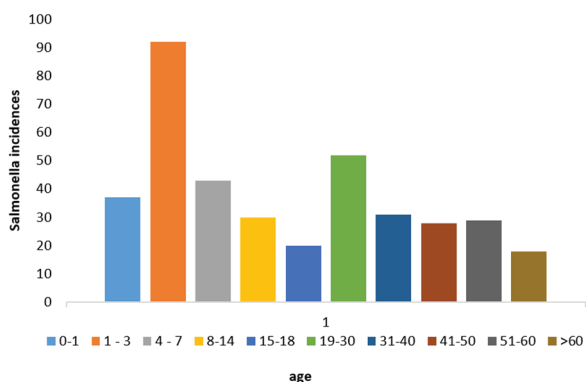


Fig. 3. Dynamics and prevalence of Salmonella in Bulgaria for the 2017–2023 study period according to the age structure of affected persons.

In a study assessing the burden of salmonellosis in different European countries, data on the actual incidence of salmonellosis showed that it ranged from 4/100 000 inhabitants for Norway to 2741/100 000 inhabitants for Bulgaria (24). However, the ways and means of combating these diseases and, above all, their prevention depend primarily on their etiology and the patterns of epidemic processes. Bulgaria, as an EU Member State, complies with and applies all directives on the control of communicable diseases and the control and monitoring of food- and waterborne infections. The results of the action of this complex of factors are illustrated by the modern characteristics of diarrhoeal diseases.

The prevalence of bacterial enterocolitis with *Salmonella* as the etiological agent in the urban population of Bulgaria is predominantly a result of the timely and accessible medical care and the possibility of an etiological diagnosis of enterocolitis in the larger cities of the country, where every proven case of salmonellosis is registered. A realistic picture of the geographic distribution of salmonellosis in the country is lacking and in practice is a complex administrative process. The reason for this is that the reported laboratory-confirmed cases of salmonellosis are registered in the RHIs, but the samples for testing in most private microbiological laboratories come from different administrative regions of the country. This should be interpreted as a weakness in the communicable disease surveillance system.

According to the epidemiological data available to us, the new *Salmonella* serotypes in the country are mostly associated with overseas travel outside the

EU: Egypt, Philippines, Zanzibar, Bali, India, Thailand, Turkey, Maldives, and according to the patients, the diarrhoeal syndrome started during their travel. Globalization, affordable transportation, and the import and export of goods have contributed to both a socioeconomic boom and the wide spread of pathogens on an everyman.

In the years of the COVID-19 pandemic, the isolation and diagnosis of bacterial enteric pathogens were neglected, which led to an elevation in the number of unspecified enteric infections, increasing the use of etiologically inadequate antimicrobial therapy, influencing the spread of new antibiotic resistance profiles, and a presumed increase in infection rates in Bulgaria (Fig. 4). The impact of the COVID-19 pandemic has similarly affected other EU Member States, according to annual analyses by the ECDC (6). Fig. 4 and Table 1 clearly show a mirrored increase in 2023 of registered *Salmonella* cases reaching their 2019 levels. This trend is a clear sign of an increase in *Salmonella* cases in a short period of time.

It is evident from the above-mentioned that *Salmonella* infections are a complex problem, which makes it necessary to know and monitor their etio-

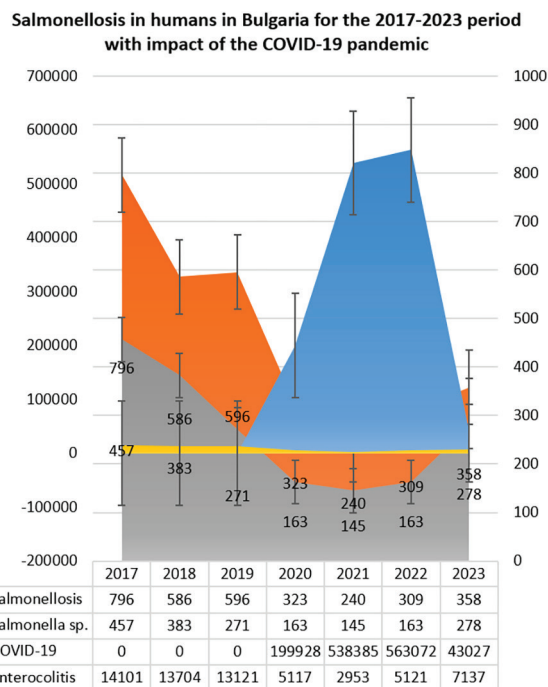


Fig. 4. Infectious enterocolitis, registered salmonellosis and laboratory-confirmed *Salmonella* strains in humans in Bulgaria before and after the COVID-19 pandemic (2017–2023).

logical structure in the fight for their containment. In addition, there is a need of regular implementation of different strategies for the control and prevention of salmonellosis in humans, related to sanitary conditions, testing of persons responsible for food handling, regular surveillance of potential pathogen-carrying animals, and treatment of both carriers and those present symptoms. In animals, all stages of the production system should be regularly tested for *Salmonella* infection, including breeding facilities, vehicles, slaughterhouses, and storage facilities (25). In a study from 2024 about antimicrobial resistance of *Salmonella* isolates in farrow-to-finish pig farms in Bulgaria, the authors report that the highest occurrence was observed in the stage of fattening pigs aged between 121–180 days (16.3%; 20/123), followed by dry sows (6.5%; 4/62), and gilts (4.8%; 12/248). About 75% of the isolated strains belonged to three serotypes: *Salmonella* Infantis (41.9%), *Salmonella* Give (16.3%), and *Salmonella* Typhimurium monophasic (16.3%) (26). Despite the high number of *Salmonella* isolates for the country, recorded outbreaks are decreasing year by year. This circumstance is attributable to several factors. Firstly, there has been a general lack of awareness of diarrhoeal syndrome within the population. Secondly, there has been an absence of timely medical assistance. Thirdly, there has been a pervasive utilisation of a differential microbiological diagnosis that is confined to bacterial species, the *Salmonella* group, and, with decreasing frequency, a limited number of serotypes. All the international *Salmonella* outbreaks presented in Table 2 have not been recorded within the country, despite data on imports of food and animal products identified as sources of the described outbreaks.

CONCLUSION

Nontyphoidal salmonellosis is a socioeconomic and public health problem that requires control of *Salmonella* infections in animals and humans. In the effort to contain them, it is necessary to know in detail and follow in dynamics the etiological structure of salmonellosis and to apply non-antibiotic-based approaches such as prophylaxis with probiotics, bacteriophages, and vaccines among farm animals.

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