

# Prevalence of *Salmonella* spp. in broiler chicken flocks in northern Poland in 2014–2016

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## Abstract

Zoonotic serotypes of *Salmonella enterica* subsp. *enterica* are an important biological factor that poses a threat to public health, and broilers are often asymptomatic carriers of these bacteria. The aim of this study was to analyze the prevalence of *Salmonella* spp. and *Salmonella* serotypes in broiler chicken flocks in northern Poland in 2014–2016. The study was conducted on commercial flocks monitored by the Institutes of Veterinary Hygiene in Gdańsk and Olsztyn in 2014–2016. A total of 4,331 samples were investigated. Identification of *Salmonella* spp. was performed by a culture-dependent method followed by biochemical and serological confirmation tests, in accordance with Polish Standards. The total percentage of infected flocks in northern Poland reached 1.57%. The prevalence of *Salmonella* spp. in broiler chickens decreased from 2.19% in 2014 to 1.22% in 2016. The percentage of flocks infected with *S. Enteritidis* and *S. Typhimurium* was 1.18% and 0.12%, respectively (*S. Enteritidis* and *S. Typhimurium* were detected in 1.29% of flocks). The most common serotypes that had not been covered by the control programme were *S. Mbandaka* (0.14%), *S. Infantis* (0.07%) and, sporadically, *S. Kentucky* and *S. Anatum*. The percentage of infected flocks was higher (by 0.26%) in the Pomeranian Region than in the Region of Warmia and Mazury, and it increased over the analyzed period – the highest number of positive samples were found in 2016 (2.04%). In the Region of Warmia and Mazury, the percentage of infected flocks was highest in 2014 (3.08%); in 2014–2016, the number of positive samples decreased steadily to reach 0.41% in 2016. In the analyzed regions, the incidence of human salmonellosis was correlated with the percentage of infected broiler flocks.

## Key words

*Salmonella*, broiler chicken, northern Poland

## INTRODUCTION

Zoonotic serotypes of *Salmonella enterica* subsp. *enterica* are an important biological factor that poses a serious threat to public health [1, 2]. Research indicates that broilers are often asymptomatic carriers of these bacteria [3]. In Poland, the first programme aimed at eliminating selected *Salmonella* serotypes from chicken farms was introduced 10 years ago (2007), and similar projects were implemented in successive years in laying hens (2008), broiler chickens (2010) and on turkey farms (2010). Despite the above, the reports of the European Food Safety Authority indicate that infections caused by *Salmonella* strains from poultry are still prevalent [4]. Poultry meat is the most common source of these bacteria, and human infections are often caused by cross-contamination or inadequate preparation of contaminated poultry meat intended for thermal processing [1, 4]. According to Radkowski [3], the vast majority of chickens supplied to abattoirs are free of infection, but even a small percentage of *Salmonella* carriers on a farm is capable of cross-contaminating other birds during transport.

According to the most recent Eurostat data [6], Poland is the leading poultry producer in the EU. In 2014, Poland had a 13.9% share of the EU poultry market where broiler chicken

meat is most widely consumed (79.8%). *Salmonella* infections in poultry pose a serious and widespread problem because poultry consumption in Poland increased from 23.4 kg *per capita* in 2005 to 29.2 kg *per capita* in 2016 [7]. Human salmonellosis is most frequently caused by *S. Enteritidis*, *S. Typhimurium*, *S. Infantis* and *S. Derby* [4]. In the EU, the prevalence of human salmonellosis decreased significantly in 2008–2011, but the decreasing trend ended during 2012–2016, and the proportion of human salmonellosis cases remains constant [4]. Salmonellosis is the most ubiquitous zoonosis in Poland, and the number of confirmed human cases has increased in recent years [5]. According to the Institute of Agricultural and Food Economics, poultry consumption has grown steadily and is expected to increase in successive years due to its wide availability and low retail prices [8]. According to EFSA [4], national programmes aiming to reduce *S. Enteritidis* and *S. Typhimurium* infections in broiler chicken flocks in the EU have been successful because the prevalence target of 1% has been met in recent years. However, the notifications submitted to the Rapid Alert System for Food and Feed (RASFF), an effective tool for exchanging information between UE Member States about serious risks detected in relation to food and feed, indicate that *Salmonella* spp. was most frequently identified in poultry products in Poland in 2017 [9].

The aim of this study was to analyze the prevalence of *Salmonella* spp. and *Salmonella* serotypes in commercial flocks of broiler chickens in northern Poland in 2014–2016.

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## MATERIALS AND METHODS

The study was conducted on commercial flocks of broiler chickens monitored by the Institutes of Veterinary Hygiene in Gdańsk and Olsztyn in 2014–2016, with the prior approval of these institutions for the use of laboratory results for scientific purposes. A total of 4,331 samples were investigated, 2,180 from Pomerania and 2,151 from Warmia and Mazury. Faecal samples for analyses were collected in accordance with the Instruction issued by the Chief Veterinary Officer [10]. Identification of *Salmonella* spp. on a weight basis/with surface faecal sampling swabs was performed by a culture-dependent method followed by biochemical and serological confirmation tests, in accordance with the *Salmonella* spp. identification protocol (Edition 1 of 3 January 2014) and Polish Standards PN-EN ISO 6579:2003+A1:2007+AC:2014-11 and PN-EN ISO 6579:2003/AC:2014-11P [11]. The average percentage of samples collected and analyzed under the national control programme carried out by the General Veterinary Inspectorate was 5% in the Region of Pomerania and 7% in the Region of Warmia and Mazury. The remaining samples were collected by local veterinarians.

Samples were pre-enriched in buffered peptone water with a temperature of 18–27°C, and incubated at 37°C ( $\pm 1^\circ\text{C}$ ) for 18 hours ( $\pm 2$  hours). Selective proliferation of *Salmonella* was carried out using the modified semi-solid Rappaport Vassiliadis (MSRV) medium (Oxoid CM1112B). The plates were incubated at a temperature of 41.5°C ( $\pm 1^\circ\text{C}$ ) for 24 hours ( $\pm 3$  hours). When negative results were noted, incubation was continued for another 24 hours ( $\pm 3$  hours). *Salmonella* colonies representing a normal growth pattern were transferred onto selective XLD (Oxoid CM0469B) and BGA (Oxoid CM0329B) media. Incubation was carried out at a temperature of 37°C ( $\pm 1^\circ\text{C}$ ), and the results were read after 24 hours ( $\pm 3$  hours).

In the next step, biochemical confirmation tests were performed (PN-EN ISO-6579:2003, item 9.5.3) and *Salmonella* isolates were identified and serotyped by slide agglutination (PN-EN ISO-6579:2003, item 9.5.4) according to the White-Kauffmann-Le Minor scheme [12]. At least one typical or suspect colony was selected from each agar plate with a selective medium. Selected individual colonies were cultured on nutrient agar and soft agar plates. Pure cultures of *Salmonella* spp. were used in biochemical confirmation tests. The plates and cultures were incubated for 24 hours ( $\pm 3$  hours) at a temperature of 37°C ( $\pm 1^\circ\text{C}$ ), and serological identification of *Salmonella* isolates was performed.

The results of the analyses are shown in Table 1, including the total number of analyzed samples and the number of samples positive for *S. Enteritidis* and *S. Typhimurium*, and the remaining *Salmonella* serotypes. The percentage of infected broiler flocks in each month of 2014–2016, and the most common *Salmonella* serotypes identified in the analyzed regions, are presented graphically in Figures 1–3.

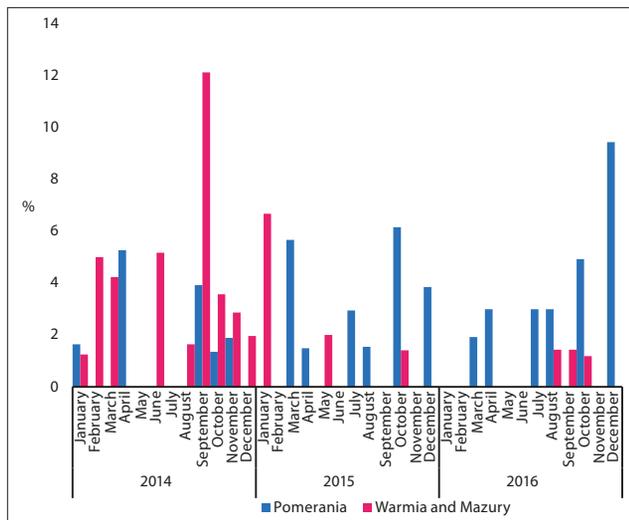
## RESULTS

Table 1 presents the results of microbiological analyses for the presence of *Salmonella* spp. in broiler chicken flocks monitored by the Institutes of Veterinary Hygiene in Gdańsk and Olsztyn in 2014–2016. In the analyzed period, a total of 4,331 samples were collected in northern Poland, and positive results were noted in 68 cases (1.57%). The prevalence of *Salmonella* spp. in broiler chickens decreased from 2.19% in 2014 to 1.1% in 2015 and 1.22% in 2016. The percentage of flocks infected with *S. Enteritidis* and *S. Typhimurium* was 1.18% and 0.12%, respectively (*S. Enteritidis* and *S. Typhimurium* were detected in 1.29% of the flocks). The most common serotypes that had not been covered by the control programme were *S. Mbandaka* (0.14%), *S. Infantis* (0.07%) and, sporadically (0.02%), *S. Kentucky*, *S. Anatum* and an unidentified *Salmonella* serotype. The percentage of infected flocks was higher (by 0.26%) in the Region of Pomerania than in the Region of Warmia and Mazury, and increased over the analyzed period – the highest number of positive samples were found in 2016 (2.04%). In the Region of Warmia and Mazury, the percentage of infected flocks was highest in 2014 (3.08%); in 2014–2016, the number of positive samples decreased steadily to reach 0.41% in 2016.

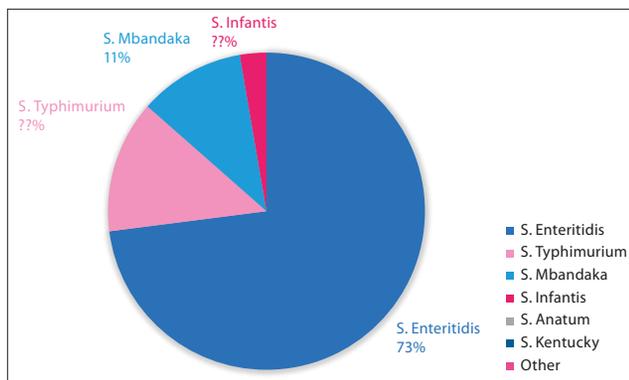
As shown in Figure 1 which illustrates the percentage of broiler flocks infected with *Salmonella* spp. in northern Poland in each month of 2014–2016, the percentage of positive samples was highest in September 2014 in the Region of Warmia and Mazury (12.1%) and in December 2016 in the Region of Pomerania (9.4%). In broiler flocks monitored by the Institute of Veterinary Hygiene in Olsztyn, *Salmonella* spp. were detected in 9 months in 2014, and in only 3 months in 2015 and 2016. In broiler flocks monitored by the Institute of Veterinary Hygiene in Gdańsk, positive samples were more evenly distributed across the months and years (5 months in 2014, and 6 months in 2015 and 2016).

**Table 1.** Prevalence of *Salmonella* spp. and *Salmonella* serotypes in broiler chicken flocks in northern Poland in 2014–2016

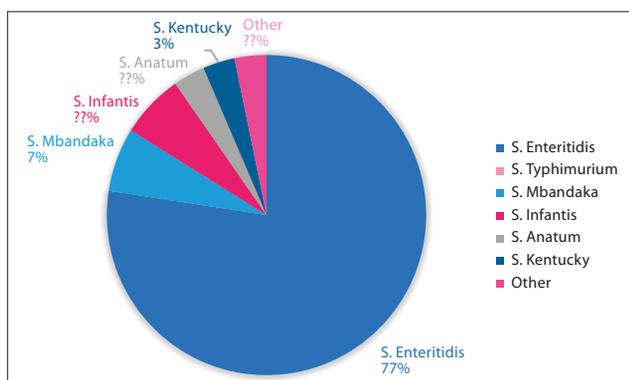
Specification	Pomerania				Warmia and Mazury				Total
	2014	2015	2016	2014–16	2014	2015	2016	2014–16	
<b>Number of analyzed samples</b>	<b>704</b>	<b>742</b>	<b>734</b>	<b>2180</b>	<b>714</b>	<b>704</b>	<b>733</b>	<b>2151</b>	<b>4331</b>
Number of positive samples	9	13	15	37	22	6	3	31	68
<b>Percentage of infected flocks (%)</b>	<b>1.278</b>	<b>1.752</b>	<b>2.044</b>	<b>1.697</b>	<b>3.081</b>	<b>0.852</b>	<b>0.409</b>	<b>1.441</b>	<b>1.570</b>
<i>S. Enteritidis</i>	8	10	9	27	17	5	2	24	51
<i>S. Typhimurium</i>	-	2	3	5	-	-	-	-	5
<i>S. Anatum</i>	-	-	-	-	-	-	1	1	1
<i>S. Kentucky</i>	-	-	-	-	-	1	-	1	1
<i>S. Infantis</i>	-	-	1	1	2	-	-	2	3
<i>S. Mbandaka</i>	1	1	2	4	2	-	-	2	6
Other ( <i>S. sp.</i> )	-	-	-	-	1	-	-	1	1



**Figure 1.** Percentage of broiler chicken flocks infected with *Salmonella* spp. in northern Poland in each month of 2014–2016



**Figure 2.** *Salmonella* serotypes identified in broiler chicken flocks in the Region of Pomerania in 2014–2016



**Figure 3.** *Salmonella* serotypes identified in broiler chicken flocks in the Region of Warmia and Mazury in 2014–2016

A higher number of different *Salmonella* serotypes were identified in the Region of Warmia and Mazury. *Salmonella* Typhimurium was not detected in the Region of Warmia and Mazury, whereas in the Region of Pomerania, this serotype accounted for 13% of *Salmonella*-positive samples. In both regions, *S. Enteritidis* was the most common serotype in broiler flocks, accounting for 77% in Warmia and Mazury and for 73% in Pomerania (Fig. 2–3).

## DISCUSSION

According to EU [4, 13, 14] and domestic reports [5], *Salmonella* spp. are among the most ubiquitous pathogens that pose a serious risk for public health. At the primary production level, the prevalence *Salmonella* spp. in poultry flocks, including broiler chickens and turkeys, decreased or stabilized across the EU in 2016, compared with previous years, but the decreasing trend in laying hen flocks since the introduction of national control programmes in 2008 was reversed, and a significant increase in *Salmonella* detections was noted in 2015–2016 [4]. The results of the presented study (Tab. 1; Fig. 1) indicate considerable variations in the number of *Salmonella* detections across regions and years. The percentage of infected broiler chicken flocks generally decreased in northern Poland in 2014–2016. In the Region of Warmia and Mazury, the proportion of positive samples from broiler chicken farms decreased by 2.7% in 2014–2016. In the Region of Pomerania, the counts of identified *Salmonella* bacteria in broiler farms increased by 0.8% between 2014 and 2016. This could be related to the seasonality of these bacteria and the differences between meteorological parameters in particular regions and years. *Salmonella* Enteritidis and *Salmonella* Typhimurium especially show strong associations with the weather conditions, for instance, high temperature [15]. On the other hand, the prevalence of *Salmonella* (Fig. 1) in flocks in Pomerania was the highest in December (2016), October (2015) and April (2014), whereas in Warmia and Mazury in September (2014) and January (2015). It should be underlined that the microclimate conditions in poultry buildings are maintained at optimal level throughout the whole year, therefore the dependence on outside temperature may be lower in the case of poultry flocks. Seasonality may be explained by a mixture of factors including, *inter alia*, climate, social, behavioural, agricultural, environmental and stochastic changes in immune populations [15]. The source of infections of *Salmonella* in poultry could be anything from a broad range of wild animals, including rats and mice [16]. In the autumn and winter seasons, rodents seek shelter in buildings, which may be the reason for the seasonality observed in the current study. Specialist chicken farms are usually closed units with a high level of bio-security. However, the occurrence of wild rodents may be difficult to exclude completely. Therefore, rodent control should be considered an important measure to provide good bio-security on farms. [16].

In the EU [4], where the prevalence of *Salmonella* in broiler flocks reached 2.6% in 2016 and was considerably higher than in this study (1.6% on average), the prevalence increased by 0.4% in comparison with 2015 and decreased by 0.8% relative to 2014. In Poland, the percentage of infected broiler chicken flocks has been decreasing steadily [17]. A study conducted in 2005–2006 (two years before the introduction of control programmes) revealed that 58.7% of broiler flocks were infected with *Salmonella* spp. [18].

Commission Regulation (EU) No. 200/2012 concerning a Union target for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of broiler chickens, as provided for in Regulation (EC) No. 2160/2003 of the European Parliament and of the Council [19], sets the target for the reduction of the maximum percentage of broiler flocks remaining positive for *Salmonella* Enteritidis and *Salmonella* Typhimurium, including monophasic *Salmonella*

Typhimurium strains with the antigenic formula 1, 4, [5], 12:i:-, to 1% or less. This target has been achieved, and the prevalence of the above serotypes in Polish broiler flocks decreased between 2010 (*S. Enteritidis* and *S. Typhimurium* – 0.72%) and 2014 (0.15%). The prevalence of *S. Enteritidis* and *S. Typhimurium* serotypes increased to 0.23% in 2015 [17], and it decreased to 0.1% in 2016 (the prevalence of *S. Typhimurium* was below 0.01% in 2014–2016). In the EU, the prevalence of *S. Enteritidis* and *S. Typhimurium* serotypes also increased from 0.2% in 2014 to 0.26% in 2015, and it reached 0.21% in 2016 [4, 13, 14]. The results obtained in the current study (Tab. 1) indicate that the reduction target has not yet been achieved in northern Poland, but the combined prevalence of *S. Enteritidis* and *S. Typhimurium* (1.29%) in poultry flocks was only somewhat higher than the recommended value. In the Region of Pomerania, the proportion of *S. Enteritidis* positive samples increased from 1.14% in 2014 to 1.23% in 2016, whereas the proportion of *S. Typhimurium* positive samples, which had not been detected in the region in 2014, was determined at 0.23% in 2015–2016. *Salmonella Typhimurium* was not detected in the Region of Warmia and Mazury in the discussed period, and the prevalence of *S. Enteritidis* decreased substantially from 2.38% in 2014 to 0.27% in 2016. The number of *S. Enteritidis* positive samples in broiler chicken flocks decreased manifold in Warmia and Mazury relative to 2001–2005 [20].

The aim of the control programme for broiler chicken flocks is to stabilize or decrease the prevalence of infections caused by the indicated *Salmonella* strains [17]. This is a very important consideration because the prevalence of foodborne diseases caused by *Salmonella enterica* serotype *Enteritidis* has increased significantly in the EU since 2006. This serotype is most frequently isolated from broiler chicken and laying hen meat [21]. Similarly to previous years, in 2016, the highest contamination with *Salmonella enterica* serotype *Enteritidis* was reported in poultry, in particular broiler chicken meat, intended for consumption after thermal processing. In contrast, the proportion of positive samples was negligent in fresh poultry meat. In 2016, the prevalence of *S. Enteritidis* also increased significantly in laying hens in the EU, and the relevant target has not been met. Poultry meat is often a source of *S. Typhimurium* (26% in the EU in 2016) [4].

In Europe, *Salmonella Enteritidis* and *S. Typhimurium* have been the most common sources of human infections for many years. These serovars are responsible for the vast majority of human salmonellosis cases in Europe (70–80% of infections caused by identified serotypes of *Salmonella*) [22]. In Poland, the proportion of *S. Enteritidis* serotypes that are most pathogenic for humans before the introduction of control programmes (in 2005/2006) was determined at 55.6%, and the proportion of ST at 4.7% [18]. According to Sadkowska-Todys and Czarkowski [23], in 2014, nearly 76% of human salmonellosis cases in Poland were caused by the *S. Enteritidis* serotype. The above authors also noted that in 2014, the number of salmonellosis cases had increased in Poland for the first time in many years. The epidemiological reports of the National Institute of Public Health – National Institute of Hygiene [24] revealed that the above trend stabilized in 2015–2016. An increase in the number of human salmonellosis cases was also observed in the Region of Pomerania where the disease incidence (number of new cases per 100,000 population) was determined at 21.6 in 2014,

23.4 in 2015 and 35.3 in 2016 [24]. In the presented study, the counts of identified *Salmonella* bacteria in broiler flocks also increased in the analyzed period (Tab. 1; Fig. 1). A similar correlation was noted between the prevalence of human salmonellosis and the number of *Salmonella* detections in broiler flocks in the Region of Warmia and Mazury (Tab. 1; Fig. 1). In the above region, the incidence of salmonellosis decreased from 25.7 in 2014 to 23.9 in 2015 and 19.5 in 2016. Human salmonellosis in Poland is the most common zoonosis, whereas in the EU *Campylobacter* infections occur most frequently (in recent years about 60%) [13, 14] and over the last 10 years the incidence and prevalence of campylobacteriosis have increased in both the developed and developing countries of the world [25]. In Poland, the number of *Campylobacter* infections is significantly lower in comparison with *Salmonella* cases. In recent years (2014–2016), the prevalence of human salmonellosis was over 90% higher than campylobacteriosis [24, 13, 14]. According to epidemiological reports [24], the prevalence of human campylobacteriosis in the Region of Pomerania in 2014–2016 were significantly lower (77–90%) than in the Region of Warmia and Mazury. Poultry is a major reservoir and source of transmission of campylobacteriosis to humans [25]. It is possible that the eradication of *Salmonella* rods from the chicken houses may promote the *Campylobacter* bacteria to colonize the emerging niche.

Similar to Europe, *S. Enteritidis* was the most common serotype in the analyzed regions of northern Poland [4, 13, 14, 22, 23], and the percentage of detections reached 77% in the Region of Warmia and Mazury and 73% in the Region of Pomerania (Fig. 2–3). *Salmonella Typhimurium* was not identified in Warmia and Mazury in 2014–2016, but it was detected in the Region of Pomerania. In recent years, *S. Mbandaka* and *S. Infantis* were identified in broiler flocks in both regions. These bacteria are less pathogenic, and they are not covered by the control programme, despite the fact *S. Infantis* is the third most common cause of human salmonellosis in Europe after *S. Enteritidis* and *S. Typhimurium* serovars. It should be noted that these bacteria are most frequently identified in broiler chicken flocks and broiler chicken meat. According to experts, *S. Infantis* could pose a growing threat to public health in the future due to high levels of drug resistance [4, 26]. Rašeta et al. [27] isolated identical strains of *S. Infantis* from broiler carcasses and human salmonellosis patients. Single cases of *S. Kentucky* and *S. Anatum* were also reported in broiler flocks in the Region of Warmia and Mazury (Fig. 3). These findings should not be underestimated because *Salmonella enterica* serotype *Kentucky* has caused food poisoning in the EU in recent years [4]. Le Hello et al. [28] observed that the number of human infections with the multidrug-resistant *S. Kentucky* has increased significantly in many European countries and in the United States in recent years. In a study by Mąka et al. [29], selected *Salmonella* serotypes isolated from poultry, including *S. Infantis* and *S. Typhimurium*, were less prevalent, but were more frequently characterized by multidrug resistance than *S. Enteritidis*. In view of the above, according to some authors [28], serovars that are less virulent, but pathogenic and capable of colonizing poultry should be regularly monitored and controlled to minimize the risk for public health. To minimize this risk the effective bio-security measures should be implemented and developed incessantly on chicken farms.

## CONCLUSIONS

*Salmonella* infections continue to affect broiler chickens despite the efforts made by international and domestic authorities and considerable improvement in the epidemiological status of poultry flocks after the implementation of *Salmonella* control programmes. In recent years, the prevalence of *Salmonella* has generally decreased in northern Poland, but considerable differences were observed between locations and years. The number of infected broiler chicken flocks has increased in the Region of Pomerania and decreased in the Region of Warmia and Mazury, and these observations are correlated with the incidence of human salmonellosis in these regions.

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