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DISTRIBUTION AND SEROLOGICAL TYPING OF *SALMONELLA* SPP. ISOLATES FROM BROILER CARCASSES IN BULGARIA

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Summary

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The studies were performed on samples of broiler carcasses from 13 different Bulgarian poultry slaughterhouses in 2008. Neck skin samples from 327 chilled broilers were examined for presence of *Salmonella* spp. All isolates were serologically typed. Positive samples were 86/327 or 26.29% of all studied broiler carcasses. Contaminated samples originated more frequently from South Bulgaria as compared to North Bulgaria (28.45% and 25.12% respectively). The most commonly encountered *Salmonella* serovars were *S.* Montevideo – 22; *S.* Enteritidis – 18; *S.* Infantis – 18; *S.* Virchow – 5; *S.* Menden – 4 isolates. Our studies showed that more than ¼ (26.29%) of all examined chilled broiler carcasses were contaminated with *Salmonella* spp. that was much more than the overall proportion of positive samples in fresh broiler meat at EU level (5.5% in 2007).

Key words: broiler carcasses, distribution, *Salmonella* spp.

INTRODUCTION

According to The Community Summary Report (Anonymous, 2010) *Salmonella* was most often found in fresh poultry meat. Most reported data on *Salmonella* in broiler meat at EU level indicated an overall proportion of 5.5% positive samples in fresh broiler meat at processing level, varying between 0% and 55.6% in different countries.

Many authors (Bailey & Maurer, 2001; Gray & Fedorka-Gray, 2002; Mølbak *et al.*, 2006) have outlined that bacteria of genus *Salmonella* are important causes of foodborne infections in humans, and the most frequent etiological bacterial agents of foodborne disease outbreaks. In particular, two *Salmonella* serotypes, *S.* Enteritidis and *S.* Typhimurium became major causes of human illness in the 1980s and 1990s, with important impact on public health and the economy in industrialized countries.

In Bulgaria, Kaloyanov *et al.* (1987) published data about the distribution and diversity of *Salmonella* spp. in poultry. Studies of Rusul *et al.* (1996) in Malaysia showed that 50% of chicken carcasses at the market and 35.5% at poultry slaughtering plants were contaminated. In Belgium, during the period 1993–1996, Uyttendaele *et al.* (1999) provided evidence of increased contamination percentages

from 19.4% in 1993 to 36.7% in 1996. The most commonly isolated serovar was *S.* Enteritidis. Capita *et al.* (2003) established that in Spain, the level of contamination of broiler carcasses was 49%. According to the observations of Harrison *et al.* (2001) and Meldrum *et al.* (2002) in Wales, UK, the contamination with *Salmonella* of fresh poultry meat ranged between 8% and 29%.

Elgroud *et al.* (2009) noted that *Salmonella* contamination was present in 37% of the broiler farms and 53% of the slaughterhouses in Constantine, Algeria. Having examined 400 whole chickens in 1999 in Poland, Mikołajchyk and Radkowski (2002) reported a relatively high percentage of *Salmonella*-positive results: 95 cases out of 400 or 23.75%. Kanashiro *et al.* (2005) reported a high incidence of *S. enterica* subsp. *enterica* serovar Enteritidis in breeders (57.5%) and broilers flocks (84.0%) in Brasil. The importance of these findings lies in the fact that *S.* Enteritidis has become the most frequent serovar responsible for foodborne outbreaks and sporadic cases of salmonellosis in humans.

The aim of the present study was to survey the distribution of *Salmonella* serovars in broiler carcasses originating from 13 slaughterhouses in Bulgaria in 2008.

MATERIALS AND METHODS

Collection and transport of broiler samples

From January to December 2008, 327 broiler carcasses were collected from 13 slaughterhouses (10 from North Bulgaria and 3 from South of Bulgaria). One whole carcass per slaughter batch was obtained and placed in a separate sterile plastic bag (Merck), immediately after chilling, avoiding cross-contamination and transported to the laboratory. During transportation, the samples were kept in cool boxes at +2 to +8 °C, free of external contamination. In most instances, samples reached the laboratory within 24 h of sampling.

Sample preparation

All samples received were examined to ensure that the transport packaging was intact before testing. With disposable gloves, the carcass were removed from the sample bag, taking care not to contaminate its outer surface. Using a sterile instrument and aseptic technique, the neck skin was removed, if present, together with the skin from one side of the carcass avoiding any fat to make a 25 g test portion that was placed into a stomacher bag.

The 25 g test portion was transferred to nine volumes (225 mL) buffered peptone water (BPW) (Merck, Darmstadt, Germany), brought to room temperature before adding. The mixture was treated in a stomacher (Stomacher® 400 Circulator, England) for approximately one minute. Foaming was avoided by removing the air from the stomacher bag.

Detection and identification methods for Salmonella spp.

The detection of *Salmonella* spp. was done according to ISO 6579-2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Anonymous, 2002). BPW dilutions were incubated at 37±1 ^{o}C for 18 ± 2 h and 1.0 mL and 0.1 mL of the cultivated BPW were added to 10 mL Tetrathionate Broth acc. to Muller-Kauffmann (MKTTn) and Rappaport-Vassiliadis broth (RV broth) (Merck),

respectively. After MKTTn and RV broth incubation for 24±3 h at 37±1 °C and 41.5±1 °C, respectively, broth cultures were streaked onto xylose lysine deoxycholate agar (XLD agar) (Merck) and brilliant-green phenol-red lactose sucrose agar (BPLS agar) (Merck). The samples were incubated at 37±1 °C for 24±3 h. Polymicrotest (BB – NCIPD Ltd. Sofia, Bulgaria) was used for rapid biochemical identification .

Serotyping of Salmonella spp..

Suspicious *Salmonella* colonies were typed by our National Reference Laboratory for Salmonella, using the Kauffmann-White Scheme (Bale *et al.*, 2007). Serotyping of all isolates was done with Salmonella O and H antisera (SIFIN, GmbH, Berlin, Germany; BB – NCIPD Ltd., Sofia, Bulgaria). For quality assurance, eleven nontypeable isolates were sent to the Community Reference Laboratory for *Salmonella* in Bilthoven, Netherlands.

RESULTS

Results of all 327 specimens of broiler carcasses, representing the same number of slaughter batches showed that 86 of them (26.29%) were positive for *Salmonella* spp. (Table 1).

Data for distribution of *Salmonella* spp. showed that 28.45% of all examined samples from South Bulgaria were positive. This percentage was higher as compared to North Bulgaria where 25.12% positive specimens were detected.

Table 1. Seasonal changes of *Salmonella* spp. isolation in different slaughterhouses in 2008

Plant No	Total samples/positive samples					% positive
	Winter	Spring	Summer	Autumn	Total	
North Bulgaria						
1	6/0	6/1	6/3	6/1	24/5	20.80%
2	–	6/2	3/0	3/0	12/2	16.65%
3	6/0	6/0	6/1	6/0	24/1	4.20%
4	–	3/2	2/1	4/4	9/7	77.70%
5	12/6	12/2	12/5	12/1	48/14	29.10%
6	–	5/0	9/1	7/3	21/4	19.00%
7	–	3/0	4/0	3/0	10/0	–
8	3/0	9/3	–	3/2	15/5	33.30%
9	–	6/0	3/1	3/0	12/1	8.30%
10	8/6	10/5	9/1	9/1	36/13	36.10%
South Bulgaria						
1	6/0	6/1	4/1	6/1	22/3	13.60%
2	–	6/5	2/1	2/0	10/6	60.00%
3	21/0	21/6	21/16	21/3	84/25	29.80%
Total samples/ positive samples	62/12	99/26	81/30	85/16	327/86	26.29%
% positive	20.0%	26.3%	37.0%	18.8%		

Table 2. *Salmonella* serovars, isolated from broiler carcasses of North and South Bulgaria

Salmonella serovars	Number of strains from slaughterhouses in:		Total number of strains
	North Bulgaria	South Bulgaria	
S. Enteritidis	13	5	18
S. Typhimurium	1	–	1
S. Infantis	18	–	18
S. Virchow	5	–	5
S. Newport	1	–	1
S. Montevideo	–	22	22
S. Give	1	–	1
S. Concord	1	–	1
S. Thompson	1	2	3
S. Irumu	1	–	1
S. Menden	4	–	4
S. Tennessee	2	1	3
S. Parkroyal	1	–	1
S. Kottbus	2	1	3
S. Bonariensis	–	1	1
S. Mbandaka	1	1	2
S. Corvalis	1	–	1
Total isolates	53	33	86
Number of *Salmonella* serovars	15	7	17

Data for different producers/slaughterhouses were extremely heterogeneous with regard to the presence of *Salmonella* spp. in examined samples/batches. They are presented in Table 1.

Data showed a clear seasonal dynamics of *Salmonella* positive samples beginning with the winter period (January-March, 2008) with 20%, increasing to 26.3% in spring time (April-June, 2008), achieving the highest level – 37% in summer time (July-September, 2008) and decreasing to 18.8% in autumn (October-December, 2008). In samples, originating from different plants, *Salmonella* spp. positive broiler carcasses ranged from 0% to 77.7%. Every studied broiler carcass represented a particular slaughter batch, and therefore, for some producers ¾ of slaughter batches were contaminated. Our studies have shown no relationship between the type of chilling and level of contamination. In fact, the plant where *Salmonella* spp. were most frequently isolated, used spray chilling of carcasses, and the slaughterhouse without any *Salmonella* isolate applied immersion cooling. Most of slaughterhouses processed broiler batches from private producers and different *Salmonella* serovars were isolated and some of plants had their own production of broilers. In this case we found a small number of different *Salmonella* serovars (1 or 2, maximum 3 for the whole period of study).

Serological typing of all 86 suspected *Salmonella* spp. isolates established 17 different *Salmonella* serovars (Table 2).

In North Bulgaria 15 serovars were found with predominance of were *S.* Infantis – 18, *S.* Enteritidis – 13, and *S.* Virchow – 5 strains. These were among the 5 most frequent *Salmonella* serovars, having provoked human salmonellosis in

2007 (*S.* Enteritidis, *S.* Infantis, *S.* Virchow, *S.* Typhimurium and *S.* Newport). In South Bulgaria, prevailing serovars were *S.* Montevideo – 22 and *S.* Enteritidis – 5 strains. The other frequently isolated serovars (*S.* Montevideo, *S.* Menden, *S.* Thompson, *S.* Tennessee and *S.* Kottbus) rarely related to human illness.

DISCUSSION

Results of our one-year study on distribution of *Salmonella* in broiler carcasses from 13 slaughterhouses in Bulgaria showed an average level of contamination 26.29%. Compared to the data for the different EU member states (from 0% to 55.6%; average 5.5%), the observed prevalence was significantly more than the average one for broiler meat at the level of slaughterhouses (Anonymous, 2010).

Studies made in developing countries as Algeria by Elgroud *et al.* (2009) reported only 55 isolates of 10 serotypes from 2490 samples or 2.2% positive of all examined specimens. In our studies the prevalence was more the 10 times higher. *Salmonella* contamination concerned 53% of the slaughterhouses in Algeria, in our case in 12 of 13 plants.

Data from some countries in Africa as Senegal and Morocco reported by Cardinale *et al.* (2003) and Abdellah *et al.* (2008) showed a high prevalence of *Salmonella*. In Senegal, in 300 chicken carcasses 96 specimens (32%) were positive. The most prevalent *Salmonella* serovars were *S.* Hadar (41.6%) and *S.* Brancaster (20.8%). In Morocco, 20.83% of the popular market samples, and 16.66% of the traditional slaughterhouses samples were positive for one or more *Salmonella*. Out of the total 57 *Salmonella* isolates, 4 different serotypes were identified of which *S.* Typhimurium (40.35%) was the most frequent followed by *S.* Newport (26.31%), *S.* Montevideo (17.54 %) and *S.* Heidelberg (15.78%). Our results are very close to these data only for *S.* Montevideo, but we didn't isolate any of other reported serovars. In fact, data from Morocco show a high prevalence of *S.* Montevideo, dominating among our serovars.

In Thailand, Boonmar *et al.* (1998) found 20 positive specimens in 200 chicken meat samples from one slaughterhouse for export. In Brasil, Tirolli & da Costa (2006) have shown 50% *Salmonella* positive samples and a high number (11) different serotypes in the city of Manaus, Amazonas – Brazil. Ozbey & Ertas (2006) in Turkey detected 12% positive chicken carcasses in the Elagiz county, Turkey.

In Poland, Mikoajczyk & Radkowski (2002) reported 13% (13 of 100) positive samples after cooling process of broiler carcasses. Our data are two times higher after this processing step. The same authors noted the following serological variants of *Salmonella* spp. isolated from whole chickens – *S.* Enteritidis, *S.* Typhimurium, *S.* Saintpaul, *S.* Agona, and *S.* Infantis. As per these results, *S.* Enteritidis was the dominant serological type in infections of slaughter chickens, as in many countries. Our observations are very similar with regard to *S.* Enteretidis and *S.* Infantis, which dominated between our isolates, but we didn't found *S.* Saintpaul and *S.* Agona.

In Lithuania, Ruzauskas *et al.* (2005) examined 43 350 poultry samples and found 409 *Salmonella* isolates or 0.9% from all specimens. The most prevalent *Salmonella* serotype was *S.* Enteritidis – 294 strains out of 409. In a survey carried out to establish baseline figures for

the contamination with *Salmonella* of raw retail chicken available within Wales, Meldrum *et al.* (2004) have obtained 739 samples between November 2001 and December 2002 and 8% of them were contaminated with *Salmonella*. These authors did not observe a seasonal pattern of *Salmonella* contamination in contrast to our results that showed a clear pick in the hottest period of year (summer) and lowest level in winter and autumn 2008.

Some authors from developed EU countries as Belgium and Spain (Uyttendaele *et al.*, 1999; Capita *et al.*, 2007) reported high rate of contamination of broiler carcasses with *Salmonella* up to 36.5% and 17.9%. Uyttendaele *et al.* (1999) noted that poultry products derived from broiler chickens running free in pine woods until slaughtering age (12 to 13 weeks) had a significantly ($P<0.05$) lower contamination rate of *Salmonella* than poultry products from enclosed broilers slaughtered at the age of 6 to 8 weeks.

Numerous authors (Boonmar *et al.*, 1998, Mikoajczyk & Radkowski, 2002; Ruzauskas *et al.*, 2005; Anonymous, 2010) have emphasized the importance of *S.* Enteritidis for the broiler meat. Our data showed that *S.* Enteritidis was the second most commonly isolated *Salmonella* serotype. The most frequently isolated serovar *S.* Montevideo was found only in samples from one slaughterhouse, receiving broilers from one and the same broiler farm.

In USA, the importance of *S.* Enteritidis in broiler production has increased from 1998 to 2007 (Anonymous, 2009). *S.* Montevideo is also reported as an important food-borne pathogen in broiler production in this period. The maximum contamination level was reported in 2000 – 4.2% from all *Salmonella* isolates from broiler carcasses.

Our results showed the significance of another *Salmonella* serovar – *S.* Infantis. The frequency of isolation was the same, as with *S.* Enteritidis. This serotype was isolated from samples of 8 plants.

In conclusion, the data of our study provided evidence for a high prevalence of *Salmonella* (26.29%) in chilled broiler carcasses in Bulgaria. In the different slaughterhouses, the level of contamination with *Salmonella* varied from 0% to 77.7%. A marked seasonal dynamics of *Salmonella* occurrence in examined samples was established. The most prevalent *Salmonella* serovars isolated from broiler carcasses were *S.* Montevideo, *S.* Enteritidis, *S.* Infantis and *S.* Virchow. Some of them (*S.* Enteritidis, *S.* Infantis and *S.* Virchow) are in the top ten of *Salmonella* serovars, said to provoke human salmonellosis (Anonymous, 2010).

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REFERENCES

Abdellah Ch., R. F. Fouzia, Ch. Abdelkader, S. B. Rachida & Z. Mouloud, 2008. Occurrence of *Salmonella* in chicken carcasses and giblets in Meknès – Morocco. *Pakistan Journal of Nutrition*, **7**, 231–233.

Anonymous, 2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. ISO 6579:2002.

Anonymous, 2009. United States Department of Agriculture, Food Safety and Inspection

Service. Serotypes Profile of *Salmonella* Isolates from Meat and Poultry Products, January 1998 through December 2007,http://www.fsis.usda.gov/PDF/Serotypes_Profile_Salmonella_Tables_&_Figures.pdf (5 July 2010 date last accessed).

Anonymous, 2010. Community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA Journal*, **8**,1496.

Bailey, J. S. & J. J. Maurer, 2001. *Salmonella* Species. In: *Food Microbiology: Fundamentals and Frontiers*, 2nd en, eds Doyle, M. P., L. R. Beuchat & T. J. Montville, ASM Press, Washington D.C., pp. 141–178.

Bale, J. A., E. de Pinna, E. J. Threlfall & L. R. Ward, 2007. Kauffmann-White Scheme - 2007: *Salmonella* Identification; Serotypes and Antigen Formulae. Centre for Infections, Health Protection Agency, UK.

Boonmar, S. A. Bangtrakulnonth, S. Pornrunangwong, N. Marnrim, K. Kaneko & M. Ogawa, 1998. *Salmonella* in broiler chickens in Thailand with special reference to contamination of retail meat with *Salmonella enteritidis*. *Journal of Veterinary Medical Science*, **60**, 1233–1236.

Capita, R., C. Alonso-Calleja & M. Prieto, 2007. Prevalence of *Salmonella enterica* serovars and genovars from chicken carcasses in slaughterhouses in Spain. *Journal of Applied Microbiology*, **103**, 1366–1375.

Capita, R., M. Alvarez-Astorga, C. Alonso-Calleja, B. Moreno & M. del Camino Garcia-Fernandez, 2003. Occurrence of *Salmonella* in retail chicken carcasses in Spain. *International Journal of Food Microbiology*, **81**, 169–173.

Cardinale, E., J. D. Perrier Gros-Claude, F. Tall, M. Cissé, E. F. Guèye & G. Salvat, 2003. Prevalence of *Salmonella* and *Campylobacter* in retail chicken carcasses in Senegal. *Révue d'élevage et de médecine vétérinaire des pays tropicaux*, **56**, 13–16.

Elgroud, R., F. Zerdoumi, M. Benazzouz, C. Bouzitouna-Bentchouala, S. A. Granier, S. Frémy, A. Brisabois, B. Dufour & Y. Millemann, 2009. Characteristics of *Salmonella* contamination of broilers and slaughterhouses in the region of Constantine (Algeria). *Zoonoses and Public Health*, **56**, 84–93.

Gray, J. T. & P. J. Fedorka-Gray, 2002. *Salmonella*. In: *Foodborne Diseases*. 2nd edn, eds Cliver, D. O. & H. P. Riemann, Academic Press, pp. 55–68.

Harrison, W. A., C. J. Griffith, D. Tennant & A. C. Peters, 2001. Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. *Letters in Applied Microbiology*, **33**, 450–454.

Kaloyanov, I., I. Slavkov & B. Likov, 1987. Diversity of *Salmonella* spp. isolated from mammals, poultry, feeds and environment during the period 1976–1980. *Veterinary Science (Sofia)*, **24**, 44–51 (BG).

Kanashiro, A. M. I., G. F. Z. Stoppa, A. L. S. P. Cardoso, E. N. C. Tessari & A. G. M. Castro, 2005. Serovars of *Salmonella* spp. isolated from broiler chickens and commercial breeders in diverse regions in Brazil from July 1997 to December 2004. *Revista Brasileira de Ciência Avícola*, **7**, 195–198.

Meldrum, R. J., D. Tucker & C. Edwards, 2004. Baseline rates of *Campylobacter* and *Salmonella* in raw chicken in Wales, United Kingdom, in 2002. *Journal of Food Protection*, **67**, 1226–1228.

Mikoajczyk, A. & M. Radkowski, 2002. *Salmonella* spp. on chicken carcasses in processing plants in Poland. *Journal of Food Protection*, **65**, 1475–1479.

Mølbak, K., J. E. Olsen & H. C. Wegener, 2006. *Salmonella* infections. In: *Foodborne Infections and Intoxications*, 3rd edn, eds Riemann, H. P. & D. O. Cliver, Academic Press, pp. 57–136.

Ozbey, G. & H. B. Ertas, 2006. *Salmonella* spp. isolation from chicken samples and identification by polymerase chain reaction. *Bulgarian Journal of Veterinary Medicine*, **9**, 67–73.

Rusul, G., J. Khair, S. Radu & R. M. Yassin, 1996. Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. *International Journal of Food Microbiology*, **33**, 183–194.

Ruzauskas, M., M. Virgailis & V. Špakauskas, 2005. Serological diversity and antimicrobial resistance of *Salmonella* isolated from different sources in Lithuania. *Veterinarski Arhiv*, **75***,* 211–221.

Tirolli, I. C. C. & C. A. da Costa, 2006. Occurrence of *Salmonella* spp. in chicken carcasses commercialized in open markets in the city of Manaus – Amazonas. *Acta Amazonica*, **36**, 205–208.

Uyttendaele, M. R., P. De Troy & J. Debevere, 1999. Incidence of *Salmonella*, *Campylobacter jejuni*, *Campylobacter coli*, and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. *Journal of Food Protection,* **62**, 735–740.

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