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THE EPIZOOTIOLOGICAL IMPORTANCE OF SALMONELLA SPP. ISOLATED IN VARIOUS ASPECTS OF POULTRY PRODUCTION IN THE SOUTHERN BAČKA AND SREM REGION*

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Abstract

Salmonella causes local and systemic infections of poultry, which may lead to substantial direct and indirect economic losses, presenting also significant risk to human health. The aim of this study was to monitor the occurrence of certain serotypes of *Salmonella spp.* isolated on poultry farms in Southern Bačka and Srem regions in the period from 2010 to 2014, as recommended by the Book of rules of early detection, diagnostic, prevention of spreading and eradication of *Salmonella spp.* We analyzed the results obtained from the laboratory for clinical bacteriology to determine number of salmonella cases. From all samples that have been submitted for bacteriology analysis, salmonellas were isolated from 7.3% samples. *Salmonella infantis* was isolated from 50.3% of all salmonella-positive samples, mostly from materials supplied from broiler farms. *Salmonella enteritidis* was most frequently isolated in broiler chickens at the rate of 48.2%. There is an increasing trend in the occurrence of *Salmonella enteritidis* and *Salmonella infantis* on poultry farms from year to year. Our research revealed the highest incidence of salmonella isolates in broilers that died during transportation or within the first three days upon arrival of chickens. During the five-year research period, 65 samples from parent flocks (63 from broiler breeders and 2 from parent flocks of layers) were salmonella positive, which ma-

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kes 8.03% of all positive isolates. It is most likely that salmonella infection occurs due to infection of parent flocks and young chickens are infected through both vertical and horizontal transmission.

Keywords: salmonella, broiler chickens, samples

EPIZOOTIOLOŠKI ZNAČAJ *SALMONELLA SPP.* IZOLOVANIH U RAZLIČITIM VIDOVIMA ŽIVINARSKJE PROIZVODNJE U JUŽNOBAČKOM I SREMSKOM OKRUGU

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Kratak sadržaj

Salmonele kod živine izazivaju infekcije lokalnog i sistemskog karaktera, dovodeći do značajnih kako direktnih tako i indirektnih ekonomskih gubitaka u industrijskom živinarstvu i predstavljaju opasnost po zdravlje ljudi. Cilj ovog rada bio je monitoring određenih sojeva *Salmonella spp.* propisanih Pravilnikom o utvrđivanju mera za rano otkrivanje, dijagnostiku, sprečavanje širenja, suzbijanje i iskorenjivanje infekcija živine određenim serotipovima salmonella, na teritoriji Sremskog i Južnobačkog okruga u periodu od 2010. do 2014. godine. Analizirali smo rezultate ispitivanja laboratorije za kliničku bakteriologiju. Od ukupno ispitanih bakterijskih infekcija iz kliničkog materijala u ovom petogodišnjem periodu, salmonele su izolovane u 7,3% slučajeva. Ustanovljeno je da je *Salmonella infantis* izolovana u 50,3% svih pozitivnih uzoraka, najčešće iz materijala dostavljenih sa farmi brojlerskih pilića. *Salmonella enteritidis* izolovana je u 48,2%, takođe najčešće kod brojlerskih pilića. Prisutan je trend porasta izolata *Salmonella enteritidis* i *Salmonella infantis* iz godine u godinu. Našim istraživanjem ustanovili smo da je najviše pozitivnih uzoraka bilo kod brojlera, u transportnim uginućima i uginućima iz prva tri dana starosti. Kod roditeljskih jata bilo je pozitivno 65 uzoraka u ovom petogodišnjem period (63 kod

teške linije i 2 kod lake linije), što iznosi 8,03 % od svih pozitivnih izolata. Pretpostavlja se da je do infekcije salmonelama moglo doći usled horizontalne i vertikalne infekcije preko roditeljskih jata.

Ključne reči: salmonela, brojlerski pilići, uzorci

INTRODUCTION

Salmonella does not cause clinical symptoms in poultry, but seldom may lead to increased mortality usually during rearing and induce a decrease in egg production. People become infected with salmonella by consuming contaminated food. These infections may be manifested in humans through lighter or heavier clinical symptoms which may be accompanied with a long convalescing period (Dačić et al., 2004). *Salmonella* are a facultative Gram - negative aerobes, rod-shaped, and classified as the *Enterobacteriaceae* family. They do not create spores and they do not encapsulate.

Salmonellas are excreted through feces, contaminating the environment so reinfections are frequent. Certain serotypes such as *Salmonella* Enteritidis (*S. Enteritidis*) and *Salmonella* Typhimurium (*S. Typhimurium*) persist inside parenchymal organs and ovaries which can cause vertical transmission (Ilić i sar., 2010). *Salmonella* can cause embryo death and early mortality in chickens (in the first seven days of life). If the parent stock has been infected, incubators for hatching chickens may also be contaminated which may lead to a large number of hatched chickens to be infected. The process of animal feed pelleting can decrease the *Salmonella* contamination only to a certain extent therefore it is crucial to maintain cleanliness in animal feed factories. Since the *Salmonella* is so widespread and difficult to eliminate from the environment, it is on the eradication program priority list throughout the world. The goal of this research was to determine the prevalence of certain serotypes of *Salmonella* spp. in Srem and Southern Backa district from year 2010 - 2014, specified by the Book of Rules for poultry salmonellosis RS number 7/10, and to briefly highlight control, prevention measures, and goals which should be achieved in order to eradicate these persistent infections.

MATERIAL AND METHODS

During the timeline from 2010 - 2014, samples for *Salmonella* examination from the Srem and Southern Bačka district were delivered to the Veterinary

Institute in Novi Sad. The following categories of poultry flocks were included in this research:

- rearing broiler breeders and rearing layer breeder flocks
- broiler breeders and layer breeders in production
- hatcheries
- Broilers
- Turkey pullets
- Layer chickens
- Pheasants raised on farms

In this research 11.044 samples from poultry were analyzed. These samples were: parenchymatous organs, embryonated chicken eggs, unhatched eggs, mortality during transport, and paper pads. *Salmonella* spp. was isolated in a laboratory for clinical bacteriology, by using liquid media for enrichment, selective media, differential media, and for serological typing the slide agglutination test with specific serums (with poly-somatic and, flagellar antigens) was done.

The results were processed by descriptive statistics and presented in percentages.

RESULTS

During the 2010 - 2014 timeline, 11.044 samples were examined, 809 (7.32 %) tested positive for *Salmonella*. Most frequently detected were *S. Enteritidis* and *Salmonella* *Infantis* (*S. Infantis*). In a smaller number of samples, *S. Typhimurium* was isolated as well (Table 1).

Table 1: The number of isolates from *Salmonella* *Enteritidis*, *Typhimurium* and *Infantis* in poultry flocks from Southern Bačka and Srem region from 2010-2014.

Year	<i>Salmonella</i> <i>Enteritidis</i>	<i>Salmonella</i> <i>Typhimurium</i>	<i>Salmonella</i> <i>Infantis</i>
2010	85	5	46
2011	32	/	39
2012	59	1	92
2013	89	2	124
2014	125	4	106
Total	390	12	407

*values in the table represent the numbers of positive samples

The results from this research show that the number of positive *S. Enteritidis* isolates had increased in the 2011-2014 timeframe. A total of 390 samples were isolated, which is approximately 48.2% of all the positive samples. Most of the positive samples originated from broilers (244) and layers (96). The prevalence of positive samples from years 2010 to 2014 is presented in Fig 1 and 2.

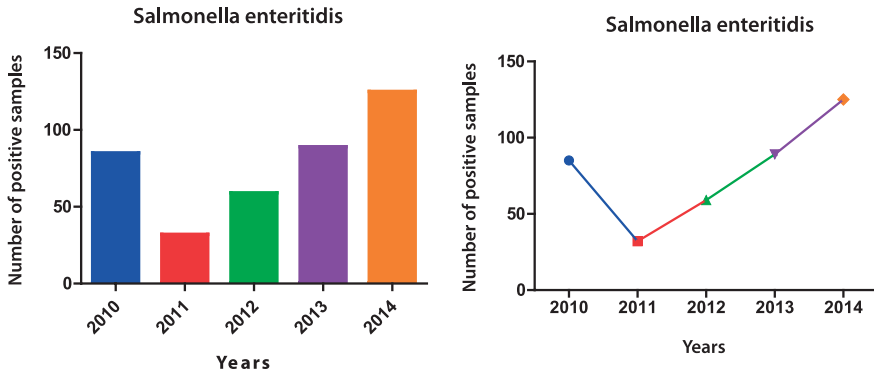


Fig 1 and 2: The prevalence of positive samples for *S. Enteritidis* from years 2010-2014.

S. Typhimurium was isolated in 12 samples at the rate of 1.48%. During the year 2011 there were no positive cases. The prevalence of positive samples throughout the years 2010-2014, is shown in Fig 3 and 4.

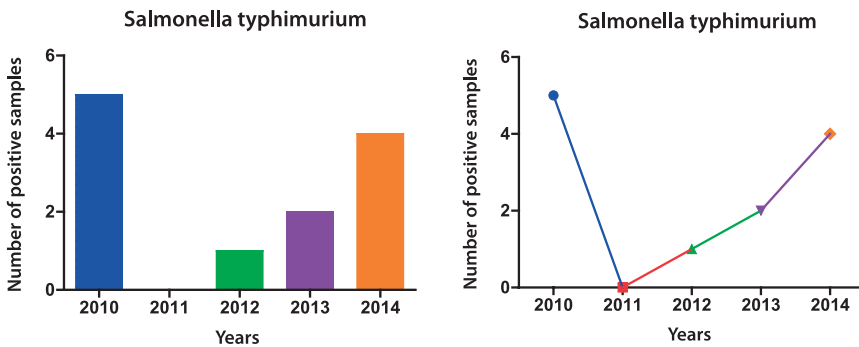


Fig 3 and 4: Prevalence of positive samples for *S. Typhimurium* from years 2010-2014.

The number of positive isolates for *S. Infantis* was the highest in 2013 and it was isolated from 50.3 % of all the positive samples. Most of the positive samples came from broiler farms (271) and layers (81), which is similar to the occurrence of *S. Enteritidis*. The prevalence for positive samples per year is shown in Fig 5 and 6.

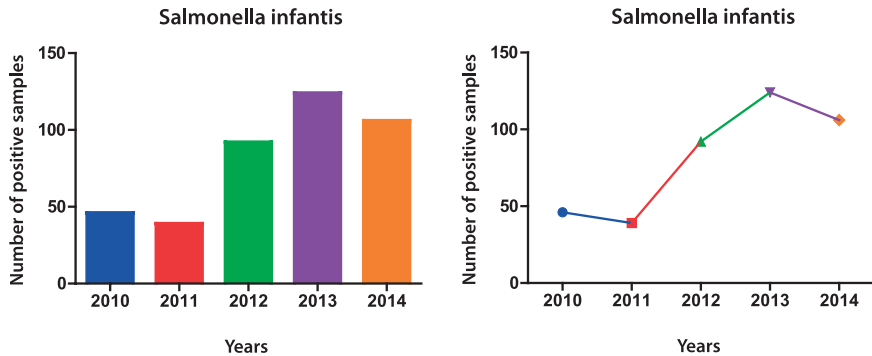


Fig 5 and 6. The prevalence of positive samples for *Salmonella* Infantis from 2010-2014.

DISCUSSION

The prevalence of *Salmonella* spp. in diagnostic materials is similar to reports from previous years (Stojanov et al., 2006). Most of the positive samples were from broilers, mortality during transportation, and mortality at three days old. From all of the positive samples, 521 were from broiler farms, which is 64.40% of the whole number of samples. Broiler breeders had 65 positive samples, which is 8.03% of all the positive samples. Sixty three positive samples were from rearing layer breeders and only two from flock of layer breeders. Samples that came from layer chickens were positive in 178 cases, which is 22.12% from the total number of *Salmonella* isolation. *Salmonella* was found in pheasants in seven samples, during this five year run, and turkey pullets had 25 positive samples.

Salmonella is isolated in high percentages in poultry flock throughout the Srem and Southern Backa district, as well as in other regions of Serbia. Stojanov et al., (2011) were examining the presence of *S. Enteritidis* and *S. Infantis* in poultry farms during 2009 and 2010. They had established that *S. Enteritidis*

was present in poultry specimens by 61.42 % in 2009 and 56.63% in 2010, in relation to the total number of positive results. During the same period, *S. Infantis* was isolated at the rate of 3% (2009) and 38.05% (2010). Our findings regarding the incidence of *Salmonella* in farms during the period from 2010-2014 are similar but the number of *S. Infantis* is increasing with regards to the period of 2010.

During the research that was done by Ilić et al., in 2010, internal organs in poultry were examined, as well as embryonated eggs and table eggs. A total of 1289 samples were examined from which 9 tested positive for *S. Enteritidis* and 9 for *S. Typhimurium*, showing the incidence of 0.7% (Ilic et al., 2010). Matović and his associates examined 48182 specimens from poultry during the years 2000-2005, in the Raška and Zlatibor epizootiological area. *Salmonella* spp, was diagnosed in 476 samples (0.98%). Similar to our finding *S. Enteritidis* was most often isolated (453 samples) and in 23 samples *Salmonella* of other serotypes was found. In their work *Salmonella* was isolated from fertile and table eggs, paper pads, mortality during the first three days, pheasant chicks, mortality during transport, carcasses and broiler chickens as well as layers and adult pheasants (Matovic et al., 2006).

Al-Nakhli and colleagues (1999) examined the presence of *Salmonella* spp. in poultry farms in Saudi Arabia, from 1988-1997. In their research 25.759 samples were examined and 1.052 i.e. (4%) were positive for *Salmonella*. These samples were taken from broiler farms, layer farms, rearing broiler breeders and rearing layer breeders. *S. Enteritidis*, *Salmonella* Virchow (*S. Virchow*) and *S. Infantis* were the most frequently found. Poppe et al., (1991), had isolated several types of *Salmonella* from a layer farm in Canada in 1991. They examined 295 samples, and isolated *Salmonella* Heidelberg 59/295 (20%), *S. Infantis* 18/295 (6.1 %), *Salmonella* Hadar 17/295 (5.8 %) and *Salmonella* Schwarzengrund 21/295 (7,2%). From 2007-2011 in a research performed by Rahmani et al., (2013), 36 serotypes of *Salmonella* were isolated from broiler farms which are located in three different provinces in north Iran. *S. Infantis* and *S. Enteritidis* were most frequently isolated. Lassing et al., (2012) performed a research on 363 broiler flocks with at least 5000 broilers in Austria. The sampling was done during the whole year. The *Salmonella* spp infections were registered in 28 flocks (7.7%). In six farms *S. Enteritidis* (1.7%) was isolated while in 2 flocks *S. Typhimurium* (0.6%) was found. In the remaining 20 flocks: *Salmonella* Montevideo (4,1%), *S. Infantis* (0,6%), *Salmonella* Senftenberg, *Salmonella* Tennessee and *S. Virchow* (0.3%) were isolated. Data shows that the risk of horizontal transmission of *Salmonella* spp. to broiler chickens is very high. The study that was conducted by Rusul and associates (1996) in

Malesia, in order to determine the prevalence of *Salmonella* in 230 samples from broiler chickens, has shown that the most prevalent are *S. Enteritidis*, *Salmonella* Muenchen, *Salmonella* Kentucky and *Salmonella* Blockley.

Salmonella are one of the most important bacteria which cause zoonoses. Worldwide, they are considered to be very significant in epidemiology, and the program for eradication is mandatory in all the EU states. In order to eliminate salmonella from the food chain it is essential to minimize the incidence of *Salmonella* in breeder flocks and in other poultry farms as well (Velhner et al., 2011). The directive, EC number 1003/2005 has required that the incidence of *S. Enteritidis* and *S. Typhimurium* as well as other significant serotypes of *Salmonellas* in breeder farms should not exceed an amount of $\leq 1\%$ (EC, 1003/2005). For these reasons, *Salmonella* monitoring needs to be more comprehensive while better management practice on farms has to become one of the primary goals in the livestock industry in Serbia.

CONCLUSION

- From 2010 – 2014, we have established an increase in *S. Infantis* in poultry samples. The highest number of samples was from broiler chickens, death during transport, and mortality in the first three days of life.
- An increase in positive samples for *S. Enteritidis* was also proven for broiler farms.
- *S. Typhimurium* was isolated in a smaller percentage, 1.48% of all the positive samples.
- The highest amount of positive samples has come from broiler chickens (64.40%).
- In layers, positive samples were found in 22.12 % of samples.
- *Salmonella* Hadar and *S. Virchow* which are category 2 according to the Book of rules number 7/10, were not isolated at this time.

In order to prevent salmonella infections in chickens, it is essential to apply the following measures: to purchase chickens only from farms that are free of salmonella; avoid mixing chickens from different flocks; thermal processing of feed ingredients; to provide water not contaminated with salmonella; prevent wild birds and rodents from accessing farms; thoroughly clean and disinfect objects between poultry production cycles; disinfect by using fumigation for hatching eggs; disinfect incubators after every hatching. Farm workers need to wear protective clothing and regularly conduct personal hygiene. Treatment

with antibiotics is not recommended in poultry, unless diarrhea and fever occur, when they can be used to reduce mortality. In laying hens antibiotics are contraindicated.

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LITERATURE

1. Al-Nakhli H. M., Al-Ogaily Z. H., Nassar T. J.: Representative Salmonella serovars isolated from poultry and poultry environments in Saudi Arabia. *Revue scientifique et technique*, 18, 3, 700-9, 1999.
2. Commission regulation (EC) No 1003/2005 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in breeding flocks of Gallus gallus and amending regulation (EC) No 2160/2003. *Official Journal of the European Union* 2005, L 170/12, 01. 07. 2005.
3. Dačić M., Petković J., Simonović Lj., Račić Z.: Salmonella enteritidis in laying hens with emphasis on presence / absence of the agent in eggs. VI Epizootiology Days with International Participation, Book of Abstracts, March 31 – April 2, 2004, Vlasinsko lake, Serbia, organized by Section of Zoonoses SVA and Depart. of Infec. Animal Disease and Bee Disease FVM, Belgrade, 2004, 159
4. Ilić Ž., Jovičić D., Žugić G., Pavlović I.: Salmonella spp. as part of the ecosystem. XII Epizootiology Days with International Participation, Book of Abstracts, April 7-10, 2010, Oplenac, Serbia, organized by Section of Zoonoses SVA et al, 2010, 222
5. Lassnig H., Much P., Schliessnig H., Osterreicher E., Kostenzer K., Kornschöber C., Köfer J.: Prevalence of Salmonella spp. in Austrian broiler flocks in the context of the EU-wide baseline survey 2005-2006. *Berl Munch Tierarztl Wochenschr*, 125, 3/4, 129-37, 2012.
6. Matović K., Žarković A., Tornić A., Šekler M., Debeljak Z., Vasković N., Kolarević M.: Prevalence of Salmonella in Raška and Zlatibor Epizootiologic region from 2000 to 2005. VIII Epizootiology Days with International Participation, Book of Abstracts, April 5-8, 2006, Beograd, Serbia, organized by Section of Zoonoses SVA and Depart. of Infec. Animal Disease and

- Bee Disease FVM, Belgrade and Scientific Institut of Veterinary Medicine, Novi Sad, 2006, 127.
7. Poppe C., Irwin R. J., Forsberg C. M., Clarke R. C., Oggel J.: The prevalence of *Salmonella enteritidis* and other *Salmonella* spp. among Canadian registered commercial layer flocks. *Epidemiology & Infection*, 106, 2, 259–270, 1991.
 8. Pravilnik o utvrđivanju mera za rano otkrivanje, dijagnostiku, sprečavanje širenja, suzbijanje i iskorenjivanje infekcija živine određenim serotipovima salmonella. „Službeni glasnik Republike Srbije”, broj 7/10, od 19. februara 2010.
 9. Rahmani M, Peighambari S. M., Svendsen C. A., Cavaco L.M., Agersø Y., Hendriksen R. S.: Molecular clonality and antimicrobial resistance in *Salmonella enterica* serovars *Enteritidis* and *Infantis* from broilers in three Northern regions of Iran. *BMC Veterinary Research*, 9:66, 2013.
 10. Rusul G., Khair J., Radu S., Cheah C. T., Yassin R. M.: Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. *International Journal of Food Microbiology*, 33, 2/3, 183-94, 1996.
 11. Stojanov I., Orlić D., Došen R., Prodanov J., Velhner M.: Finding of *Salmonella* in materials originating from Poultry and Pigs during 2002-2005. VIII Epizootiology Days with International Participation, Book of Abstracts, April 5-8, 2006, Beograd, Serbia, organized by Section of Zoonoses SVA and Depart. of Infec. Animal Disease and Bee Disease FVM, Belgrade and Scientific Institute of Veterinary Medicine, Novi Sad, 2006, 141-142.
 12. Stojanov I., Potkonjak D., Kapetanov M., Ratajac R., Maljković M., Pušić I., Jovičin M.: Changes in prevalence of some *Salmonella* serotypes in materials originating from poultry. First International Epizootiology Days, Book of Abstracts, April 6-9, 2011, Sijarinska spa, Serbia, organized by Section of Zoonoses SVA et al, 2011, 86.
 13. Velhner M., Potkonjak D., Stojanov I., Petrović J., Kozoderović G.: *Salmonella* control in poultry production and resistance monitoring in Serbia. *Arhiv Veterinarske Medicine*, 4, 2, 11-22, 2011.

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