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Original Article

Occurrence of *Salmonella* spp. in backyard poultry in Bosnia and Herzegovina

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Abstract

Background: Infected poultry is one of the most important reservoirs of *Salmonella*. **Aims:** The investigation presented here was conducted to examine the occurrence of *Salmonella* in fecal samples among selected flocks of backyard poultry in Bosnia and Herzegovina (B&H). **Methods:** Isolation and identification of *Salmonella* was performed in accordance with BAS EN ISO 6579/AMD 1:2007. When genus *Salmonella* was confirmed, the determination of the antigenic formula of *Salmonella* isolates was performed in accordance with BAS CEN ISO/TR 6579-3:2015. After that, *Salmonella* serotypes were subjected to antibiotic susceptibility testing using EUVSEC sensititre microtiter plates impregnated with different concentrations of antibiotics. At the end, real-time PCR was used to detect extended spectrum β-lactamases (ESBL) and carbapeneamase encoding genes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{CMY}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$ and *bla*$_{GES}$). **Results:** S*almonella* spp. was detected in pooled feces from four backyards, housed by chickens only. Three isolates were confirmed by slide agglutination as serotype Enteritidis and one as serotype Typhimurium. Antibiotic susceptibility testing by microdilution did not reveal phenotypical resistance among these four isolates. Real-time PCR used to detect ESBL and carbapeneamase encoding genes revealed the *bla*$_{TEM}$ gene in one *S.* Enteritidis isolate. **Conclusion:** Data presented in this study provide further evidence on the circulation of different *Salmonella* serotypes in backyard poultry in B&H. These findings emphasize the potential role of backyard poultry in the epidemiology of salmonellosis and the risks it poses for keepers, consumers, and general public health.

Key words: Antimicrobial resistance, Backyard poultry, *Salmonella*

Introduction

A large number of different hosts, including wild and domestic animals as well as humans can be infected or act as carriers of *Salmonella*. Bacteria from the genus *Salmonella* are divided into two species: *Salmonella enterica* and *S. bongori* (Le Minor and Popoff, 1987; Grimont and Weill, 2007; Guibourdenche *et al*., 2010). The species *enterica* is further divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*) (Lamas *et al*., 2018). The largest number of *Salmonella* serotypes belongs to the species *S. enterica* (2587), of which the subspecies *S. enterica* subsp. *enterica* has 1547 serotypes (Grimont and Weill, 2007; Guibourdenche *et al*., 2010). The global distribution of *Salmonella* is the result of their mechanism of adaptation, phenotypic modifications and genetic mutations. *Salmonella* successfully survives in the environment in the presence of organic material, as well as in different species of living organisms (Hulaj, 2008).

Infected poultry is one of the most important reservoirs of *Salmonella* that can be transmitted to humans through the food chain production. The prevention of salmonellosis is complicated by their ability to spread vertically and horizontally in poultry farms and to contaminate the environment (Holt *et al*., 1994). Salmonellosis remains the second most commonly reported gastrointestinal infection in humans after *Campylobacter*, and an important cause of food-borne outbreaks in the European Union (EU) (EFSA, 2018).

Due to large financial investments, the emergence of human and avian salmonellosis results in significant economic losses in egg and meat production, as well as those caused by efforts in the prevention and control of the diseases (McMullin, 2020). Given the importance for public health, the prevention of salmonellosis in many countries holds a significant place in the strategy for the prevention of infectious diseases (Holt *et al*., 1994;

Rešidbegović and Kavazović, 2008).

Some *Salmonella* serotypes are host-specific, while others can infect different animal species. Serotypes, which are highly specific and adapted to poultry are *S.* Gallinarum and *S.* Pullorum (Emadi *et al*., 2009; Dale and Brown, 2013). The most common *Salmonella* serotypes that are of particular public health importance are *S.* Enteritidis and *S.* Typhimurium (Anonymous, 2007; Christensen *et al*., 2011). Aiming at preventing and reducing cases of human salmonellosis, national control programs for *Salmonella* are being implemented in EU Member States (MS) in *Gallus* species poultry and turkeys, with special reference to serotypes of importance for public health: *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow, and *S.* Hadar (EFSA, 2018).

Intensive poultry farming constitutes the most important part of modern livestock production. On the other hand, in developing countries, backyard poultry remains still a significant source of animal proteins (Pym *et al*., 2006; Sonaiya, 2008). However, this type of breeding often goes with insufficient biosecurity measures leading to a higher risk of zoonotic disease outbreaks such as salmonellosis. Given the low profit and high costs which are associated with the implementation of the biosecurity principles, protection of backyard poultry health is of little interest (Permin and Detmer, 2007). However, wide distribution of this production type warrants continuous monitoring programs in order to prevent the potential spread of *Salmonella* to intensive poultry facilities. In this regard, considering the lack of continuous control programs in backyard flocks in Bosnia and Herzegovina, objectives of our research were:

a) To investigate the presence of *Salmonella* in pooled chicken feces from backyard poultry from wider areas of Bosnia and Herzegovina (B&H)

b) To study serotyping and antibiotic susceptibility of obtained *Salmonella* isolates

c) To examine the presence of ESBL genes using multiplex real-time PCR

Materials and Methods

During 2013, pooled fecal samples from 738 animals housed in 65 backyards throughout B&H were collected. Fifty-five backyards had chickens only. A mixed population of chickens, turkeys, ducks, and geese were present in eight backyards, and two backyards had only turkeys.

Isolation and serotyping of *Salmonella*

The procedure for the isolation and identification of *Salmonella* was performed in accordance with the standard BAS EN ISO 6579/AMD 1:2007 (ISO, 2007). When genus *Salmonella* was confirmed, the determination of the antigenic formula of *Salmonella* isolates was performed in accordance with BAS CEN ISO/TR 6579-3:2015 (ISO, 2015). The commercial *Salmonella* antisera used for slide agglutination were from SIFIN (Berlin, Germany).

Antibiotic susceptibility testing (AST)

Obtained *Salmonella* serotypes were subjected to AST using EUVSEC sensititre microtiter plates (Trek Diagnostic Systems, England) impregnated with different concentrations of antibiotics (sulfamethoxazole 8-1024 µg/ml, gentamicin 0.5-32 µg/ml, ciprofloxacin 0.015-8 µg/ml, ampicillin 1-64 µg/ml, cefotaxime 0.25-4 µg/ml, ceftazidime 0.5-8 µg/ml, tetracycline 2-64 µg/ml, trimethoprim 0.25-32 µg/ml, chloramphenicol 8-128 µg/ml, colistin 1-16 µg/ml, meropenem 0.03-16 µg/ml, azithromycin 2-64 µg/ml, nalidixic acid 4-128 µg/ml, and tigecillin 0.25-8 µg/ml) to determine the minimum inhibitory concentration (MIC). The reference strain *E. coli* ATCC 25922 was used as an internal control. When interpreting the results, the growth of *E. coli* strain ATCC 25922 (two positive controls) in the provided microtiter plate fields was considered. Interpretation of the results was performed in accordance with the guidelines of the National Food Institute (DTU, 2012). No interpretative criteria were available for azithromycin.

Bacterial DNA extraction for real-time PCR

Single *Salmonella* colonies from nutrient agar (Nutrient agar Condalab, Madrid, Spain) were inoculated into 5 ml of buffered peptone water (BPW Condalab, Madrid, Spain) and incubated overnight at 37°C. Later on, 1 ml of each culture was centrifuged at 14,000 × g for five min. The supernatant was removed and the remaining bacterial pellet was resuspended in 300 µL of sterile TE-buffer (10 mM Tris, 0.1 mM EDTA, pH = 8). Aiming at the lysis of the bacterial cell and the release of DNA, heating to 99°C for 10 min followed. The suspension was cooled on ice for 3 min, vortexed, centrifuged at 14,000 × g for two min, and cooled again. Finally, 100 µL of isolated supernatant was used for real-time PCR. For further molecular testing, samples were stored at -20°C.

Four *Salmonella* isolates were subjected to multiplex real-time PCR to detect the presence of the ESBL genes. The protocol included specific sequences of genes encoding β-lactamases belonging to class A (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}), AmpC β-lactamases from class C (bla_{CMY}), and genes encoding carbapenemases (bla_{KPC}, bla_{NDM}, bla_{GES}, bla_{OXA-48}, and bla_{VIM}).

The sequences of the specific primers and fluorescent probes used to detect ESBL and carbapenemase encoding AMR genes, as well as the protocols based on which the PCR were performed, are shown in Table 1.

Real-time PCR for detection of EBSL-encoding bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{CMY} genes

A CFX96 Real-time System (Bio-Rad, Germany) was used for multiplex real-time PCR, while the analysis of the results was performed in the Bio-Rad CFX Manager 3.1 software. The real-time PCR protocol used for the detection of bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{CMY} genes was performed according to the protocol of

Table 1: Sequences of specific primers and fluorescent probes used to detect individual AMR genes

Target gene	Primer-probe name	Sequence (5´-3´)	Reference
bla$_{TEM}$	TEM_fwd.	GCATCTTACGGATGGCATGA	Roschanski *et al*. (2014)
	TEM_rev.	GTCCTCCGATCGTTGTCAGAA	
	TEM_probe	6-FAM-CAGTGCTGCCATAACCATGAGTGA-BHQ1	
bla$_{SHV}$	SHV_fwd.	GTCCTCCGATCGTTGTCAGAA	
	SHV_rev.	TCCTGCTGGCGATAGTGGAT	
	SHV_probe	Cy5-TGCCGGTGACGAACAGCTGGAG-BBQ650	
bla$_{CTX-M}$	CTX_A_fwd.	CGGGCRATGGCGCARAC	
	CTX_A_rev.	TGCRCCGGTSGTATTGCC	
	CTX_A_probe	VIC-CCARCGGGCGCAGYTGGTGAC-BHQ1	
	CTX_B_fwd.	ACCGAGCCSACGCTCAA	
	CTX-B_rev.	CCGCTGCCGGTTTTATC	
	CTX_B_probe	Quasar705-CCCGCGYGATACCACCACGC-BHQ1	
bla$_{CMY}$	CMY_fwd.	GGCAAACAGTGGCAGGGTAT	
	CMY_rev.	AATGCGGCTTTATCCCTAACG	
	CMY_probe	ROX-CCTACCGCTGCAGATCCCCGATG-BHQ2	
bla$_{KPC}$	KPC_fwd.	TGCAGAGCCCAGTGTCAGTTT	van der Zee *et al*. (2014)
	KPC_rev.	CGCTCTATCGGCGATACCA	
	KPC_probe	Cy5-TTCCGTCACGGCGCGCG-BBQ-650	
bla$_{NDM}$	NDM_fwd.	CATTAGCCGCTGCATTGATG	
	NDM_rev.	GTCGCCAGTTTCCATTTGCT	
	NDM_probe	6-FAM-CATGCCCGGTGAAATCCGCC-BHQ1	
bla$_{OXA-48}$	OXA-48_fwd.	GCGTGGTTAAGGATGAACAC	
	OXA_48_rev.	CATCAAGTTCAACCCAACCG	
	OXA_48_probe	ROX-AGCCATGCTGACCGAAGCCAATG-BHQ2	
bla$_{VIM}$	VIM_fwd.	GAGATTCCCACGCAYTCTCTAGA	
	VIM_rev.	AATGCGCAGCACCAGGATAG	
	VIM_probe	Yakima Yellow-ACGCAGTGCGCTTCGGTCCAGT-BHQ1	
bla$_{GES}$	GES_fwd.	CGGTTTCTAGCATCGGGACACAT	Swayne *et al*. (2011)
	GES_rev.	CCGCCATAGAGGACTTTAGCMACAG	
	GES_probe	ATTO700-CGACCTCAGAGATACAACTACGCCTATTGC-DDQ1	

Roschanski *et al*. (2014) in a final reaction of 25 µL, which contained 12.5 µL absolute qPCR Mix (Thermo Scientific, St. Leon Roth, Germany), 1 µL of each upstream and downstream primer (10 pmol), 0.1 µL of TEM TaqMan probes (5 pmol), 0.2 µL of each of the other four TaqMan probes (10 pmol), 0.6 µL of DNA free water and 1 µL of bacterial DNA sample. PCR amplification was performed under optimized conditions as follows. To achieve maximum polymerase activity, it was necessary to perform a preliminary preheating step at 95°C for 15 min. This was followed by 30 cycles: 95°C for 15 s, 50°C for 15 s, and 70°C for 20 s. At the end of the run, the cycle threshold (Ct) was calculated by determining the signal strength at which the fluorescence exceeded the threshold value that was automatically set in Bio-Rad CFX Manager 3.1.

Real-time PCR to detect carbapenemase-encoding *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$, and *bla*$_{GES}$ genes

The real-time PCR protocol used for the detection of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$, and *bla*$_{GES}$ genes was performed according to the adapted protocol of Swayne *et al*. (2011) in a final reaction of 25 µL which contained 12.5 µL Platinum® Quantitative PCR SuperMix-UDG (Invitrogen, Paisley, UK), 1 µL of each upstream and downstream primer (10 pmol/L), 0.2 µL of each of the other 5 TaqMan probes (10 pmol/L), 0.5 µL of free water DNA and 1 µL of DNA sample. The thermoprofile was performed according to the adapted protocol of van der Zee *et al*. (2014) and included an initial denaturation lasting 15 min at 95°C, followed by 30 cycles at 95°C for 15 s and 60°C for 1 min.

Results

From a total of 65 backyards examined, *Salmonella* spp. was detected in pooled feces from four backyards, housed by chickens only (Fig. 1). Three isolates were confirmed by slide agglutination as serotype Enteritidis and one as serotype Typhimurium.

The antibiotic susceptibility testing by microdilution did not reveal phenotypical resistance among these four isolates. Real-time PCR to detect extended spectrum β-lactamases (ESBL) and carbapeneamase encoding genes such as *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{CMY}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{VIM}$, and *bla*$_{GES}$, revealed the presence of the *bla*$_{TEM}$ gene in one *S.* Enteritidis isolate (Fig. 2). The CT value for this sample (specific probe of a portion of the *bla*$_{TEM}$ gene) was: 22.88.

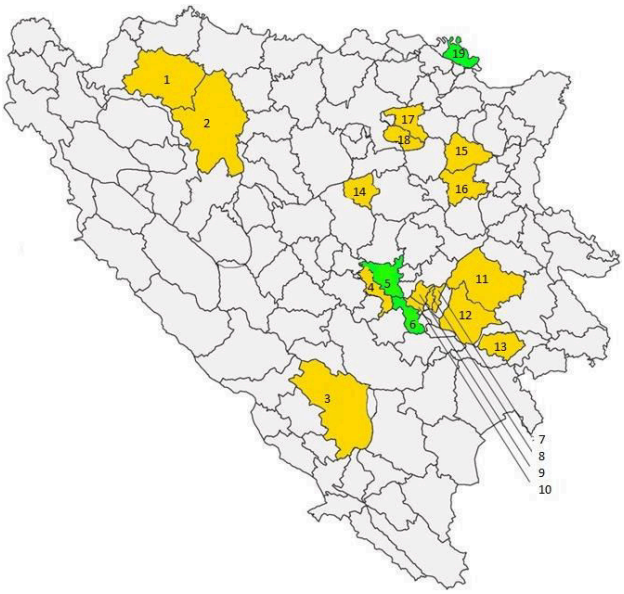

Fig. 1: A map of B&H with depicted municipalities showing the geographical distribution of *Salmonella* spp.-positive and -negative poultry backyard farms denoted with numbers 1-19. Green municipalities with *Salmonella* spp.-positive backyard farms (number: 5, 6, and 19) were subjected to serotyping and detection of AMR genes. Yellow (numbered), municipalities contained farms that were *Salmonella* spp.-negative during the sampling period

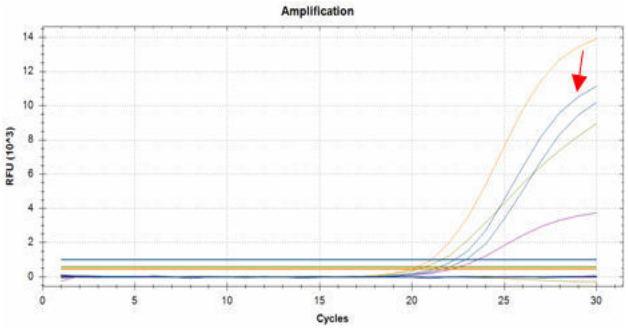

Fig. 2: Real-time PCR amplification of the *bla*TEM gene detected in one *S.* Enteritidis strain (marked with red colored arrow) along with positive controls (orange (*bla*SHV), blue (*bla*TEM), green (*bla*CTX-A/B), and purple (*bla*CMY))

Discussion

The demand of the growing human population for poultry products is mostly met by large scale industrial farming. Prior to the introduction of high production hybrids, poultry was raised in backyards, and poultry eggs and meat were consumed mainly by the farmers themselves (Milošević *et al*., 2005a). Nowadays, regardless of the price, poultry products supplied by alternative raising systems are increasingly on demand. High quality, safety, and absence of biological and chemical residues are paramount aspects to be fulfilled (Milošević *et al*., 2005a; Milošević *et al*., 2005b).

Studies on the presence of pathogens with zoonotic implications were sporadic in B&H (Rešidbegović and Kavazović, 2007; Hadžiabdić, 2014; Elezaj, 2015). In addition, the presence of *S.* Infantis and *S.* Hadar evident in two backyards inhabited by different species of domestic poultry indicated the potential role of backyard poultry as a reservoir of *Salmonella* spp. in B&H (Rešidbegović, 2008). The detection of four *Salmonella* spp. isolates in the present work further supports these observations. Worldwide, the prevalence of *Salmonella* spp. in backyard poultry differs among countries and ranges from 3% in the United States (Shah *et al*., 2020) to 10.4% in Australia (Manning *et al*., 2015), and 12.7% in China (Zhao *et al*., 2016). It is also observed to be significantly lower than in commercial poultry farming (35%) (Zhao *et al*., 2016).

The results of the present study support the observation regarding the predominance of *S.* Enteritidis and *S.* Typhimurium in backyard poultry previously reported in southern Iran (Jafari *et al*., 2007; Emadi *et al*., 2009) and the United States (Shah *et al*., 2017; Shah *et al*., 2020). A similar pattern was observed in China, where more than 80% of the isolates belonged to the *S.* Enteritidis serotype (Zhao *et al*., 2016).

Similar to our study, *Salmonella* serotypes from the United States exhibited susceptibility to a wide range of antimicrobials (Shah *et al*., 2017; Shah *et al*., 2020). However, higher antimicrobial resistance of various *Salmonella* serotypes recovered from backyard flocks was observed in China where twenty-two (57.9%) of the 38 isolates were resistant to ampicillin, while the percentage of resistance to cefazolin, cefotaxime, and ceftazidime was 7.9%. The multidrug resistance (MDR) rate was 26.3% (Zhao *et al*., 2016). These findings imply that poultry from the free-range system can serve as a potential reservoir of antibiotic-resistant *Salmonella*, which poses a public health problem (Zhao *et al*., 2016). The antimicrobial sensitivity of *Salmonella* to the tested antibiotics supports Emadi *et al*. (2009) in that the use of antibiotics in the treatment of backyard poultry diseases is less frequent, resulting in the emergence of AMR. Nevertheless, it should be mentioned that only four strains from our study were submitted to AST, hence there is insufficient data regarding the developing trends of AMR in backyard poultry.

Keeping backyard poultry in the vicinity of commercial flocks is an important factor in understanding the broader epidemiology of backyard poultry salmonellosis. The occurrence of *Salmonella* in backyard poultry in areas with a large conglomeration of poultry may be an indicator of the circulation of dominant serotypes within a particular geographical area (Hadžiabdić, 2014). Our results could be viewed in this light since three flocks in which *S.* Enteritidis was detected were located in areas with large concentrations of intensively kept poultry indicating the potential spread of this serotype from commercial facilities into backyard poultry. According to previous research on the epidemiology of salmonellosis in B&H, it has been observed that commercial flocks of poultry can be carriers of different *Salmonella* serotypes, which has significant importance for public health (Rešidbegović and Kavazović, 2007; Hadžiabdić, 2014). Similar to the

present work, *S.* Enteritidis, *S.* Typhimurium, and *S.* Infantis serotypes were detected in these studies. Furthermore, the emergence of very similar or identical PFGE genotypes of *S.* Enteritidis originating from a flock of commercial poultry suggested the prevalence of a homogeneous group of genotypes in B&H (Hadžiabdić, 2014). However, it should be mentioned that for many years, pulsed-field gel electrophoresis (PFGE) has been considered as "gold standard" for molecular bacterial typing (Neoh *et al*., 2019). Given the comparison of the molecular profiles of large DNA fragments, higher resolutions and thus the diversity of *S.* Enteritidis isolates might be achieved using the techniques of next generation sequencing, which would potentially indicate the greater diversity of these strains (Neoh *et al*., 2019).

The presence of *Salmonella* in the yards inhabited with chickens only may have been the sequel of a long-term persistence of *Salmonella* in the surroundings of these backyards. Another reason could be the introduction of well-exploited laying hens purchased directly from commercial facilities. Furthermore, a common practice observed from the anamnestic data is the lack of quarantine for the newly acquired individuals brought for the repopulation of the flocks.

In the present work, we observed some of the specifics of backyard poultry such as the cohabitation of different types of poultry, age diversity, inadequate housing and nutrition, insufficient biosecurity measures, treatment of poultry as pets and keeping the birds for several years. In addition to the above, very close contacts of wild ducks (*Anas platyrhynchos*) with the raised poultry were recorded in one household. All of these make the introduction of successful prevention and control measures more challenging and difficult, which in turn increases the biosecurity risk for keepers, their family members, and the larger public. Outbreaks of human salmonellosis associated with live poultry have been reported since the 1950s, and the incidence has increased in recent years due to the popularity of keeping flocks in private farms (Behravesh *et al*., 2014).

Prevention measures require the introduction of a One Health approach at all levels to achieve optimal health and well-being for all people and animals. Such an initiative implies the design and adoption of effective regulatory framework and application of national control programs, with a focus on the possible persistence of zoonotic pathogens in flocks of backyard poultry and their spread to commercial poultry and potential risks to public health (Özdemir, 2020). Scientific support to find continuous, comprehensive, and applicable biosecurity systems in flocks of extensively kept poultry would certainly contribute to our knowledge of this matter.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Behravesh, CB; Brinson, D; Hopkins, BA and Gomez, TM (2014). Backyard poultry flocks and salmonellosis: a recurring, yet preventable public health challenge. Clin. Infect. Dis., 58: 1432-1438.

Christensen, LS; Josefsen, MH; Pedersen, K; Christensen, J; Bonnichsen, L; Sørensen, G and Hoorfar, J (2011). Real-time monitoring of *Salmonella enterica* in free-range geese. Appl. Environ. Microbiol., 77: 3160-3162.

Dale, E and Brown, C (2013). Zoonotic diseases from poultry. Braz. J. Vet. Pathol., 6: 76-82.

EFSA (2018). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. EFSA Journal. 16: e05182.

Elezaj, I (2015). The study of *Salmonella* spp. in poultry in an extensive farming system. Master thesis, Veterinary Faculty of the University of Sarajevo, Bosnia and Herzegovina. PP: 34-37.

Emadi, CS; Hasanzadeh, M; Bozorg, MM and Mirzaei, S (2009). Characterization of the Salmonella isolates from backyard chickens in north of Iran, by serotyping, multiplex PCR and antibiotic resistance analysis. Archives of Razi Institute. 64: 77-83.

Grimont, PA and Weill, FX (2007). *Antigenic formulae of the Salmonella serovars*. WHO Collaborating Centre for Reference and Research on Salmonella. Institut Pasteur. 9th Edn., Institut Pasteur, France. PP: 1-166.

Guibourdenche, M; Roggentin, P; Mikoleit, M; Fields, PI; Bockemühl, J; Grimont, PA and Weill, FX (2010). Supplement 2003-2007 (No. 47) to the white-Kauffmann-Le minor scheme. Res. Microbiol., 161: 26-29.

Hadžiabdić, S (2014). Prevalence and antibiotic susceptibility of certain serotypes of *Salmonella* spp. in poultry. Master thesis. Veterinary Faculty of the University of Sarajevo, Bosnia and Herzegovina. PP: 47-75.

Holt, JG; Krieg, NR; Sneath, PH and Staley, JT (1994). *Bergey's manual of determinative bacteriology*. 9th Edn., Baltimore, Maryland, USA, Williams and Wilkins. PP: 368-383.

Hulaj, B (2008). Findings of *Salmonella* spp. on laying hen farms in Kosovo. Master thesis, Veterinary Faculty of the University of Sarajevo, Bosnia and Herzegovina. P: 43.

ISO (2015). BAS CEN ISO/TR 6579-3:2015. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 3: Guidelines for serotyping of *Salmonella* spp. The Institute for Standardization of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina.

ISO (2007). BAS EN ISO 6579/AMD 1:2007. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Amendment 1: Annex D: Detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage. The Institute for Standardization of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina.

Jafari, RA; Ghorbanpour, M and Jaideri, A (2007). An investigation into *Salmonella* infection status in backyard chickens in Iran. Int. J. Poult. Sci., 6: 227-229.

Lamas, A; Miranda, JM; Regal, P; Vázquez, B; Franco, CM and Cepeda, A (2018). A comprehensive review of non-enterica subspecies of *Salmonella enterica*. Res. Microbiol., 206: 60-73.

Le Minor, L and Popoff, MY (1987). Designation of *Salmonella enterica* sp. nov., nom. rev., as the type and only species of the genus *Salmonella*: request for an opinion. Int. J. Syst. Evol. Microbiol., 37: 465-468.

Manning, J; Gole, V and Chousalkar, K (2015). Screening for *Salmonella* in backyard chickens. Prev. Vet. Med., 120: 241-245.

McMullin, PF (2020). *Diseases of poultry*. 14th Edn., Hoboken, New Jersey, USA, John Wiley & Sons. P: 719.

Milošević, N; Perić, L; Strugar, V; Žikić, D and Pavlovski, Z (2005a) Rearing of fattening chickens on free range and extensively in chicken coop. Biotechnol. Anim. Husb., 21: 5-6.

Milošević, N; Perić, L; Strugar, V; Žikić, D and Pavlovski, Z (2005b). Rearing of fattening chickens on free range and extensively in chicken coop. Biotechnol. Anim. Husb., 21: 217-221.

National Food Institute (DTU) (2012). The 13th EURL-AR proficiency test *Salmonella*, *Campylobacter* and genotypic characterization 2012. [online]. Website: https://www.eurl-ar.eu/CustomerData/Files/Folders/19-reports-eqas-reports/255_report-2012-salm-camp-isbnfinal.pdf [accessed 02.02. 2013].

Neoh, HM; Tan, XE; Sapri, HF and Tan, TL (2019). Pulsed-field gel electrophoresis (PFGE): A review of the "gold standard" for bacteria typing and current alternatives. Infect. Genet. Evol., 74: 103935.

Özdemir, D (2020). The structural characteristics, management, and challenges of backyard poultry farming in residential areas of Turkey. Animals. 10: 2336.

Permin, A and Detmer, A (2007). Improvement of management and biosecurity practices in smallholder poultry producers. FAO, Rome. [online]. Website: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.520.9557&rep=rep1&type=pdf [accessed: 12.07.2019].

Pym, RAE; Guerne Bleich, E and Hoffmann, I (2006). The relative contribution of indigenous chicken breeds to poultry meat and egg production and consumption in the developing countries of Africa and Asia. *In Proceedings of the XII European Poultry Conference*. Vol. 1014., Verona, Italy, 10-14 September. P: 197.

Rešidbegović, E (2008). Monitoring for avian influenza, Newcastle disease and salmonellosis in extensively kept poultry in the Federation of B&H. Project Final Report, Sarajevo, Bosnia and Herzegovina. The project is funded by Ministry of Agriculture, Water Management and Forestry of the Federation of Bosnia and Herzegovina.

Rešidbegović, E and Kavazović, A (2007). Finding of bacteria from the genus *Salmonella* spp. in poultry. *21st Symposium of Infectious Diseases of Bosnia and Herzegovina with international participation*. Mostar, Bosnia and Herzegovina. P: 60.

Rešidbegović, E and Kavazović A (2008). *Fungal and bacterial diseases of birds*. 1st Edn., Sarajevo, Bosnia and Herzegovina, Promocult d.o.o., PP: 45-56.

Roschanski, N; Fischer, J; Guerra, B and Roesler, U (2014). Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in Enterobacteriaceae. PloS One. 9: e100956.

Shah, DH; Board, MM; Crespo, R; Guard, J; Paul, NC and Faux, C (2020). The occurrence of *Salmonella*, extended-spectrum β-lactamase producing *Escherichia coli* and carbapenem resistant non-fermenting Gram-negative bacteria in a backyard poultry flock environment. Zoonoses Public Hlth., 67: 742-753.

Shah, DH; Paul, NC; Sischo, WC; Crespo, R and Guard, J (2017). Population dynamics and antimicrobial resistance of the most prevalent poultry-associated *Salmonella* serotypes. Poult. Sci., 96: 687-702.

Sonaiya, F (2008). Smallholder family poultry as a tool to initiate rural development. In International Conference Poultry in the Twenty-first Century: Avian Influenza and beyond, Bangkok, Thailand, held 5-7 November 2007. PP: 529-547.

Swayne, RL; Ludlam, HA; Shet, VG; Woodford, N and Curran, MD (2011). Real-time TaqMan PCR for rapid detection of genes encoding five types of non-metallo-(class A and D) carbapenemases in Enterobacteriaceae. Int. J. Antimicrob. Agents. 38: 35-38.

van der Zee, A; Roorda, L; Bosman, G; Fluit, AC; Hermans, M; Smits, PH; van der Zanden, AG; Te Witt, R; van Coppenraet, LEB; Stuart, JC and Ossewaarde, JM (2014). Multi-centre evaluation of real-time multiplex PCR for detection of carbapenemase genes OXA-48, VIM, IMP, NDM and KPC. BMC Infect. Dis., 14: 1-5.

Zhao, X; Gao, Y; Ye, C; Yang, L; Wang, T and Chang, W (2016). Prevalence and characteristics of *Salmonella* isolated from free-range chickens in Shandong Province, China. Biomed. Res. Int., 2016: 1-6.