P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article
https://doi.org/10.47278/journal.ijvs/2022.169

Isolation and Serological Identification of Current *Salmonella* Species Recovered from Broiler Chickens in Egypt

Nagwa S Rabie[1], Hanaa S Fedawy[1]*, Dalia M Sedeek[1], MA Bosila[1], Abdelbaki MM[1], Aly M Ghetas[1], Kh M Elbayoumi[1], Eman R Hassan[1], Zeinab MS Amin Girh[1], Hoda M Mekky[1], Ashraf MA Barakat[2] and AA Samy[3]

[1]Poultry Diseases Department, National Research Centre, P.O. 12622, Giza, Egypt
[2]Zoonotic Diseases Department, National Research Centre, P.O. 12622, Giza, Egypt
[3]Microbiology and Immunology Department, National Research Center, P.O. 12622, Giza, Egypt
***Corresponding author:** hanaafedawy@yahoo.com

Article History: 22-571 Received: 13-Mar-22 Revised: 27-Apr-22 Accepted: 25-May-22

ABSTRACT

This study was carried out to identify and serotype Salmonella (S.) species from suspected broiler chickens and healthy chickens in seven Egyptian governorates (KafrEl-sheikh, Fayuom, Qalubia, Menofiya, Garbia, Cairo, and Giza). Five hundred specimens (liver, spleen, caecum, and bone marrow) were collected from poultry farms and slaughterhouses. Samples were only collected from slaughterhouses in Cairo and Giza governorates. Forty-nine Salmonella isolates were recovered, and their colonies were characterized on specific media. Those Salmonella isolates were morphologically and biochemically identified. Furthermore, 33 out of 500 samples were serologically confirmed as Salmonella serovars by the slide agglutination test based on O and H antigen using 140 commercial polyvalent and monovalent diagnostic Salmonella antisera with a prevalence rate of 6.6%. They were serotyped as *S. blegdam* (39.4%), *S. typhimurium* (21.2%), *S. montevideo* (9.1%), *S. agama* (6.1%), *S. gueuletapee* (6.1%), *S. salamae* (6.1%), *S. enteritidis* (3%), *S. infantis* (3%), *S. kentucky* (3%), and *S. virchow* (3%). The prevalence of Salmonella serovars in KafrEl-sheikh, Fayoum, Qalubia, Menofiya, Garbia, Cairo, and Giza was 8.5%, 4%, 8%, 12%, 8%, 0%, and 0%, respectively. Finally, continuous monitoring of Salmonella serovars in chickens is essential for better prevention and reduction of its zoonotic risk.

Key words: *Salmonella*, Serovars, Isolation, Biochemical identification, Serological identification, Broilers.

INTRODUCTION

Salmonellosis has a great economic impact in poultry industry, due to high morbidity, mortality and reduced production (Khan et al. 1998; Rostagno et al. 2006; Abd El-Hack et al. 2021).

Also, Salmonellosis has a great zoonotic and public health importance in Egypt and worldwide. It causes an important zoonotic food borne disease characterized by mortalities, gastroenteritis, and/or septicemia in humans (Majowicz et al. 2010; Newell et al. 2010; Eng et al. 2015; Balasubramanian et al. 2019; Girh et al. 2019; Haley et al. 2019; Wang et al. 2020). Chickens were considered the main source of *Salmonella* outbreaks because they act as carriers of this pathogen in their guts. The main reservoir for *Salmonellae* is contaminated poultry and eggs (Antunes et al. 2016). Salmonellosis in poultry may occur by one or more strains of genus *Salmonella* either in acute or chronic form (Hofstad et al. 1992). The disease in chickens characterized by significant economic losses due to high morbidity, mortality especially in young chicks, and reduced production (Khan et al. 1998; Rostagno et al. 2006). Additionally, Salmonellae can be transmitted vertically from broiler breeder chickens to their progeny (Lister 1988; Barbour et al. 1999; Wibisono et al. 2020).

Salmonellae are gram-negative, facultatively anaerobic, and non-spore-forming bacteria belong to family Enterobacteriaceae. They biochemically produce carbon dioxide and hydrogen gases from D-glucose, and typically hydrogen sulfide is produced by most *Salmonellae* species. All *Salmonellae* are aerogenic except for *Salmonella* serovar Typhi which never produces gas which does not produce urease, oxidase, and indole (Popoff and LeMinor 2001). About 2600 different *Salmonella* serovars have been described worldwide (Mezal et al. 2014). These serovars were serologically identified according to their somatic (O) and flagellar (H) antigens (Amagliani et al. 2012). European Food Safety Authority

Cite This Article as: Rabie NS, Fedawy HS, Sedeek DM, Bosila MA, Abdelbaki MM, Ghetas AM, Elbayoumi KM, Hassan ER, Girh ZMSA, Mekky HM, Barakat AMA and Samy AA, 2022. Isolation and serological identification of current *salmonella* species recovered from broiler chickens in Egypt. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2022.169

(EFSA) stated the prevalence of *S. infantis* (29.2%) and *S. enteritidis* (13.6%), followed by *S. kentucky* (6.2%) and *S. typhimurium* (4.4%) as the most isolated *Salmonella* serotypes in broiler chickens in European union (EFSA, 2010). Furthermore, different *Salmonella* serovars have been reported in Egypt (Abd El-Ghany et al. 2012; Halawa et al. 2016; El-Sharkawy et al. 2017; Mahmoud et al. 2018; Elkenany et al. 2019; Awad et al. 2020). Thus, continuous isolation and characterization of the most prevalent serovars are essential for improvement of salmonellosis prevention and reduce its public health hazard.

Thus, this study aimed to isolate and characterize the most prevalent *Salmonella* serovars from broiler chicken's farms and slaughter houses in 7 governorates in Egypt in the period between December 2019 till May 2021.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Ethical Committee for Medical Research at the National Research Centre, Egypt (19157).

Chicken Flocks

This study was conducted on 500 samples of freshly dead chickens showed diarrhea from 5 governorates (KafrEl-sheikh, Fayuom, Qalubia, Menofiya, and Garbia), and freshly slaughtered broiler chickens from 2 governorates (Cairo and Giza). All birds were subjected to postmortem examination.

Bacterial isolation and identification

Sampling

For bacteriological examination, a total number (no.) of 500 samples were collected from (liver, spleen, caecum, and bone marrow) from freshly dead broiler chickens in 5 governorates: KafrEl-sheikh (no.200), Fayuom (no.50), Qalubia (no.50), Menofiya (no.50) and Garbia (no.50). Samples from freshly slaughtered broiler chickens were also collected from Cairo and Giza governorates (no.100). All samples were labeled and transported in cool boxes to the laboratory.

Culture Media

Bacterial enrichment was carried out using pre-enrichment buffered peptone water (Oxoid, UK) and incubated at 37°C for 18 hours for *Salmonella* spp. A total amount of 0.1 ml of the pre-enriched culture of suspected *Salmonella spp*. were inoculated into enrichment Rappaport Vassiliadis (RV) broth (Oxoid, UK) at 41°C for 24 h. A loopful of the enriched broth was streaked onto (S.S) agar (Oxoid) and (XLD) agar (Oxoid, UK) and incubated at 37°C for 24 h. Then, the growing colonies were examined for bacterial growth according to (Quinn et al. 1994) and (Collee et al. 1996). The suspected colonies (pink colonies with or without black center on XLD and colorless colonies with black centre on S.S agar) were picked up and purified for further investigation (Quinn et al. 1994). A loopful of the colony was stained with Gram stain and examined under a microscope according to Merchant and Packer (1967). Then kept in semi-solid agar for biochemical analysis (Quinn et al. 2002).

Biochemical Identification

The proper biochemical characterization was carried out using triple sugar iron agar (TSI), urea splitting ability in Christensen's urea agar, carbon utilization in Simmon's citrate agar, lysine iron agar and indole production in tryptone broth (Sneath et al. 1986).

Serological Typing of *Salmonella* Species

The obtained *Salmonella* isolates were subjected to serological identification according to Edward and Ewing (1972) through the application of a standard slide agglutination test based on O and H antigen using 140 commercial polyvalent and monovalent diagnostic *Salmonella* antisera (SINIF Co., Germany).

Statistical Analysis for Prevalence Calculation

The prevalence (P) in percentage was calculated using the formula P = d/n, where d is the number of positive samples analyzed at that point in time and n is the total number of chickens sampled at that point in time according to Thrusfield (2005).

RESULTS

Isolation of *Salmonella* spp. was attempted from 500 field samples (liver, spleen, caecum, and bone marrow) that were collected from poultry farms and poultry slaughterhouses of broiler chickens from seven Egyptian governorates (KafrEl-sheikh, Fayuom, Qalubia, Menofiya, Garbia, Cairo and Giza). Postmortem examination of the collected samples from freshly dead birds was shown nodular myocarditis, pericarditis and hepatitis. However, samples which collected from slaughterhouses had no lesions.

Salmonella suspected isolates showed smooth red colored colonies with black center on XLD and (S-S) agar. *Salmonella* produces colorless colonies with black centers due to H_2S production. Our result revealed that a suspected 49 *Salmonella* isolates out of 500 collected samples were detected by morphological and bacteriological examination. The isolated bacteria were Gram-negative, non-spore-forming and short rod-shaped single or paired in an arrangement under the microscope. These isolates were subjected to further biochemical identification where the suspected *Salmonella* isolates were positive to TSI, carbon utilization in Simmon's citrate agar and lysine iron agar but negative to urea and indole tests as in Table 1. Results of biochemical identification revealed that 49 suspected *Salmonella* isolates were biochemically positive.

Further serological identification using the slide agglutination test based on O and H antigen using 140 commercial polyvalent and monovalent diagnostic *Salmonella* antisera revealed that 33 *Salmonella* erovars were confirmed as *Salmonella* with a prevalence rate of 6.6% (33/500) as in Table 2. A total number of 33 *Salmonella* serovars were serotyped as *S. blegdam* (13 isolates), *S. typhimurium* (7 isolates), *S. montevideo* (3 isolates), *S. agama* (2 isolates), *S. gueuletapee* (2 isolates), *S. salamae* (2 isolates), *S. enteritidis* (1isolate), *S. infantis* (1isolate), *S. kentucky* (1 isolate), and *S. virchow* (1 isolate) as shown in Fig. 1. Results of serotyping demonstrated that the most predominant serotype was *S. blegdam* followed by *S. typhimurium* then *S.* Montevideo. On the

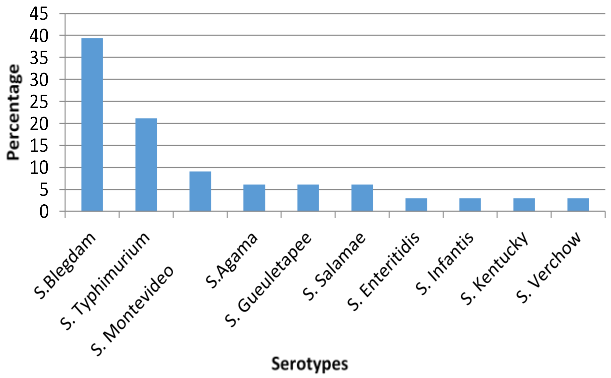

Fig. 1: Percent of serotyped *Salmonella* serovars.

Table 1: Biochemical profile of *Salmonella* isolates

Test	Result
TSI	+ (Alkaline\Slant\Acid bed\H_2S)
Urease	-
Simmons citrate	+
Lysine	+
Indoie	-

TSI: triple sugar iron agar, H_2S: Hydrogen sulfide production, +: positive reaction,-: negative reaction

Table 2: Prevalence of *Salmonella* isolates in different governorates

Governorates	Number of samples	Identified Salmonellae	Percent
KafrElsheikh	200	17	8.5
Fayoum	50	2	4
Qalubia	50	4	8
Menofiya	50	6	12
Garbia	50	4	8
Cairo & Giza Poultry Slaugter houses	100	0	0
Total	500	33	6.6

other hand, *S. agama*, *S. gueuletapee*, and *S. salamae* had the same ratio. As well as *S. enteritidis*, *S. infantis*, *S. kentucky* and *S.* Verchow had the same and the least ratio of isolation.

The prevalence of *Salmonella* spp. in each governorate was shown in Table 2. Our result revealed that the high prevalence rate was detected in Kafr El-sheikh governorate (8.5%) and the lowest prevalence rate was detected in Cairo and Giza poultry slaughterhouse (0%).

DISCUSSION

Salmonella infection in poultry is considered one of the most important bacterial diseases causing heavy economic losses through mortality and reduced production (Haider et al. 2004; Rajan et al. 2017). Chickens can be infected with a wide variety of *Salmonella* serovars. Some serovars are host-specific for chicken such as *S.* Pullorum and *S.* Gallinarum, while other serovars are capable of infecting a wide range of hosts as *S. typhimurium* and *S. enteritidis* (Foley et al. 2008; Girh et al. 2018). Although there were more than 2600 identified serovars, identification and classification of *Salmonella* isolates into different serovars remain as a critical topic for studying the prevalence and surveillance. On the other hand, Salmonellosis is considered a major food borne pathogen in most countries of the world especially in developing countries (Soultose et al. 2003; Carraminana et al. 2004; Chai et al. 2017; Wessels et al. 2021; Ehuwa et al. 2021; EL-Saadony et al. 2022).

In the current study, isolation of *Salmonella* spp. was carried out from 500 field samples (liver, spleen, caecum, and bone marrow). All samples were collected from poultry farms and slaughterhouses of broiler chickens from seven Egyptian governorates. *Salmonella* suspected isolates showed smooth red colored colonies with black center on XLD agar while on (S-S) agar. *Salmonella* produces colorless colonies with black centers due to H_2S production. It has been previously reported that pink colonies with or without black centers were typical for *Salmonella* on XLD. Many cultures of *Salmonella* spp. may also produce large colonies with glossy black centers or may appear as almost completely black (Sujatha et al. 2003; Ramya et al. 2012; Rabie et al. 2012; Islam et al. 2016). Our results revealed that a suspected 49 *Salmonella* isolates out of 500 collected samples were detected by morphological and biochemical examination (Table 1).

Previously, *Salmonella* were recovered from 4 broiler chicken flocks from Qalubia governorate in an incidence rate 3.84%, 4.15%, 5.06% and 5.18% (Abd El-Ghany et al. 2012). Furthermore, an incidence rate 7.5% of *Salmonella* have been detected in broiler chicks during 1st week of life in 5 Egyptian provinces (Sedeik et al. 2019). Our results were also in accordance to Samanta et al. (2014) who identified 22 isolates (6.1%) of *Salmonella* out of the 360 samples from drinking water, cloacal swabs, feed, and eggs. Additionally, *Salmonella* prevalence was recorded as 6%, 5.8 % and 3.5% in Iran, Belgium, and Paraguay respectively (Jafari et al. 2007; Namata et al. 2009; Leotta et al. 2010). In contrast, an incidence rate 14.7%, 10.9% and 12.4% was reported by others (Murugkar et al. 2005; Abd El-Tawab et al. 2015; El-Sharkawy et al. 2017) respectively. Moreover, prevalence rate of 29% have been reported from broiler chickens in Lithuania, 20% in Italy, and 11% in the Netherlands (Van Overbeke et al. 2006; Pieskus et al. 2008). Additionally, a considerably higher prevalence rate was described in other studies in China (52.2%) (Yang et al. 2011), South Africa (51%) (Zishiri et al. 2016) and India (46%) (Srinivasan et al. 2014). It has been reported that the prevalence of *Salmonella* affected by geographical location, age of chickens, seasons of the year, the hygienic status of the farms, or antibiotic using regime (Sikder et al. 2005).

Serological identification demonstrated that 33 *Salmonella* serovars were confirmed as *Salmonella* with a prevalence rate of 6.6% (33 out of 500) as shown in Table 2. In our study, the most predominant serotypes were *S. blegdam* (39.4%) followed by *S. typhimurium* (21.2%) which belongs to O antigen serogroups D1 and B respectively. While, *S. enteritidis* (D1 serogroup), *S. infantis* (C1 serogroup), *S. kentucky* (C3 serogroup), and *S. virchow* (C1 serogroup) had the lowest ratio (3%) (Fig. 1). Both *S. enteritidis* and *S. typhimurium* were the most frequent serotypes isolated in Egyptian poultry farms (Abd El-Ghany et al. 2012; Halawa et al. 2016; El-Sharkawy et al. 2017; Elkenany et al. 2019). Moreover, it was confirmed that *S. kentucky* (C3 serogroup) had been identified as one of the most prominent *Salmonella* serovars isolated from broilers causing diarrhea and high mortalities in Egypt (Mahmoud et al. 2018). Additionally, it was reported that both *Salmonella enteritidis* and *Salmonella kentucky* were

the dominant serovars with 22.6% each (Awad et al. 2020). However, the percent of *S.* enteritidis, *S. virchow* and *S. kentucky* was reported to be 2.4%, 1.4% and 0.8% respectively (Sedeik et al. 2019). Finally, continuous monitoring of *Salmonella* serovars in chickens is essential for better prevention and reduction of its zoonotic risk.

Acknowledgments

We would like to thank Prof. Dr. Ebtehal Abd El Aty Elsayed, chief researcher in Microbiology and Serology Unit, Animal Health Research Institute for her technical support. This work was funded by National Project number (12010140) titled (Control of Salmonellosis in chicken) in National Research Centre. All authors are members of this project.

Author's Contribution

Nagwa S. Rabie, AA Samy and Kh M Elbayoumi carried out the research design, supervision for all the steps and revised the manuscript. Nagwa S Rabie, Hanaa S Fedawy and Ali M Ghetas: wrote and revised the manuscript. Hanaa S Fedawy, Dalia M Sedeek, Abdelbaki MM, MA Bosila, Ali M Ghetas, Hoda M Mekky: isolation and identification of the microbial agent. Eman R Hassan, Zeinab MS Amin Girh, AA Samy and Ashraf MA Barakat: Collection of samples from different poultry farms and Cairo and Giza Poultry Slaughterhouses.

REFERENCES

Abd El-Hack ME, El-Saadony MT, Shafi ME, Alshahrani OA, Saghir SAM, Al-Wajeeh AS, Al-Shargi OYA, Taha AE, Mesalam NM and Abdel-Moneim AE, 2021. Prebiotics can restrict Salmonella populations in poultry: A review. Animal Biotechnology 19: 1-10. https://doi.org/10.1080/10495398.2021.1883637

Abd El-Tawab AA, Fatma EH, Ahmed MA, Soad AN and Nehal MN, 2015. Studies on different *Salmonella* serotypes isolated from poultry in different governorates in Egypt. Benha Veterinary Medical Journal 28: 169-175. https://doi.org/10.21608/BVMJ.2015.32498

Abd El-Ghany WA, El-Shafii SS and Hatem ME, 2012. A survey on *Salmonella* species isolated from chicken flocks in Egypt. Asian Journal of Animal and Veterinary Advances 7: 489-501. https://doi.org/10.3923/ajava.2012.489.501

Amagliani G, Brandi G and Schiavano GF, 2012. Incidence and role of *Salmonella* in seafood safety. Food Research International 45: 780-788. https://doi.org/10.1016/j.foodres.2011.06.022

Antunes P, Mourão J, Campos J and Peixe L, 2016. Salmonellosis: the role of poultry meat. Clinical Microbiology Infection 22: 110–121. https://doi.org/10.1016/j.cmi.2015.12.004

Awad A, Gwida M, Khalifa E and Sadat A, 2020. Phenotypes, antibacterial-resistant profile, and virulence-associated genes of Salmonella serovars isolated from retail chicken meat in Egypt. Veterinary World 13: 440-445. https://doi.org/10.14202/vetworld.2020.440-445

Balasubramanian R, Im J, Lee JS, Jeon HJ, Mageni OD, Kim JH, Rakotozandrindrainy R, Baker S and Marks F, 2019. The global burden and epidemiology of invasive non-typhoidal Salmonella infections. Human Vaccine and Immunotherapeutic 15: 1421-1426. https://doi.org/10.1080/21645515.2018.1504717

Barbour E, Jurdi LH, Talhouk R, Qatanani M, Eid A, Sakr W, Bouljihad M and Spasojevic R, 1999. Emergence of Salmonella enteritidis outbreaks in broiler chickens in the Lebanon: epidemiological markers and competitive exclusion control. Revue Scientifique et Technique 18: 710-718. https://doi.org/10.20506/rst.18.3.1184

Chai S, Cole D, Nisler A, Mahon B, 2017. Poultry: The most common food in outbreaks with known pathogens, United States, 1998-2012. Epidemiology & Infection 145: 316-325. https://doi.org/10.1017/S0950268816002375

Haley BJ, Kim SW, Haendiges J, Keller E, Torpey D, Kim A, Crocker K, Myers RA and Van Kessel JAS, 2019. Salmonella enterica serovar Kentucky recovered from human clinical cases in Maryland, USA (2011-2015). Zoonoses Public Health 66: 382-392. https://doi.org/10.1111/zph.12571

Carraminana JJ, Agustin I and Herrera A, 2004. High prevalence of multiple resistances to antibiotics in *Salmonellae* serovars isolated from a poultry slaughterhouse in Spain. Veterinary Microbiology 104: 133-139. https://doi.org/10.1016/j.vetmic.2004.08.010

Collee JG, Fraser AG, Marmion BP and Simmons A, 1996. Practical Medical Microbiology. 14th Ed. Churchill Livingstone.

Edward PR and Ewing WH, 1972. Identification of Enterobacteriaceae.3rd Edition. Burgess Publishing Co, Minneapolis, USA, pp: 709.

EFSA 2010. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and food-borne outbreaks in the European Union in 2008. European Food Safety Authority 8: 1496.

Ehuwa O, Jaiswal AK and Jaiswal S, 2021. Salmonella, food safety and food handling practices. Foods 10: 907. https://doi.org/10.3390/foods10050907

Elkenany R, Elsayed MM, Zakaria AI, El-Sayed SA and Rizk MA, 2019. Antimicrobial resistance profiles and virulence genotyping of *Salmonella* 384 Enterica serovars recovered from broiler chickens and chicken carcasses in 385 Egypt. BMC Veterinary Research 15: 124. https://doi.org/10.1186/s12917-019-1867-z

El-Saadony MT, Salem HM, El-Tahan AM, Abd El-Mageed TA, Soliman SM, Khafaga AF, Swelum AA, Ahmed AE, Alshammari FA and Abd El-Hack ME, 2022. The control of poultry salmonellosis using organic agents: an updated overview. Poultry Science 101(4): 101716. https://doi.org/10.1016/j.psj.2022.101716

El-Sharkawy H, Tahoun A, El-Gohary A, El-Abasy M, El-Khayat F, Gillespie T, Kitade Y, Hafez HM, Neubauer H and El-Adawy H, 2017. Epidemiological, molecular characterization and antibiotic resistance of *Salmonella* Enterica serovars isolated from chicken farms in Egypt. Gut Pathogen 9: 1-12. https://doi.org/10.1186/s13099-017-0157-1

Eng SK, Pusparajah P, MutalibN AB, Ser HL, Chan KG and Lee LH, 2015. *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science 8: 284-293. https://doi.org/10.1080/21553769.2015.1051243

Foley S, Lynne A and Nayak RJ, 2008. *Salmonella* challenges: prevalence in 392 swine and poultry and potential pathogenicity of such isolates. Journal of Animal Science 86 (E. Suppl): E149-E162. https://doi.org/10.2527/jas.2007-0464

Girh ZMSA, Rabie NS and Zaki MS, 2018. Avian Salmonellosis: A review. World Rural Observations 10: 19-24. https://doi.org/10.7537/marswro100218.04

Girh ZMSA, Rabie NS and Zaki MS, 2019. Avian zoonotic diseases. Stem Cell 10: 12-17. https://doi.org/10.7537/marsscj100219.03

Haider MG, Hossain MG, Hossain MS, Chowdhury EH, Das PM and Hossain MM, 2004. Isolation and characterization of enterobacteria 400 associated with health and disease in

Sonali chickens. Bangladesh Journal of Veterinary Medicine 2: 15-21. https://doi.org/0.3329/bjvm.v2i1.1928

Halawa M, Moawad A, Eldesouky I and Ramadan HJ, 2016. Detection of antimicrobial phenotypes, ß-lactamase encoding genes and class L INTEGRONS in *Salmonella* serovars isolated from broilers. International Journal Poultry Science 15: 1-7. https://doi.org/10.3923/ijps.2016.1.7

Hofstad MS, John BH, Calnek BW, Reid WN and Yoder HW, 1992. Diseases of Poultry, 8th Ed; Panima Education Book Agency, New Delhi, India, pp: 65-123.

Islam JAT, Mahbub-E-Elahi AT and Kamrul H, 2016. Isolation and identification of *Salmonella* spp. from broiler and their antibiogram study in Sylhet. Bangladesh. Journal of Applied Biology & Biotechnology 4: 46–51. https://doi.org.10.7324/JABB.2016.40308

Jafari RA, Ghorbanpour M and Jaideri A, 2007. An investigation into *Salmonella* infection status in back-yard chickens in Iran. International Journal of Poultry Science 6: 227-229. https:// doi.org/10.3923/ijps.2007.227.229

Khan MA, Bari AM, Islam MR, Das PM and Ali MY, 1998. Pullorum disease in semi-mature chicks and its experimental pathology. Bangladesh Veterinary Journal 32: 124-128.

Lister S, 1988. *Salmonella enteritidis* infection in broilers and broiler breeders. Veterinary Record 123(13): 350. https://doi.org/ 10.1136/vr.123.13.350

Leotta G, Suzuki K, Alvarez FL, Nunez L, Silva G, Castro L, Faccioli L, Zarate N, Weiler N, Alvarez M and Copes J, 2010. Prevalence of *Salmonella* spp. in backyard chickens in Paraguay. International Journal of Poultry Science 9: 533-536. https://doi.org/10.3923/ijps.2010.533.536

Mahmoud M, Askora A, Barakat AB, Rabie OF and Hassan SE, 2018. Isolation and characterization of polyvalent bacteriophages infecting multi drug resistant *Salmonella* serovars isolated from broilers in Egypt. International Journal of Food Microbiology 266: 8-13. https://doi.org/10.1016/j.ijfoodmicro.2017.11.009

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A and Hoekstra RN, 2010.The global burden of nontyphoidal *Salmonella* gastroenteritis. Clinical Infectious Diseases 50: 882-889. https://doi.org/10.1086/650733

Merchant IA and Packer RA, 1967. Veterinary bacteriology and virology.7th Ed. The Iowa University Press, Ames, Iowa, USA, pp: 286-306.

Mezal EH, Sabol A, Khan MA, Ali N, Stefanova R and Khan AJ, 2014. Isolation and molecular characterization of *Salmonella enteric* serovar Enteritidis from poultry house and clinical samples during 2010. Food Microbiology 38: 67-74. https://doi.org/10.1016/j.fm.2013.08.003

Murugkar H, Rahman H, Kumar A and Bhattacharyya D, 2005. Isolation, phage typing & antibiogram of *Salmonella* in man & animals in northeastern India. Indian Journal of Medical Research 122: 237-242.

Namata H, Welby S, Meroc E and Mintiens K, 2009. Identification of risk factors for the prevalence and persistence of *Salmonella* in Belgian broiler chicken flocks. Preventive Veterinary Medicine 90: 211-222. https://doi.org/10.1016/j.prevetmed.2009.03.006

Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, Opsteegh M, Langelaar M, Threfall J, Scheutz F, Van der Giessen J and Kruse H, 2010. Food-borne diseases-the challenges of 20 years ago still persist while new ones continue to emerge. International Journal Food Microbiology 139: S3-S15. https://doi.org/10.1016/j.ijfoodmicro.2010.01.021

Pieskus J, Franciosini MP, Proietti PC, Reich F and Kazeniauskas E, 2008. Preliminary investigations on *Salmonella* spp. Incidence in meat chicken farms in Italy, Germany, Lithuania and the Netherlands. International Journal of Poultry Science 7: 813-817. https://doi.org/10.3923/ijps.2008.813.817

Popoff MY and LeMinor L, 2001. Antigenic formulas of the *Salmonella* serovars, 8th Ed. World Health Organization Collaborating Centre for Reference and Research on Salmonella, Paris.

Quinn PJ, Carter ME, Markey BK and Carter GR, 1994. Clinical Veterinary Microbiology. Welfe Publishing.

Quinn PJ, Carter ME, Markey BK, Donnoly WJ and Leonard FC, 2002. Veterinary Microbiology and Microbial Diseases, 166-1117 Osney Mead. Oxford First Ltd, UK.

Rabie NS, Khalifa NO, Radwan MEI and Afify JSA, 2012. Epidemiological and Molecular Studies of *Salmonella* Isolates from Chicken, Chicken Meat and Human in Toukh, Egypt. Global Veterinaria 8: 128-132.

Rajan K, Shi Z and Ricke SC, 2017. Current aspects of Salmonella contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. Critical Reviews in Microbiology 43: 370-392. https://doi.org/10.1080/1040841X.2016.1223600

Ramya P, Madhavarao T and Venkateswara RL, 2012. Study on the incidence of *Salmonella* Enteritidis in poultry and meat samples by cultural and PCR methods. Veterinary World 5: 541-545. https://doi.org/10.5455/vetworld.2012.541-545

Rostagno M, Wesley I, Trampel D and Hurd HS, 2006. *Salmonella* prevalence in 481 market-age turkeys on farm and at slaughter. Poultry Sciences 85: 1838-1842. https://doi.org/10.1093/ps/85.10.1838

Samanta I, Joardar S, Das P, Sar T, Bandyopadhyay S, Dutta T and Sarkar UJ, 2014. Prevalence and antibiotic resistance profiles of *Salmonella* 491 serotypes isolated from backyard poultry flocks in West Bengal, India. Journal Applied Poultry Research 23, 536-545. http://dx.doi.org/10.3382/japr.2013-00929

Sedeik ME, El-shall NA, Awad AM, Elfeky SM, Abd El-Hack M, Hussein EOS, Alowaimer AN and Swelum AA, 2019. Isolation, conventional and molecular characterization of *Salmonella spp.* from newly hatched broiler chicks. AMB Express 9: 136. https://doi.org/10.1186/s13568-019-0821-6

Sikder AJ, Islam MA, Rahman M and Rahman MMB, 2005. Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. International Journal of Poultry Science 4: 905-910.

Sneath PHA, Mair NS, Sharpe ME and Holt JG, 1986. Bergey's Manual of Systematic Bacteriology Vol. 2. Williams and Wilkins Co. Baltimore

Soultose N, Koidis P and Madden RH, 2003. Prevalence of *Listeria* and *Salmonellae* in retail chicken in Northern Ireland. Letters in Applied Microbiology 37: 421-423. https://doi.org/10.1046/j.1472-765x.2003.01423.x

Srinivasan P, Balasubramaniam GA, Gopala TR, Murthy K, Saravanan S and Balachandran PJ, 2014. Prevalence and Pathology of Salmonellosis in 497 Commercial Layer Chicken from Namakkal, India. Pakistan Veterinary Journal 34: 324-328.

Sujatha K, Dhanalakshmi K and Rao AS, 2003. Isolation and characterization of *Salmonella gallinarum* from chicken. Indian Veterinary Journal 80: 473–474.

Thrusfield M, 2005. Veterinary Epidemiology, 2nd Ed, Blackwell Science, UK, pp: 178-187.

Van Overbeke I, Duchateau L, De Zutter L, Albers G and Ducatelle R, 2006. A comparison survey of organic and conventional broiler chickens for *Salmonella* contamination for infectious agents affecting health and food safety. Avian Disease 50: 196-200. https://doi.org/10.1637/7448-093005R.1

Wang X, Wang H, Tingting L, Liu F, Cheng Y, Guo X, Wen G, Luo Q, Shao H, Pan Z and Zhang T, 2020. Characterization of Salmonella spp. isolated from chickens in Central China.

BMC Veterinary Research 16: 299. https://doi.org/10.1186/s12917-020-02513-1

Wessels K, Rip D and Gouws P, 2021. *Salmonella* in Chicken Meat: Consumption, Outbreaks, Characteristics, Current Control Methods and the Potential of Bacteriophage Use. Foods (Basel, Switzerland) 10: 1742. https://doi.org/10.3390/foods10081742

Wibisono FM, Wibisono FJ, Effendi MH, Plumeriastuti H, Hidayatullah AR, Hartadi EB and Sofiana ED, 2020. A review of Salmonellosis on poultry farms: public health importance. Systematic Reviews in Pharmacy 11: 481-486. https://doi.org/10.31838/srp.2020.9.69

Yang B, Xi M, Wang X, Cui S, Yue T, Hao H, Wang Y, Cui Y, Alali W and Meng JJ, 2011. Prevalence of *Salmonella* on raw poultry at retail markets in China. Journal of Food Protection 74: 1724-1728. https://doi.org/10.4315/0362-028X.JFP-11-215

Zishiri OT, Mkhize N and Mukaratirwa SJ, 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella spp*. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort Journal of Veterinary Research 83: 1-11. https://doi.org/10.4102/ojvr.v83i1.1067