Bacteriological characterization of *Salmonella* species isolated from laying ducks

Ashraf. A. Abdeltawab[1]; Ehab. M. El- Nahas[2]; Ahmed. A. Askora[3]; Hayam. S. Abdelaziz[4]

[1]Bacteriology, Immunology and Mycology Dep., Fac. Vet. Med. Banha Univ. [2] virology Dep., Fac. Vet. Med. Banha Univ.[3] Microbiology, Botany Dep., Fac. Science. Zagazig Univ. and [4]Vet. Fac. Vet. Med. Banha Univ.

A B S T R A C T

The current study was aimed to investigate the incidence and bacteriological characterization of *Salmonella* serovars in laying ducks**.** A total of 52 samples of laying ducks were collected from different farms in Kalubia governorate, Egypt**.** Samples were isolated from liver, spleen, ovaries, oviducts and intestine. *Salmonella* isolation revealed a total percentage of 2%; ovaries & oviducts revealed a high incidence among the examined samples (4%), followed by liver & intestine with incidence of 3 and 1%, respectively. The results revealed one isolate of *Salmonella* strain which subjected to biochemical and serological identification. The isolated *Salmonella* was identified as being a non-lactose fermenting, (NLFs) Gram negative rod-shaped organism, oxidase negative, catalase positive, indole and Voges Proskauer (VP) negative, methyl red and Simmons citrate positive, H_2S producing and urea negative. The isolated Salmonella serotyped as S. Typhimurium O1, 4, **[**5**]**, 12: i : 1, 2 at Serological Department of Animal Health Research Center. Antimicrobial sensitivity test was conducted on the isolated *Salmonella* Typhimurium which showed resistance to doxycycline, ampicillin, gentamycin, colistin, vancomycin and neomycin.

Keywords: *Salmonella* Typhimurium**,** Ducks, Antibiotics.

(BVMJ, 34 (1), 2018)

1. INTRODUCTION

Salmonella species live in the intestinal tracts of warm and cold-blooded animals. Some species are ubiquitous; It is the major causes of food-borne disease throughout the world (Altekruse *et al*., 1999; Humphrey, 2002; Schlundt, 2002; Wang *et al*., 2008). *Salmonella* infected poultry represent a source of pathogens for humans, causing severe illness and even death. It is estimated that 16 million new cases of typhoid fever occur each year around the world, mostly in developing country (D'Aoust, 1994; Parry *et al*., 2002; Dimitrov *et al*., 2007); the infection is characterized by a variety of clinical manifestations ranging from high-grade fever to complications including "encephalopathy, peritonitis, perforation and hemorrhage". The commonest serotypes causing disease in humans are *Salmonella* Enteritidis and *Salmonella* Typhimurium (Baggesen *et al*., 2002; Aktas *et al*., 2007).

S. Typhimurium is a common contaminant of poultry and eggs, causing

food-borne diseases and mortalities (Borie *et al*., 2008).

Multidrug resistant *S.* Typhimurium due to the indiscriminate use of antibiotics, changes in food production, food rejection, and preventive measures have incurred significant economic losses to poultry producers (Tsonos *et al*., 2013).

Furthermore, up to 90% of antibiotics given orally, are not fully absorbed in the poultry gut, and can be excreted in the feces without changing (Kumar *et al*., 2005).

The external and internal egg contamination by *Salmonella* during poultry production is a complex issue, influenced by many variables. As a result, implementation of appropriate control measures is extremely difficult (Whiley and Ross, 2015). Egg contamination can occur by two routes, vertical or horizontal. Vertical transmission is a result of reproductive organ colonization (ovary and oviduct) before shell formation, whereas horizontal transmission occurs due to external egg shell contamination (De Reu *et al*., 2006).

Oral challenge of both *S.* Enteritidis and *S.* Typhimurium has the potential to invade the reproductive organs. However, only *S.* Enteritidis has been recovered from egg contents (Gantois *et al*., 2009). The intrinsic properties and resistance to antibacterial compounds enabling *S.* Enteritidis to colonize the oviduct and contaminate egg internal contents are well-known (Gantois *et al*., 2009).

There is, however, limited information on the long term shedding, colonization of reproductive organs and egg contamination by *S.* Typhimurium. the majority of previous studies examined the capability of *S.* Typhimurium to colonize reproductive organs and/or egg contamination frequency up to 3 weeks post-infection, which could fail to unveil the ability of *S.* Typhimurium to cause egg contamination over a prolonged period (Davies and Wales, 2013).

The current study aimed to bacteriological characterization of *Salmonella* species isolated from laying ducks and to achieve that the followings must be done by Isolation of different *Salmonella* serovars from laying ducks, investigation the incidence and antimicrobial sensitivity test for the isolated *Salmonellae*.

2. MATERIAL AND METHODS

2.1. Sampling:

A number of 52 samples were collected from diseased living and freshly dead laying ducks were obtained from different farms located in Kalubia governorate, Egypt. Samples for *Salmonella* are taken from liver, spleen, ovaries, oviducts and intestine. The standard microbiological techniques for detection of different *Salmonella* serovars conducted according to ISO 6579 (2002); 25 g of poultry composite samples were homogenized in a stomacher, for 1 to 2 min in 225 ml of buffered peptone water (BPW) and then incubated under aerobic conditions at 37°C for 16 - 20 hr followed by selective enrichment of 0.1 in 10 ml of Rappaport – Vassiliadis (RV) broth. The RV broth was incubated at 42°C for 18-24 h. The broth was then subcultured onto Xylose Lysine Desoxycholate agar (XLD) agar, then

incubated at 37°C for 18 - 24 h. Presumptive positive colonies (non-lactose fermentative with suitable colony morphology) were identified morphologically, biochemically, serologically by slide agglutination test using polyvalent and monovalent somatic (O), virulence (Vi) and tube agglutination test for flagellar (H) antigens (Serological Department of Animal Health Research Center). The stock cultures were stored in nutrient broth containing 20% V/v glycerol at refrigerator

2.2. Media (ISO 6579, 2002):

2.2.1. Peptone water medium:

Samples were first pre-enriched in buffered peptone water (non- selective liquid broth) which is necessary for transferring swab specimens and to permit the detection of few number or injured *Salmonella*.

2.2.2. Rappaport Vassiliadis broth (RV):

RV broth is a selective enrichment liquid broth that make proliferation of *Salmonella* and inhibition the growth of other competing micro-organisms.

2.2.3. Xylose Lysine Desoxycholate agar (XLD agar):

XLD agar is a selective medium that used for isolation of *Salmonella*.

2.2.4. Nutrient broth medium:

This medium was used for propagation of isolated *Salmonella*.

2.2.5. Nutrient agar medium:

Nutrient agar medium important for the growth of isolated bacteria.

2.2.6. Macconkey agar medium:

This medium was used for differentiating between lactose fermenting (as *E. coli*) and non-lactose fermenting (as *Salmonella*) enterobacteriacae.

2.3. *Identification of Salmonella spp.:*

According to ISO 6579, (2002).

2.3.1. Biochemical identification of isolates:

According to Quinn et al. (2002).

2.3.1.1. H2S production using Triple Sugar Iron agar(TSI):

Isolated colonies were stabbed into the bottom of TSI tubes and then the slants were streaked in zigzag like. Inoculated tubes were incubated at 37°C for 24 hr.

2.3.1.2. Urea hydrolysis test:

Isolated colonies were inoculated on to Christensen urea agar slant and incubated at 37°C, then examined after four hours. If there was no change it was left for 24 hrs at 37°C.

2.3.1.3. Lysine decarboxylation test:

Isolated organisms were stabbed into the bottom of the Lysine decarboxylation broth tubes then the needles were drawn over the slant to produce sufficient surface growth. The inoculated tubes were incubated at 37°C for 24 hr. The colors of the butt and slant were observed.

2.3.1.4. Indole production test:

Indole production test was done by inoculating the test medium with the isolated organism and incubated at 37°C for 48 hr. at the end of incubation, 0.5 ml of Kovac‘s reagent was added. Production of red ring in the alcohol layer indicate a positive reaction.

2.3.1.5.Voges-Proskauer reaction:

Broth was inoculated with the isolated organism and incubated at 37°C for 48 hr, then 0.6 ml of α-naphthol 5% was added to a test tube containing 1ml of the incubated broth followed by 0.2 ml of potassium hydroxide 40%. The tube was gently shaken in which expose the medium to atmospheric oxygen, and then the tube was allowed to remain undisrupted for 10-15 mins. The production of orange red color within 15 mins indicated positive result.

2.3.1.6. Methyl Red test:

The Methyl Red broth was inoculated with the isolated organism and incubated at 37°C for 48 hr, then 5 drops of Methyl Red reagent were added to the broth then the results were taken immediately. Production of red color indicated positive result.

2.3.1.7. Citrate utilization test:

A Simmon‘s citrate agar slope was inoculated as a single streak on the surface with the tested isolates then incubated at 37°C for 48 hr. Production of deep blue color indicated positive result.

2.3.1.8. Sugar fermentation test:

Sugar media as 1% peptone water containing 1% Andrade‘s indicator plus 1% of the following required sugars: (glucose, lactose, sucrose, mannitol, salicin and adontil) and Durham‘s tube.

2.3.2. Serological identification of Salmonella:

The isolates which were identified biochemically as Salmonella were subjected to serological identification and carried out according to Kauffman- white scheme as described by Kauffman (1974) by slide agglutination test using polyvalent and monovalent somatic (O), virulence (Vi) and tube agglutination test for flageller (H) antigens (Serological Department of Animal Health Research Center).

2.4. Sensitivity of isolated Salmonella spp to different antimicrobials:

S. Typhimurium which isolated from laying ducks was tested for the sensitivity to 15 antimicrobial agents. *Salmonella* strain was spread on nutrient agar plates and disks containing different antibiotics were placed on culture. After incubation the halos of inhibition were measured Table (1).

Table (1): Inhibition zone diameter standard to antimicrobials: (CLSI,2011)

Antibiotic	Antibiotic code	Disc Potency *Mg/disc*	Zone diameter standard (mm)		
			Resistant (R)	Intermediat (I)	Susciptable (S)
Ampicillin	*AM*	10	≤ 13	14-16	≥ 17
Amoxicillin	*AX*	25	≤ 11	12-13	≥ 14
Doxycycline	*DO*	30	≤ 10	11-13	≥ 14
Gentamycin	*CN*	10	≤ 12	13-14	≥ 15
Trimethoprim/ sulphamethoxazole	*SXT*	25	≤ 10	11-15	≥ 16
Chloramphenicol	*C*	30	≤ 12	13-17	≥ 18
Norfloxacin	*NOR*	10	≤ 12	13-16	≥ 17
Ciprofloxacin	*CIP*	5	≤ 14	15-17	≥ 18
Colistin Sulphate	*CL*	10	≤ 8	9-10	≥ 11
Imipenem	*IPM*	10	≤ 13	14-15	≥ 16
Tetracycline	*TE*	30	≤ 11	12-14	≥ 15
Streptomycin	*S*	10	≤ 11	12-14	≥ 15
Vancomycin	*VA*	30	≤ 14	15-16	≥ 17
Nalidixic acid	*NA*	30	≤ 13	14-18	≥ 19
Neomycin	*N*	30	≤ 12	13-16	≥ 17

3. RESULTS

3.1. Isolation and Identification of Salmonella spp.:

Salmonella organism on XLD agar appear as smooth colonies with black center. The highest percentage of recovery was ovaries followed by liver and finally intestine 45%, 35% and 20% respectively.

3.2. Biochemical identification:

Biochemical identification of the isolated *Salmonella* using standard laboratory tests give the results in Table (2)

3.3. Results of serological identification of the isolated Salmonella:

Serological identification of *Salmonella* recovered from different organs revealed that isolation of *S.* Typhimurium which serotyped to O1, 4, **[**5**]**, 12: i: 1, 2.

3.4. Results of antimicrobial sensitivity test for the isolated Salmonella using disc diffusion method:

The isolated *Salmonella Typhimurium* was resistant to doxycycline, ampicillin, gentamycin, colistin, vancomycin and neomycin while, sensitive to amoxicillin, trimethoprim/ sulphamethoxazole, chloramphenicol, norfloxacine, ciprofloxacin, impenem, naldixic acid, tetracycline and streptomycin antimicrobial drugs (Table 3).

Table (2): Results of biochemical identification of the isolated Salmonellae using standard laboratory tests:

Type of media	Result of biochemical identification
Urea agar	Negative result – the color of urea agar was yellow.
Triple sugar iron agar	Positive result – alkaline slant (red), acid butt(yellow) with H2S and gas production.
Lysine iron agar	Positive result – Deep purple (alkaline) slant and alkaline butt, No gas production, No H2S production.
Simmons Citrate	Positive result – Blue color.
Indole reaction	Negative result – Yellow ring.
Methyl Red test	Positive result – Red color at the surface.
Voges-Proskauer reaction	Negative result – No bright red color.

Table (3): Antimicrobial sensitivity of isolated Salmonella:

Antibiotic discs / Strain	Doxycycline	Amoxicillin,	Ampicillin	Trimethoprim/Sulphamethoxazole	Chloramphenicol	Norfloxacin	Gentamycin	Ciprofloxacin	Imipenem	Colistin	Naldixic acid	Tetracycline	Vancomycin	Streptomycin	Neomycin
S. Typhimurium	R	S	R	S	S	S	R	S	S	R	S	S	R	S	R

S = represent sensitive strains, and **R** = resistant strains to antibiotics tested.

4. DISCUSSION

Salmonella organisms is a leading cause of foodborne illness in many countries which poultry being important vehicle of transmission (Threlfall *et al*., 2014). In the present study one isolate recovered from internal organs (as ovaries, oviducts, liver and intestine) of laying ducks had symptoms of salmonellosis. The isolate was defined as Salmonella Typhimurium where Zoo El Fakar and Rabie (2009) recovered S. Gallinarum and Abd El Fatah (2014) recovered S. Gallinarum from intestine and oviduct of layers. The highest percentage of recovery was ovaries followed by liver and finally intestine 45%, 35% and 20% respectively. Salmonella were recovered from liver samples of diseased poultry with incidence of 5.4% by Sharawy (2006) and 2% by Abd El Fatah (2014). On the other hand Akond *et al.* (2012) found that the highest proportion of Salmonella contamination was in the intestinal fluid samples 60%.

The isolation frequency of *Salmonella* strains resistant to one or more antibiotics have increased in the Saudi Arabia, United States, United Kingdom and other countries of the world. This is due to the increased and uncontrolled use as well as easy accessibility to antibiotics in many countries of the world (Gross *et al*., 1998; Yu *et al*., 2008). Emerging resistance in *Salmonella* Typhi has been described especially in Africa and Asia and the appearance of *Salmonella* Typhimurium DT104 in the late 1980s raised main public health concern, thereby threatening the lives of infected individuals (Grob *et al*., 1998; Montville and Matthews, 2008). Van *et al*. (2007) stated that multi-resistance occurred in *Salmonella* serotypes including Albany, Anatum, Havana, London and Typhimurium. The resistance towards the traditional first-line antibiotics such as ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole define multidrug resistance (MDR) in *Salmonella Enterica* (Crump and Mintz, 2010).

In our study the isolated *Salmonella* Typhimurium was resistant to doxycycline, ampicillin, gentamycin, colistin, vancomycin and neomycin while, sensitive to amoxicillin, trimethoprim/ sulphamethoxazole, chloramphenicol, norfloxacine, ciprofloxacin, impenem, naldixic acid, tetracycline and streptomycin antimicrobial drugs.

5. REFERENCES

Abd Elfatah, A.A. 2014. Detection of multidrug resistant Salmonellae. M.V.Sc thesis, Fac. of Vet. Med. Cairo univ.

Akond, M.A., Shirin, M., Alam, S., Hassan, S.M.R., Rahman, M.M., Hoq, M. 2012. Frequency of drug resistant *Salmonella spp.* isolated from poultry samples in Bangladesh. Stamford Journal of Microbiology 2(1): 15-19.

Aktas, Z., Day, M., Kayacan, C.B., Diren, S.; Threlfall, E.J., 2007. Molecular characterization of Salmonella Typhimurium and Salmonella Enteritidis by plasmid analysis and pulsed-field gel electrophoresis. *International journal of antimicrobial agents*, *30*(6): 541- 545.

Altekruse, S.F., Stern, N.J., Fields, P.I., Swerdlow, D.L. 1999. Campylobacter jejuni--an emerging foodborne pathogen. *Emerging infectious diseases*, *5*(1): 28.

Baggesen, L.S., Aarestrup, F.M. 2002. Characterization of Salmonella enterica Serovar Typhimurium DT104 Isolated from Denmark and Comparison with Isolates from Europe and the United States. J. Clin. Microbiol. pp. 1581-1586.

Borie, C., Albala, I., Sànchez, P., Sánchez, M. L., Ramírez, S., Navarro, C., Robeson, J. 2008. Bacteriophage treatment reduces Salmonella colonization of infected chickens. *Avian diseases*, *52*(1): 64-67.

Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S21, 2011 Wayne, PA, USA CLSI

Crump, J.A., Mintz, E.D. 2010. Global trends in typhoid and paratyphoid fever. *Clinical Infectious Diseases*, *50*(2): 241- 246.

D'Aoust, J.Y. 1994. Salmonella and the international food trade. *International journal of food microbiology*, *24*(1-2): 11 - 31.

Davies, R.H., Wales, A.D. 2013. Salmonella contamination of cereal ingredients for animal feeds. Vet Microbial. 25; 166(3-4):543-9.

De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J., Herman, L. 2006. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis. *International journal of food microbiology*, *112*(3): 253-260.

Dimitrov, T., Udo, E.E., Albaksami, O., Kilani, A.A., Shehab, E.D.M. 2007. Ciprofloxacin treatment failure in a case of typhoid fever caused by Salmonella enterica serotype Paratyphi A with reduced susceptibility to ciprofloxacin. *Journal of medical microbiology*, *56*(2): 277 - 279.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T. J., Van Immerseel, F. 2009. Mechanisms of egg contamination by Salmonella Enteritidis. *FEMS microbiology reviews*, *33*(4): 718-738.

Gross, U., Tschäpe, H., Bednarek, I., Frosch, M. 1998. Antibiotic resistance inSalmonella enterica serotype typhimurium. *European Journal of Clinical Microbiology and Infectious Diseases*, *17*(6), 385-387.

Humphrey, T. 2000. Public-health aspects of Salmonella infection. *Salmonella in domestic animals*, *1*, 245-263.

ISO 6579, 2002. E 4rd ed. Microbiology - General guidance on methods for the detection of Salmonella, International Organization for Standardization, Geneve, Switzerland.

Kauffman, G. 1974. Kauffmann white scheme. *J. Acta. Path. Microbiol. Sci*, *61*, 385.

Kumar k., Gupta S. C., Chander Y., Singh A. K. 2005. Antibiotic use an agriculture and its impact on the terrestial environment. *Adv. Agron.* 87 1–54.

Montville, T.J., Matthews, K.R. 2008. Food Microbiology: An Introduction, 2nd (edn).

Parry, C.M., Hien, T.T., Dougan, G., White, N.J., Farrar, J.J. 2002. Typhoid fever. N. Engl. J. Med. 347: 17-82.

Quinn, G.P., Keough, M.J. 2002. Experimental design and data analysis for biologists. Cambridge University Press.

Schlundt, J. 2002. New directions in foodborne disease prevention. International journal of food microbiology, 78(1-2): 3-17.

Sharawy, H.I. 2006. new approaches for studying different aspects of Salmonellosis. Ph.D. in microbiology cairo university.

Threlfall, E.J.J., Wain, T., Peters C., Lane, E., De Pinna, C.L., Little, A.D. Wales, R.H., Davies. 2014. Egg-borne infections of humans with salmonella: not only an S. Enteritidis problem. World's Poultry Science Journal 70 (01):15-26.

Tsonos J., Vandenheuvel D., Briers Y., De Greve H., Hernalsteens J.P., Lavigne R. 2013. Hurdles in bacteriophage therapy: deconstructing the parameters. *Vet. Microbiol;* 171: 460–469.

Van, T.T.H., Moutafis, G., Istivan, T., Tran, L.T., Coloe, P.J. 2007. Detection of Salmonella spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Applied and environmental microbiology*, *73*(21): 6885-6890.

Wang, L., Shi, L., Alam, M. J., Geng, Y., Li, L. 2008. Specific and rapid detection of foodborne Salmonella by loop-mediated isothermal amplification method. *Food Research International*, *41*(1): 69-74.

Whiley, H., Ross, K. 2015. Salmonella and eggs: from production to plate. *International journal of environmental research and public health*, *12*(3): 2543-2556.

Yu, C.Y., Chou, S.J., Yeh, C.M., Chao, M.R., Huang, K.C., Chang, Y.F., Chu, C. 2008. Prevalence and characterization of multidrug-resistant (type ACSSuT) Salmonella enterica serovar Typhimurium strains in isolates from four gosling farms and a hatchery farm. *Journal of clinical microbiology*, *46*(2): 522-526.

Zoo El-fakar Saher, A., Rabie Nagw, S. 2009. Immunogenic properties of outer membrane protein of Salmonella in chicken Global Veterinaria 3(2): 75-79.