Kafrelsheikh Vet. Med. J. Vol. 12 No. 1 (2014) (329-347)

INVESTIGATION OF SALMONELLA ENTERITIDIS COLONIZATION IN THE REPRODUCTIVE TRACT, FORMING EGG, FRESHLY LAID EGGS OF NATURALLY INFECTED DUCKS

Torky, H. A.[1] *and Hefnawi, S.*[2]

[1] Professor of Microbiology, Faculty of Veterinary Medicine, Alexandria University.

[2] Master student, Microbiology department, Faculty of Veterinary Medicine, Alexandria University.

ABSTRACT

Salmonella enterica serovar enteritidis the "hot strain" infected the ovary, intestine, forming egg of naturally infected ducks proved to have unique DNA allele by ERIC-PCR. The present study sought to determine whether several Salmonella Enteritidisisolates obtained from forming egg associated with reproductive tissue by naturally infected duckshad unique DNA allele or not? Ninety five ducks fromdifferent three farms in this study (thirty six from farm one, thirty one from farm two and twenty eight from farm three) and 135 freshly laid eggs collected from the same farms.Nine ducksout of theninety fiveducks were positive for detection of Salmonella Enteritidis bacteriologically, typed serologically and confirmed with MultiplexPCR on gene OMPC (204 bp) for genus Salmonella and gene ENT (304 bp) for Salmonella serotype Enteritidis. Salmonella Enteritidiswasdetectedin ovaries; oviduct, forming eggs and intestine of one duck were 4, 2, 2 and 4cases respectively out of 95ducks tissue organs. Salmonella Enteritidis in outer eggs shell was 3 and 0 in internal eggs contents out of 135 freshly laid eggs of the same farms.

INTRODUCTION

Salmonella infections are important as a cause of clinical disease in duck and as a source of food-borne transmission of disease to humans. Heavy economic losses occur due to morbidity, mortality, reduced egg and meat production in duck. Mortality may vary from 10% to 80% or higher in severe outbreaks ***Kleven and Yoder, (1998).***

Although ducks are very resistant to systemic infection caused by Salmonella, they are potential reservoirs of this organism and may shed it in the faeces, contaminating the environment ***Barrow et al., (1999).*** There was an outbreak of *S. Enteritidis* linked to eggs contaminated by this pathogen ,In British Columbia between 2007 and 2010 and identified eggs as the most likely source of illness ***Taylor et al., (2012).*** The deposition of *S. Enteritidis*within eggs seems to result from the colonization of reproductive organs, particularly the ovary and upper oviduct, in systemically infected hens ***(Okamura et al., 2001a, b and De Buck et al., 2004).*** The site of *S. Enteritidis*deposition in eggs (albumen or yolk) may be determined by the region of the duck's reproductive tract that is colonized ***Gast and Holt (2000b).***

Egg contamination by S. Enteritidis can be caused by penetration through the egg shell from contaminated faeces after or during oviposition ***Humphrey et al., (1991b)*** or by direct contamination of yolk, albumen, egg shell membranes or eggshells before oviposition originating from the infection of reproductive organs with S. Enteritidis ***Shivaprasad et al., (1990).***

Lysozymes are one of the major antimicrobial molecules of the egg albumen, ***(Skaar, 2010).*** Lysozyme acts on bacteria mainly by hydrolyzing the beta-1, 4 glycosidic bonds between N-acetylmuramic

acid and N-acetylglucosamine, resulting in degradation of peptidoglycan, and subsequent cell lysis ***(Bera et al., 2005). Naknukool et al., (2006)*** supposed that reduced Duck lysozyme could be the alternative antimicrobial agent for food industry to supply the customers who are concerned about natural foods, as it proposed higher activity than reduced chicken lysozyme.

Traditional detection methods for Salmonella species which based on culture using selective media and characterization of suspicious colonies by biochemical and serological tests are generally time consuming ***Wallance et al., (1999)*** while polymerase chain reaction (PCR) is considered a rapid and sensitive method for detection of Salmonella species ***(Stone et al., 1994 and Abouzeed et al., 2000).***

Among the molecular methods for typing bacteria, ERIC-PCR genomic fingerprinting have been found to be extremely reliable, reproducible, rapid and highly discriminatory ***Versalovic et al., (1994).*** ERIC-PCR useful for differentiation of field-isolated Salmonella strains and epidemiological studies ***Hyungkun et al., (2005).***

Experimentally many authors tried to prove that egg infection occurred as ascending infection from the cloaca ***(Keller et al., 1995 and Miyamoto et al., 1997, 1999)*** or descending infection of the reproductive tract and lining of the oviduct of hen duck ***Hoop and Pospischil,(1993)***

The present study was aimed to prove the unique of DNA of Salmonella species isolated in reproductive tract, intestine, and egg in naturally infected duck using molecular technique by ERIC-PCR.

MATERIALS AND METHODS

A total number of 95hen ducks from different three farms from kafr El-Sheikh province,after slaughtering were examined for detection of *Salmonella Enteritidis* (from different duck tissue; Ovary, Oviduct, Forming eggs and Intestinal tissue). And a total number of 135 freshly laid eggs were collected from the same farms separately. The tissue samples were taken as portions of the ovary, oviduct, forming eggs and intestine from each duck were aseptically removed, inoculated in Tetrathionate broth and Rappaport Vassiliadis broth then transferred rapidly to the laboratory. Each broth culture was incubated for 24 hours at 37 °C. Egg samples were taken as follow: A- Outer shell: cotton swabs were immersed in peptone water and passed on all outer shell surfaces then pre-enriched in tube containing 5 ml peptone water ***Stephenson et al., (1991).*** B- Internal eggs contents: entire liquid contents of each egg were thoroughly mixed with sterile pipette separately and aspired by sterile pipette 0.6 ml transferred to 15 ml of TS broth and incubated for 24 hour at 37 °C ***Waltman et al., (1998)*** then cultured on selective media like Xylose Lysine Deoxycholate agar (XLD), Salmonella-Shigella Agar (SSA) and MacConkey Agar. Petridishes were incubated at 37°C for 24 hours ***(Oxoid, 1982).*** The presumptive colonies of Salmonella were taken for further identification by biochemical tests like IMViC (Indole, Methyl red, Voges-Proskauer, Citrate), Triple sugar iron test, and sugar fermentation tests ***Edward and Ewing (1972).*** Then serologically diagnosed by polyvalent and monovalent O and H Salmonella antisera, Multiplex-PCR analysis for confirmation of *Salmonella Enteritidis* using primers specific for *Salmonella* genus with 204 bp according to ***Kwang et al., (1996)*** wasused:

(i) OMPCF: ATC GCT GAC TTA TGC AAT CG.

(i) OMPCR: CGG GTT GCG TTA TAG GTC TG

And primersspecific for *Salmonella* serotype Enteritidiswith 304 bp according to ***Agron et al., (2001)*** wereused:

(ii) ENTF: TGT GTT TTA TCT GAT GCA AGA GG.

(ii) ENTR : TGA ACT ACG TTC GTT CTT CTG G.

The primers used for Enterobacterial Repetitive Intergenic Consensus (ERIC) fingerprinting ***Versalovic et al., (1991)*** have been previously applied to epidemiological studies of Salmonella according to ***Millemann et al., (1996).*** There are two primer used in ERIC-PCR.

(i) DG111 (ERIC1R): ATGTAAGCTCCTGGGGATTCAC.

(ii) DG112 (ERIC2): AAGTAAGTGACTGGGGTGAGCG.

About 1.5 ml of enriched broths were taken in eppendorf tubes and centrifuged at 8000 rpm for 10 min and the supernatant was discarded. Fifty µl of sterile distilled water was added to the tubes and boiled in water bath at 100 °C for 10 min and immediately snap chilled to release DNA. Then centrifuged at 13,000 rpm for 5 min and the supernatantcontaining DNA from respective samples were used as templatefor PCR analysis. Mixture containing 2 µl of 10x Taq DNA polymerase buffer containing 100 mMtris with pH 9.0, 500 mMKCl, 15 mMMgCl and 1% triton X-100), 2 µl of 10 mMdNTP mix, 0.9 U/µl of Taq DNA polymerase (Genei), 2 µl each of forward and reverse primer (4 pmol/µl) and 5 µl of crude bacterial cell lysate. This mixture was made up to 20 µl using molecular grade water. Amplification was done

in thermal cycler each cycle consisted of a 1-min denaturation step (94 °C), a 2-min annealing step (35 °C) and a 2-min extension step (72 °C). Products were extended for7 min (72 °C) after 45 amplification cycles. For ERIC, each cycle consisted of a 45-s denaturation step (94 °C), a 45-s annealing step (55°C) and a 45-s extension step (72 °C). After 30 amplification cycles, products were extended for 7 min (72 °C). ***Soumetet al., (1999).***

The amplified DNAfragments were resolved by agarose gel electrophoresis, stained with ethidium bromide (0.5µg/ml) and visualized with an UV transilluminator, ***Sambrook et al., (1989).***

RESULTS AND DISCUSSION

The results of current study were tabulated in tables (1-2) and illustrated with figures (1 and 2):

Table (1): Show the number of positive hen ducks for Salmonella Enteritidis.

No. of farm	Total no. of ducks in farmeach	No. of examined duck for Salmonella	No. of positive Ducks for Salmonella	Percent of positive ducks for Salmonella
Farm A	10000	36	4	11.1 %
Farm B	8000	31	3	9.7 %
Farm C	5000	28	2	7.6 %
Total	23000	95	9	9.47 %

Table (2): Salmonella Enteritidis isolates fromduck reproductive organs, intestine eggs shell and internal egg contents of ducks at slaughter.

No. of farm	Reproductive organs			Intestine	Eggs	
	Ovary	Oviduct	Forming eggs	Iliocecal junction	Outer shell	Eggs contents
Farm A	3	-	2	3	-	-
Farm B	-	2	-	-	2	-
Farm C	1	-	-	1	1	-
Total	4	2	2	4	3	-

*No= number

Fig (1) show multiplex PCR amplification, lane 1: Salmonella Enteritidis isolated from oviduct, lane 2: Salmonella Enteritidis isolated from intestine, lane 3: Salmonella Enteritidis isolated from ovary, lane 4: Salmonella Enteritidis isolated from forming egg and lane 5: Salmonella Enteritidis isolated from freshly laid egg.

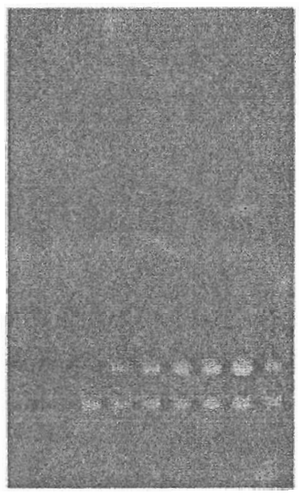

FIG. 1. Multiplex PCR amplification; Lane M, molecular weight marker (100 bp ladder); ct1 = *S. typhi* control, ct2. = *S. enteredis* control, lane 1: Salmonella Enteritidis isolated from (oviduct) ; lane 2: Salmonella Enteritidis isolated from (intestine) ; lane 3: Salmonella Enteritidis isolated from (ovary); lane 4: Salmonella Enteritidis isolated from (forming egg) ;lane 5: Salmonella Enteritidis isolated from (freshly laid egg)

Fig (2) show ERIC fingerprinting patterns of Salmonella Enteritidis strains lane 1: Salmonella Enteritidis isolated from oviduct, lane 2: Salmonella Enteritidis isolated from intestine, lane 3: Salmonella Enteritidis isolated from ovary, lane 4: Salmonella Enteritidis isolated from forming egg and lane 5: Salmonella Enteritidis isolated from freshly laid egg.

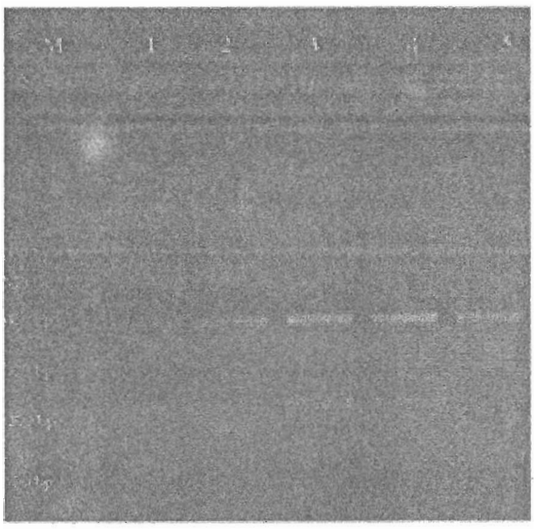

Fig. 2. ERIC fingerprinting patterns of Salmonella Enteritidis strains. Lane M, molecular weight marker (100 bp ladder); lane 1: Salmonella Enteritidis isolated from (oviduct) ; lane 2: Salmonella Enteritidis isolated from (intestine) ; lane 3: Salmonella Enteritidis isolated from (ovary); lane 4: Salmonella Enteritidis isolated from (forming egg) ; lane 5: Salmonella Enteritidis isolated from (freshly laid egg)

The rational basis for salmonellosis control in the duck industry relies heavily on research into salmonellosis in chickens, which may not always be appropriate. Very little recent information is available on the characteristics and pathogenesis of salmonella infections in commercial ducks ***Pomeroy et al., (1991).***

Infection with *Salmonella* usually starts by ingestion, followed by colonization in the intestine. After colonization, *Salmonella* is able to penetrate the mucosal epithelium which results in a systemic infection,

with colonization of the spleen, liver and ovaries ***Carroll et al., (2004).*** Evidently some strains of *Salmonella Enteritidis* had become more invasive in chickens reaching the ovary, oviduct and eggs ***Mason (1999).***

During this study out of 95 duck samples 9 were found to be positive to Salmonella Enteritidis and among the positive samples, 4 were from farm A, 3 were from farm B and 2 were from farm C. The percentages of positive samples from these farms were 11.1%, 9.7% and 7.6%, respectively **(Table 1).**

Table (2) show the number of positive sample for*Salmonella Enteritidis* in ovaries, oviduct and forming eggs were 4, 2 and 2 respectively. While, in intestine were 4, zeroin internal eggs contents and on outer shell three were positively of one hundred and thirty five freshly laid eggs.

*Salmonella Enteritidis*in ovaries were 4 out of 95 examined ovaries; ***Gast et al., (2011b)*** isolated *Salmonella Enteritidis* by percentage of 20.8% form ovaries, ***Wael et al., (2011)*** *S.E* isolates from ovaries of layer hens were 22% and ***Gast et al., (2013)*** detected *Salmonella Enteritidis* from ovaries were 10.4%.

Colonization of *Salmonella Enteritidis* in oviducts were 2 out of 95examined oviducts of ducks this result agree with ***Gast et al., (2013)*** who found it at 2.1%. but, very lower than ***Wael et al., (2011)*** who found it at 27.9% and ***Gast et al., (2011b)*** who found its frequencies ranged from 8.3% to 33.3% for oviducts.

*SalmonellaEnteritidis*isolated fromforming eggs were 2 positive samplesthis result disagree with ***Keller et al.,(1995)*** whofound it at a

percentage of 27.1%; observed a higher contamination rate of forming eggs as compared with laid eggs. They suggested a heavier colonization of the eggs during their development, diminished by factors within the eggs,such as antibodies, antibacterial enzymes, iron-sequestering and bacterial protease-inhibiting proteins, controlling the pathogen before the eggs are laid.

Salmonella Enteritidis in **table (2)** was 4 out of 95 examined intestine of ducks at 4.21%. ***Abd El-gawad (2010)*** isolated *Salmonella Enteritidis* from cloacal swabs at 7.8%. While ***Temelli et al., (2010)*** found that isolated *Salmonella Enteritidis* in intestine were 50.0% and ***Wael et al., (2011)*** reported that isolates from caeca were 30.8%. This variation may be referred to variation in degree of preventive control measures applied in layer farms.

Three freshly laid duck eggs out of 135 eggs had S. *Enteritidis* contamination in its outer shell surface with a percentage 2.22%. ***Gast et al., (2004)*** were recovered it in 40% of chicken egg shell and **Wael *et al.*, *(2011)*** on 59.28% while ***EFSA (2007)*** rate was 0.8% and ***Schroeder et al., (2005)*** at 0.005%. The correlation between the presence of Salmonella in faeces and egg shell contamination ***Gast and Beard (1990b)*** explain the difference in percentage concerning duck eggs it is not present in the available literature

Eggs may be contaminated with *Salmonella Enteritidis* during formation in the ovary and oviduct (on egg contamination) ***Gast and Beard (1990a)***. Several lines of evidence support the view that egg contamination with *Salmonella Enteritidis* is more likely to take place

during the formation of the egg in the reproductive organs than by egg shell penetration ***Michailidiset al.,(2010).*** The result in this investigation contests these views.

Isolation of *Salmonella Enteritidis* from egg shell as a result from intestinal carriage, was expected as there is direct correlation between the presence of *Salmonella* in faeces and egg shell contamination ***Gast and Beard (1990b).***

The natural associations of serovar Enteritidis with tubular gland cells of the oviduct, eggs, both after natural infection reports arerare ***Hoop and Popischil(1993).*** And the predilection for invasion of ovarian tissue and eggs is not fully explained, it thought to virulence factors such as binding of type 1 fimbrial to secretions of shell membrane producing glands, high molecular weight lipopolysaccharide and high density growth ***Guan et al.,(2006).***

The genome of bacteria is composed of conserved core of genes for essential cellular processes and flexible gene pool enables the bacterium to adapt to special environment conditions ***Dobrindt, (2005).***

There is indication that *Salmonella Enteritidis* survive the attack with the help of antimicrobial molecules during the formation of the egg in the hen's oviduct and inside the egg. This appears to require a unique combination of genes encoding for improved cell wall protection and repairing cellular and molecular damage, among others ***InneGantois et al., (2008).*** In thisinvestigation using ERIC-PCR confirm its unique DNA allele.

REFERENCES

- ***Abdel-Gawad, M. H. (2010):*** Detection of Salmonella Enteritidis in chicken environment. M. V. SC. Thesis (Bacteriology) Fac. Vet. Med. Alexandria University.
- ***Abou-Zeed, Y.M.; Hariharan, H.; Poppe, C. and Kibenge, F.S. (2000):*** Characterization of Salmonella isolate from beef, broiler chicken and human sources on Prince Edward Island. Comp. Immunol. Microbiol. Infect. Dis., 23(4):253-266.
- ***Agron, P. G., R. L. Walker, H. Kinde, S. J. Sawyer, D. C. Hayes, J. Wollard, and G. L. Andersen. (2001):*** Identification by subtractive hybridization of sequences specific for Salmonella enterica serovars Enteritidis. Appl. Environ. Microbiol., 67:4984–4991.
- ***Barrow, P.A; Lovell, M.A; Murphy, C.K and Page, K. (1999):*** Salmonella infection in a commercial line of ducks; experimental studies on virulence, intestinal colonization and immune protection. Epidemiology and Infection; 123(1):121-32.
- ***Bera, A., Herbert, S., Jakob, A., Vollmer, W., Götz, F., (2005):*** Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferaseOatA is the major determinant for lysozyme resistance of Staphylococcus aureus. Mol. Microbiol. 55:778-787.
- ***Carroll, P., R. M. La Ragione, A. R. Sayers, and M. J. Woodward. (2004):*** The O-antigen of Salmonella enterica serotype Enteritidis PT4: a significant factor in gastrointestinal colonization of young but not newly hatched chicks. Vet. Microbiol. 102:73–85.

- ***De Buck, J., Van Immerseel, F., Haesebrouck, F., Ducatelle, R., (2004):*** Colonization of the chicken reproductive tract and egg contamination by Salmonella. J. Appl. Microbiol. 97, 233–245.
- ***Dobrindt, U. (2005):*** Genomics of Escherichia coli. Int. J. Med Microbiol 295, 357- 371.
- ***Edwards, P.R and Ewing, W.H (1972):*** Identification of Enterobacteriaceae. 3rd ed. Burgers publishing Company USA.
- ***EFSA (2007):*** The community summary report on trends and sources of zoonosis, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA J 130: 34–117.
- ***Gast R. K., and Holt, P. S. (2000b):*** Deposition of phage type.4 and 13a Salmonella enteritidis strains in the yolk and albumen of eggs laid by experimentally infected hens. Avian Dis. 44:706–710.
- ***Gast, R. K.; Guard-Bouldin, J. and Holt, P. S. (2004):*** Colonization of reproductive organs and internal contamination of eggs after experimental infection of laying hens with Salmonella Heidelberg and Salmonella enteritidis. Avian Dis. 48:863–869.
- ***Gast, R.K. & Beard, C.W. (1990a):*** Isolation of *Salmonella Enteritidis* from internal organs of experimentally infected hens. Avian Diseases, 34:991- 993.
- ***Gast, R.K. & Beard, C.W. (1990b):*** Production of Salmonella enteritidis contaminated eggs by experimentally infected hens. Avian Diseases, 34, 438–446.
- ***Gast, R.K.; Guraya, R.; Guard, J. and Holt, P.S. (2011b):*** Frequency and magnitude of internal organ colonization following exposure of laying hens to different oral doses of *Salmonella Enteritidis*. Int. J. Poult. Sci., 10: 325-331.

- ***Gast, R.K.; Rupa, Guraya, Deana, R. ;Jones and Kenneth, E. Anderson (2013):*** Colonization of internal organs by *Salmonella* Enteritidis in experimentally infected laying hens housed in conventional or enriched cages Poult. Sci. vol. 92 no. 2 468-473.

- ***Guan, J.; Grenier, C. and Brooks, B.W. (2006):*** In vitro study of Salmonella enteritidis and Salmonella Typhimurium definitive type 104: survival in egg albumen and penetration through the vitelline membrane. Poultry Science; 85:1678-1681.

- ***Hoop, R.K. &Popischil, A. (1993):*** Bacteriological, serological, histological and immunohistochemical findings in laying hens with naturally acquired *Salmonella enteritidis* phage type 4 infection. Veterinary Record, 133:391-393.

- ***Humphrey, T. J.; Chart, H., Baskerville, A. and Rowe, B. (1991b):*** The influence of age on the response of SPF hens to infection with Salmonella Enteritidis PT4. Epidemiology and Infection 106: 33–43.

- ***Hyungkun, L.; Kyung H. L.; Chong-Hae, H.; Gyung-Jin B. and Weon S. C. (2005):***Comparison of four molecular typing methods for the differentiation of Salmonella spp. International Journal of Food MicrobiologyVolume 105, Issue 3, 15 December 2005, Pages 411–418.

- ***Inne Gantois1, Richard Ducatelle1, Frank Pasmans1, Freddy Haesebrouck1, RichardGast 2, (2008):*** Mechanisms of egg contamination by Salmonella Enteritidis Tom J. Humphrey3 &Filip Van Immerseel Ghent University, Salisburylaan133, B-9820

- ***Keller, L.H.; Benson, C.E.; Krotec, K. and Eckroade, R.J. (1995):*** *Salmonella* Enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. Infect Immun. 63:2443-2449.

- ***Kleven, S.H and Yoder, H.W (1998):*** Mycoplasmosis. In: HG Purchase, LH Arp, CH Domermuth and JE Pearson (Eds): A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th ed., American Association of Avian Pathologists, Kenett Square. Pp: 74-88.

- ***Kwang, J., E.;Littledike, T.and Keen, J. E. (1996):*** Use of the polymerase chain reaction for Salmonella detection. Lett. Appl. Microbiol. 22:46–51.

- ***Mason J (1999):*** Introduction: *Salmonella enterica* serovar Enteritidis current status and a recommendation for its prevention and control. In: Saeed, A.M, Gast, R.K, Potter, M.E, Wall, P.G, editors. *Salmonella enterica* serovar Enteritidis in humans and animals. Ames, IA: Iowa State University Press: 267–9.

- ***Michailidis, G.A. M.; Avdi A. A. and Argiriou, B. (2010):*** Transcriptional profiling of antimicrobial peptides avian b-defensins in the chicken ovary during sexual maturation and in response to Salmonella enteritidis infection Institute of Agro-biotechnology, CERTH, 57001.

- ***Millemann, Y., LesageDescauses, M.C., Lafont, J.P., Chaslus-Dancla, E., (1996):*** Comparison of random amplified polymorphic DNA analysis and Enterobacterial Repetitive Intergenic Consensus-PCR for epidemiological studies of Salmonella. FEMS Immunology and Medical Microbiology 14:129-134.

- ***Miyamoto, T.; Baba, E.; Tanaka, T.; Sasai, K.; Fukata, T. and Arakawa, A. (1997):*** Salmonella Enteritidis contamination of eggs from hens inoculated by vaginal, cloacal and intravenous routes. Avian Diseases, 41:296-303.

- ***Miyamoto, T.; Kitaoka, D.; Withanage, G. S.; Fukata, T.; Sasai, K. and Baba, E. (1999):*** Evaluation of the efficacy of Salmonella Enteritidis oil-emulsion bacterin in an intravaginal challenge model in hens. Avian Diseases, 43: 497-505.

- ***Naknukool, S.; Hayakawa, S.; Uno, T. and Ogawa, M. (2006):*** Antimicrobial Activity of Duck Egg Lysozyme against Salmonella enteritidis. IUFoST, 1783-1794.

- ***Okamura, M., Kamijima, Y., Miyamoto, T., Tani, H., Sasai, K. and Baba, E. (2001a):*** Differences among six Salmonella serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. Avian Diseases 45, 61–69.

- ***Oxoid (1982):*** Manual of culture media, ingredients and other laboratory service. Wade Road, Basingtoke Hampshire, UK.

- ***Pomeroy, B. S; Nagaraja, K. V; Fowl Typhoid .In: Calnek, B .W. ;Barnes, H. J ; Beard, C. W ;Reid, W. M and Yoder, H. W , eds (1991):*** Diseases of poultry , 9thed . Iowa: Iowa State University Press, 87–99.

- ***Sambrook, J.; Fritsh, E.F.; and Maniatis, T. (1989):*** Molecular cloning .A laboratory Manual.2nd ed., Cold Spring Habor Laboratory Cold Spring. New York.

- ***Schroeder, C.M.; Naugle, A.L.; Schlosser, W.D. ; Hogue, A.T.; Angulo, F.J. ; Rose, J.S. ; Ebel, E.D. ; Disney, W.T.; Holt, K.G. and Goldman, D.P. (2005):*** Estimate of illnesses from *Salmonella enteritidis* in eggs, United States, 2000. Emerg.Infect. Dis., 11: 113-115.

- ***Shivaprasad, H. L., Timoney, J. F.; Morales, S., Lucio, B. and Baker, R. C. (1990):*** Pathogenesis of Salmonella Enteritidis infection in laying chickens .I. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses. Avian Diseases 34: 548–557.

- ***Skaar, E.P., (2010):*** The battle for iron between bacterial pathogens and their vertebrate hosts. Plos.Pathog. 6: 1000949.

- ***Soumet, C.; Ermel, G.; Rose, N.; Drouin, P.; Salvat, and Colin, P. (1999):*** Identification by a multiplex PCR-based assay of Salmonella typhimurium and Salmonella Enteritidis strains from environmental swabs of poultry houses. Lett Appl. Microbiol. 29(1): 1-6.

- ***Stephenson P., Satchell F.B., Allen G. & Andrews W.H. (1991):*** Recovery of Salmonella from shell eggs. J. Assoc. off. anal. Chem., 74:821-826.

- ***Stone, G.G.; Oberst, R.D.; Hays.M. ; MCVEYS, S. and Chengappa, M.M. (1994):*** Detection of Salmonella Serovars from clinical samples by enrichment broth cultivation-PCR Procedure.J. CLin.Microbiol., 32(7):1742-1749.

- ***Taylor, M.; Leslie, M.; Ritson, M.; Stone, J.; Cox, W.; Hoang, L.; Galanis, E. and Outbreak Investigation Team. (2012):*** Investigation of the concurrent emergence of Salmonella Enteritidis in humans and poultry in British Columbia, Canada, 2008-2010. Manuscript pending publication in Zoonoses and Public Health (doi: 10.1111/j.1863-2378.2012.01500.x).

- ***Temelli , S.; Kahya , S.A.; Eyigor and Carli K. T. (2010):*** Incidence of *Salmonella* Enteritidis in chicken layer flocks in Turkey: Results by real-time polymerase chain reaction and International Organization for Standardization culture methods Poultry Science 89 :1406–1410 doi:10.3382/ps.2010-00796

- ***Versalovic, J., Koeuth, T., Lupski, J.R., (1991):*** Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Research 19, 6823-6831.

- ***Versalovic, J.; Schneider, M.; Bruijn, F.J.; Lupski, J.R. (1994):*** Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods Mol. Cell. Biol., 5, 25-40.

- ***Wallance, H.A.; June, G.; Sherrod, P.; Hammack, T.S. and Amaguana, R.M. (1999):*** Salmonella in Food and Drug Administration, Bacteriological Analytical Manual. Tomlison, L.A. (ed).

- ***Waltman, W. D., R. K. Gast, and E. T. Mallinson. (1998):*** Salmonellosis. In: A laboratory manual for the isolation and identification of avian pathogens, 4th ed. D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 4–13.

- ***Wael F. El-Tras; Ph.D, Ahmed, A. Tayel, Ph.D and Dalia Khater, PhD (2011):*** Egg Packagers Exposure to *Salmonella Enteritidis* in Egyptian Laying FarmsAnimal and Veterinary Sciences, 5: 1-5.

تواجد ميكروب السالمونيلا أنترتيدس فى الجهاز التناسلى والبيض غير المكتمل والبيض وأمعاء البط المعدي طبيعى

أ.د. حلمى تركى[1] ، صفاء الحفناوى[2]

تعتبر السالمونيلاإنترتيدس من أهم أنواع السالمونيلا التى تصيب البط وهذه الدراسة ترى ما اذا كان ميكروب السالمونيلا أنترتيدس المعزول من البيض الغير مكتمل والقادر على التواجد فى الجهاز التناسلى والبيض الخاص بالبط المعدي طبيعى بالميكروب له نفس التتابع الجينى ام لا. خمس وتسعون بطة فى هذه الدراسة فقط تسعة منها كانت موجبة العدوى بميكروب السالمونيلا أنترتيدس عن طريق العزل البكتيري والتصنيف السيرولوجى وتم تأكيد ذلك بعمل تفاعل البلمرة المتسلسل.

وكان تواجد ميكروب السالمونيلا أنترتيدس فى المبيض وقناة البيض والبيض الذى لم يكتمل نموه وموجود فى قناة البيض والامعاء كالآتى 4,2,2و4على التوالى من 95 بطة.

وكان تواجده على القشرة الخارجية للبيض 3وفى المحتوى الداخلى للبيض لا يوجد ميكروب من أصل 135بيضه من نفس المزارع.

هذه العترة من ميكروب السالمونيلا أنترتيدس المعديه للمبيض والأمعاء والبيض الغير ناضج داخل قناة البيض للبط المعدي طبيعا ثبت انها لها نفس التتابع الجينى.