Veterinary Medical Journal –Giza (VMJG), Vol. 60(2), 23-31, December 2014
http://www.vetmedjcu.com/
ISSN 1110-1423

Isolation and Molecular Typing of Isolated *Salmonella* Species from Poultry in Egypt

Mai Ibrahim[a], Salah El din Salem[a], Mahmoud El Hariri[a*]
[a]Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Giza Egypt

Abstract

The aim of this study is to determine the prevalence of pathogenic *Salmonella*isolates recovered from poultry farms in Egypt.A total of 36 (29%) Salmonella isolates were recovered from 124 samples of poultry (broilers, layers, and breeders) from different governorates in Egypt. The Salmonella isolates were confirmed by *inv*A gene detection. The molecular characterization was done by using *spv*A gene to identify *Salmonella*Enteritidisand *fliC* gene to identify *Salmonella*Typhimurium by PCR. The 36 isolates (29%, 36/124) were genotyped by sequence analysis of *inv*A gene PCR product. The sequence analysis revealed that, the isolates belonged to three different serotypes, including S. Heidelberg (n = 17), S. Gallinarum (n = 6), and S. Newport (n = 1). Whereas *spv*A gene was used for detection of S. Entritidis (n = 8).
Keywords: *Salmonella*- Serotyping – Molecular – Prevalence - Egypt.

Introduction

Salmonellosis is considered as one of the major bacterial problems in the poultry industry worldwide (Duchet-Suchaux et al., 1995). Most of the outbreaks in developed and developing countries produced by this bacterium indicate its importance and impact(Bell and Kyriakides,2002) *Salmonella* spp. cause asymptomatic intestinal infections in birds but acute outbreaks exhibiting clinical disease along with high levels of mortality occur in chicks younger than 2 weeks old (Duchet-Suchaux et al., 1995).*Salmonella*specific PCR with primers for *inv*A and/or *hil*A is rapid, sensitive and specific for detection of *Salmonella* in many clinical samples(Lampel et al., 2000). The rapid detection of *Salmonella enterica*, coupled a culture procedure with PCR amplification of the genus-specific *invE/invA* genes was done by(Schrank et al., 2001)
There are, little information about the prevalence of Salmonella in broiler carcasses from Egypt. The objectives of this study were (i) to determine the prevalence of Salmonella in poultry farms in Egypt; and (ii) to determine Salmonella isolatesserotype by molecular technique using sequence analysis method.

Material and Methods

Samples:

One hundred twenty four chicken tissue (liver) samples were collected randomly from different local farms (broiler, layer & breeder) in Alexandria, Cairo, Monofya, Behera, Sharqia and Fayoum in Egypt (Table 1).

Microbiological analysis:

According to OIE Manual 2010(OIE, 2010) samples were enriched directly in two different selective enrichment broths, Rappaport Vassiliadis (RV) (Oxoid) at 41.5°C, tetrathionate (TT) (Oxoid) at 37°C for 18-24 hr., then plating on XLD (Oxoid) and brilliant green (BG) agar (Oxoid). All plates were aerobically incubated at 37°C overnight (24-48 hr). The colonies of *Salmonella*appearas shiny pink with black centers and sometimes glossy large 3-5mm in diameter on XLD agar or reddish/pink and slightly convex on BGA.Five typical colonies from each agar plate were picked and streaked on nutrient agar and incubated for 24 hours at 37°C to confirm the purity of the culture, then submitted to biochemical tests.

Salmonella isolates genomic DNA extraction:

Positive samples were confirmed by PCR of *inv*A gene(Rahn et al., 1992)An extraction of isolated *Salmonella*: Bacteria were cultured on nutrient agar for 24 hrs at 37°C. Extraction of DNA was performed by dry heat at 98°C for 10 min and cooled at freezer (-20°C) for 10 min and centrifuged at 6000 rpm for 5min. supernatant were used for amplification by PCR of *Salmonella* specific primers.

Molecular characterization of Salmonella isolates:

Salmonella specific primers, have respectively the following nucleotide sequence based on the *inv*A gene of *Salmonella* 5′ GTG AAA TTA TCG CCA CGT TCG GGC AA 3′and 5′ TCATCG CAC CGT CAAAGG AAC C -3′ (Rahn et al. 1992). Reaction with these primers were carried out in a 25µl amplification mixture consisting of 12.5µl of PCR Master mix (Thermo Scientific), 0.5 µl of each primer, 9 µl of grade water and 3 µl of extraction for each isolate were used in the reaction.The cycle conditions were as follow: An initial denaturation at 94 C for 5 min. Followed by 35 cycles of denaturation at 94°C for ٣٥ sec, annealing at 64°C for 30 sec and elongation at 72°C for ٤٥ sec, followed by 7 min final extension period at 72°C.The*Spv* A gene F5'-GCCGTACACGAGCTTATAGA-3' and *Spv* A gene R 5'-ACCTACAGGGGCACAATAAC-3'for *Salmonella*Entritidis according to(Pan and Liu, 2002)while, the used *fli* C gene, *Fli* C gene F 5'-CGGTGTTGCCCAGGTTGGTAAT-3' and *Fli* C gene R5'-ACTGGTAAAGATGGCT-3' according Olivera et.al. 2003 for detection of *Salmonella*Typhimurium(Oliveira et al. 2003).

The thermal cyclic conditions were as *inv*A but annealing was at 56°C for 2 min. The amplified DNA products from Salmonella specific-PCR were analyses with electrophoresis on 1.2% agarose w/v gels stained with ethidium bromide and visualized by UV illumination. A current of 120 V was applied to each gel.

Molecular genotyping by sequence analysis of invA gene:

Other isolates than S. Entritidis and S. Typhmuriumserovars were identified by sequence analysis of invA gene sequencing and Identification of homologies between nucleotide sequences of the detected Salmonella strains and other strains

published on GenBank was done using BLAST 2.0 search programs (National Center for Bio-technology Information, 'NCBI'; http://www.ncbi.nlm.nih.gov/).The maximum scores designated in the BLAST search have a well-defined statistical interpretation, making matches easier to distinguish from random background hits(Altschul et al. 1997).

Results

Salmonella was detected in 36 samples of the 124 examined chicken liver samples (29 %). Total number of positive samples of *Salmonella* isolation from chicken liver from (broiler, layer and breeder) with 57 % from broilers, 2 % from layers and 0 % from breeders. Showed in table (2).

The molecular identification of *Salmonella*Entritidis isolates by amplificationof *spv*A gene specific for *Salmonella*Entritidis. Eight isolates were identified as *S.*Entritidis by amplification of PCR product at 250 bp Fig (2). All examined isolates were negative for *fli*C gene of *Salmonella*Typhimurium. *Salmonella* was detected in 36 samples of the 124 examined chicken liver samples (29 %). Total number of positive samples of *Salmonella* isolation from chicken liver from (broiler, layer and breeder) with 57 % from broilers, 2 % from layers and 0 % from breeders Table (2).

Sequencing of PCR products for identify the other *Salmonella* spp. and genotypes. 3serovars of *Salmonella* were identified by sequencing for 25 isolates ,*Salmonella* Heidelberg was the most identified serovar (50 %), then *Salmonella*Gallinarum (16 %) and *Salmonella* Newport (3 %) Table (3). Fourteen *Salmonella* strains identified by sequencing of *inv*A gene and obtained accession number inGenBank Showed in table (4)

Table (1) Number of examined samples and its governmental distribution:

Governorates	Chicken type	Age	Collected	Total
Behera	broiler	1-8 days	22	
Monofya		17-days	9	
		24-days	5	
Alexandria		1-11 days	25	
Sharqia	layer	28 weeks	41	
Fayoum		55 weeks	2	
Cairo	breeder	4 weeks	20	124

Table (2) Number of positive Salmonella and prevalence percent:

Type	No. of samples	Positive samples	Prevalence %
Broiler	61	35	57
Layers	43	1	2
Breeder	20	0	0
Total no.	124	36	29

Table (3): Number of identified *Salmonella* isolates from sequencing

Serovars	No. of isolates	%
S. Gallinarum	6	16
S. Heidelberg	18	50
S. Newport	1	3

Table (4): accession number of Salmonella isolates:

Samples No.	Code	Serovars
KJ718882	2Alx-B	*S.* Gallinarum
KJ718883	7Alx-B	*S.* Gallinarum
KJ718884	11Alx-B	*S.* Gallinarum
KJ718885	42Beh-B	*S.* Heidelberg
KJ718886	47Beh-B	*S.* Gallinarum
KJ718887	50Beh-B	*S.* Heidelberg
KJ718874	52Beh-B	*S.* Heidelberg
KJ718875	54Beh-B	*S.* Heidelberg
KJ718876	55Beh-B	*S.* Heidelberg
KJ718877	56Beh-B	*S.* Heidelberg
KJ718878	58Beh-B	*S.* Heidelberg
KJ718879	70Shar-L	*S.* Newport
KJ718880	105Mo-B	*S.* Heidelberg
KJ718881	107Mo-B	*S.* Heidelberg

Veterinary Medical Journal –Giza (VMJG), Vol. 60(2), 23-31, December 2014
http://www.vetmedjcu.com/
ISSN 1110-1423

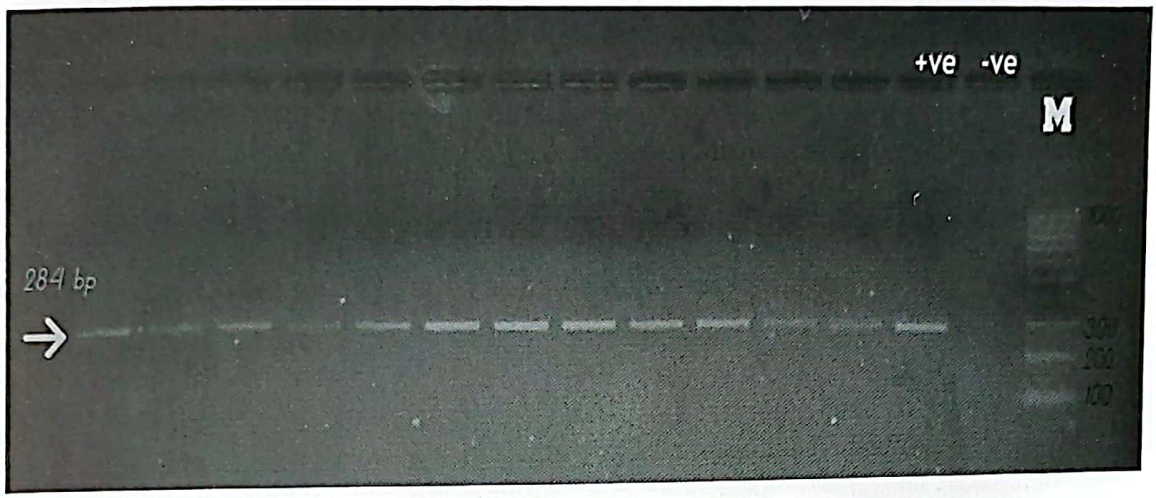

Fig : (1) PCR of *inv* A gene: Lane M: DNA ladder from 100-1000, lane C-: *E.coli* ATCC 25922, lane C+:*S.Entritidis* ATCC 13076, and other lanes positive *inv* A gene (284 bp) *Salmonella* isolates.

Fig : (2) PCR of *spv*A gene: Lane M: DNA ladder from 100-1000, lane C-: *E.coli* ATCC 25922, lane C+:*S.Entritidis* ATCC 13076, and other lanes : positive *S.* Entritidis isolates (250bp).

Discussion

One of the most problematic zoonosis in terms of public health all over the world not only because of the high endemicity, but also because of the obstacles in controlling the disease in addition to significant morbidity and mortality rates(Tessari et al. 2013). Avian salmonellosis is responsible for considerable loss in poultry industry through the death of birds and loss in production(Martin et al., 2011and Nogrady et al., 2012). Salmonellosis can result in several disease symptoms including gastroenteritis, bacteremia, typhoid fever and focal infection(Rotimi et al., 2008 and Gordon, 2011). Depending on molecular techniques (PCR and sequencing) after conventional isolation is a very useful method to identify *Salmonella* strains. Skwark and coworkerproved that PCR is a very useful

method to identify *Salmonella* strains and to determine their virulence factors by amplification of characteristic genetic markers **(Fig 1 and 2)**(Skwark et al. 2004). In the present study, the rate of *Salmonellae* isolation in poultry farms (liver organs) was investigated. *Salmonellae* were isolated from different types (broiler, breeder & layer) with incidence of 29 %, these results in agree with(Authman, 1992 and Hegazi, 2002)as 26 % and 30 % respectively (Table 2).

The contamination rates were observed in other countries, 23-34% in Belgium(Uyttendaele et al. 1998), 25% in United Kingdom(Plummer et al. 1995), 26% in Ireland(Duffy et al. 1999) and 36% in Malaysia(Rusul et al. 1996). The difference in the prevalence rates may be due to socio-economic factors. Moreover, the highest percent of isolation was from broiler 57% **(Table 2),** it might be attributed to horizontal and/or vertical transmission of *Salmonella* to the chicks.

The horizontal transmission of *Salmonellae* can be mediated by mechanisms including direct bird-to-bird contact, ingestion of contaminated feeds, water or litter, or using contaminated equipment. While vertical transmission to the progeny of infected breeder flocks can result from internal or external contamination of eggs (Gast, 2003), as they were small in age and Salmonella is high in ages less than 2 weeks. The highest percentage of isolation was *S.* Heidelberg serovar (50 %), then *S.*Entritidis (22 %), *S.*Gallinarum (16 %) and S. Newport (3 %). The most frequently isolated serotypes from chickens were *Salmonella* Heidelberg, and *Salmonella*Enteritidis (CDC, 2004). The result of this study may indicate the challenge, intensification of poultry production may face in the future in the country from salmonellosis disease unless due attention is given to the prevention and control of these diseases. Therefore concerted efforts such as organized national regulatory programs should be established to mitigate the losses and control the diseases.

References

Altschul, F., Madden, L., Schäffer, A., Zhang, J., Zhang, Z., Miller, W., Lipman, J., 1997, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res.25:3389–3402.

Authman Magda, M. M., 1992 Studies of bacterial causes of early poultry mortality in Sharqia governorate. M.V.Sc thesis (Microbiology) Fac. Of Vet. Med. Zagazig University.

Bell, C. and Kyriakides, A. 2002, *Salmonella*: A practical approach to the organism and its control in foods. Blackwell Science Oxford.

CDC, 2004, *Salmonella* Surveillance: Annual Summary 2003. Centers for Disease Control and Prevention Atlanta GA.

Duchet-Suchaux, M., Le chopier, P., Marly, J., Bernardet, P., Delaunay, R. and Pardon, P., 1995, Quantification of experimental *Salmonella*Enteritidis carrier state in B13 leghorn chicks. Avian Dis. 39 796–803.

Duffy, G. O. M., Cloak, M. G., O'Sullivan, A., Guillet, J. J., Sheridan, I. S., et al., 1999, The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. Food Microbiology 16: 623- 631.

Gast, R. K., 2003, Paratyphoid infections. In: Diseases of poultry 11th edn.(EdsSaif Y.). Iowa State University Press Ames 583-613.

Gordon, M.A., 2011, Invasive non typhoidal*Salmonella* disease: epidemiology pathogenesis and diagnosis. Current Opinion in Infectious Disease 24: 484-489.

Hegazi, A.M., 2002, Epidemiological studies on salmonellosis in chickens with special reference to *Salmonella*Entritidis. Phd. Thesis Alex. University Fac. Vet. Med. Scientific Central Library.

Lampel, K.A., Orlandi, P.A. and Kornegay, L., 2000, Improved template preparation for PCR-based assay for detection of food-borne bacterial pathogens.Appl. Environ. Microbiol. 66: 4539-4542.

Martin, C., Balasch, S., Vega, S. and Lainez, M., 2011, Sources of *Salmonella* contamination during broiler production in Eastern Saipan. Preventive Medicine. Vol. 98 Issue 1 1 Jan. 2011 Pages 39-45.

Nogrady, N., Kiraly, M., Davies, R., Nagy, B., 2012, Multidrug resistant *Salmonella* Infants of Broiler in Europe. International Journal of Food Microbiology 108-112.

OIE, Terrestrial Manual 2010: Salmonellosis Version adopted by the World Assembly of Delegates of the OIE in May 2010. Vol. 1-19.

Oliveira, S.D. C.R., Rodenbusch, M.C. Ce., Rocha, S.L.S. and Canal, C.W. , 2003, Evaluation of selective and non-selective enrichment PCR. Letters in Applied Microbiology 36(4): 217-221.

Pan, T.M. and Liu, Y.J., 2002, Identification of *Salmonella* Enteritidis isolates by polymerase chain reaction and multiplex polymerase chain reaction. J. Microbial. Immunol. Infect. 35: 147-151.

Plummer, R. A., Blissett, S. S. J. and Dodd, C. E. R., 1995, *Salmonella*contamination of retail chicken products sold in the UK. Journal of Food Protection 58: 843- 846.

Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galán, J.E., Ginocchio, C., Curtiss, R., Gyles, C.L., 1992,Amplification of an *inv*A gene sequence of *Salmonella*Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*.Mol. Cell Probes 6(4): 271-279.

RotimI,V. O., Jamal, W. Y., Pal, T., Sonnevend, A., Dimitrov, T. S., Albert, M. J., 2008, Emergence of multidrug resistance *Salmonella* spp. and isolates with reduced susceptibility to ciprofloxacin in Kuwait and the United Arab Emirates. Diagnostic Microbiology and Infectious Disease 8: 71-77.

Rusul, G., Khair, J., Radu, S., Cheah, C.T. and Yassin, R.M., 1996, Prevalence of *Salmonella* in broilers at retail outlets processing plants and farms in Malaysia.Int. J. Food Microbiol 33: 183–194.

Schrank, I.S., Mores, M.A.Z., Costa, J.L.A., Frazzon, A.P.G. and Sonicini, R. *et al.* 2001. Influence of enrichment media and application of a PCR based method to detect *Salmonella* in poultry industry products and clinical samples. Vet. Microbiol. 82: 45-53.

Skwark, M., Nawrotek, P. and Czyzewska, I., 2004, The detection and determination of potential virulence of *Salmonella* spp. using PCR method Pol. J. Vet. Sci.7(1):33-37.

Tessari E. N. C., Kanashiro, A. M. I., Stoppa, G. F. Z., Renato, L. L., De Castro, A. G.M. and Cardoso, AL. S. P., 2013, Important Aspects of *Salmonella* in the Poultry Industry and in Public Health *Salmonella* A Dangerous Foodborne Pathogen : 978-953-307-782-6.

Uyttendaele, M. R., Debevere, J. M. , Lips, R. M., and Neyts, K.D., 1998, Prevalence of *Salmonella* in poultry carcasses and their products in Belgium. International Journal of Food Microbiology 40: 1-8.

Veterinary Medical Journal –Giza (VMJG), Vol. 60(2), 23-31, December 2014
http://www.vetmedjcu.com/
ISSN 1110-1423

العزل والتوصيف الجزيئي والنوعي السالمونيلا المعزوله من الدواجن في مصر

مي ابراهيم ، صلاح الدين سليم ، محمود الحريري

قسم الأحياءالدقيقة ، كلية الطب البيطرى،جامعة القاهرة،الجيزة مصر

الهدف من هذه الدراسة هو تحديد مدى انتشار السالمونيلا المسببة للأمراض من مزارع الدواجن في مصر. تم عزل عدد 36 (29٪) من عزلات السالمونيل امن واقع124 عينه من عينات الدواجن (الدجاج اللاحم ،والبياض ، والامهات) من مختلف المحافظات في مصر. تم تأكيدا لعزلات السالمونيلا التي تم عزلها عن طريق الكشف الجيني لجين *inv*A. تم الانتهاء من التوصيف الجزيئي بالكشف الجيني لجين *spv*A الجين لتحديد السالمونيلا انترتيديس واستخدم *fli*C جين لتحديد السالمونيلا التيفية بواسطةPCR. تم تحديد النوع الجيني لعدد (29٪، 124/36) من العزلات عن طريق تحليل التسلسل لمنتج PCR لجين *inv*A. وكشف تحليل التسلسل ذلك، ان العزل انتمي إلى ثلاثة أنماط مصلية مختلفة،بما في ذلك S. Heidelberg(ن = 17)، S. Gallinarum(ن = 6)،و S. Newport (ن = 1). فيحين *pv* A *s* جين استخدم للكشف عن S. Entritidis (ن = 8).