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A Survey on *Salmonella* Species Isolated from Chicken Flocks in Egypt

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ABSTRACT

This study was carried out to investigate serologically and molecularly the prevalent species of *Salmonella* present in four broiler chicken flocks in Kalubia governorate, Egypt. A bacteriological examination was done on a total of 1073 samples that collected from 293 chickens (127 apparently healthy, 62 diseased with whitish watery diarrhea and 104 dead broiler chickens). These samples included cloacal swabs, gall bladders, yolk sacs, spleens and livers. The colonial morphology, microscopical and biochemical identifications of isolates revealed presence of 51 strains of *Salmonella* representing; 10 (2.55%) from apparently healthy chickens, 18 (7.03%) from diseased broilers and 23 (4.69%) from dead birds. Positive *Salmonella* strains were present as percentages of 3.84, 4.15, 5.06 and 5.18% from flocks 1, 2, 3 and 4, respectively. Using slide agglutination test, serotyping of the isolated strains according to somatic (O) and flagellar (H) antigens detected presence of 19 *S. enteritidis* (37.25%); 10 *S. infantis* (19.60%); 1 *S. chiredzi* (1.96%); 4 *S. kentucky* (7.84%); 15 *S. typhimurium* (29.41%) and 2 *S. tsevie* (3.92%). There was a variation in the presence of *Salmonella* species serotypes in each of the examined flocks. Molecular characterization using Polymerase Chain Reaction (PCR) produced positive amplification of 284 bp fragments of *invA* genes (100%) specific for all members of *Salmonella* species. From the previous results, it could be concluded that there are different *Salmonella* serotypes including *S. enteritidis*, *S. infantis*, *S. chiredzi*, *S. kentucky*, *S. typhimurium* and *S. tsevie* circulating in broiler chicken farms in Kalubia governorate, Egypt and the most prevalent ones are *S. enteritidis* and *S. typhimurium*.

Key words: *Salmonella*, chickens, Egypt, broiler chicken

INTRODUCTION

More than 2500 serotypes of salmonellae have been described, mostly belonging to the species *Salmonella enterica* (Brenner *et al*., 2000). Some of these serotypes, such as *S. typhimurium* and *S. enteritidis*, can infect humans while other serotypes are host-specific, infecting a single species and generally causing severe, typhoid-like symptoms sometimes leading to death (for instance, *S. infantis* and *S. kentucky* in poultry). These serotypes can be responsible for disease outbreaks leading to severe economic losses (Calenge *et al*., 2010). European Food Safety Authority (EFSA, 2007) accounted *S. enteritidis* and *S. typhimurium* for most zoonotic salmonellosis associated with food of animal origin. The latest mentioned species were accounted for almost 80% of identified serovars in human in 2006 (Collard *et al*., 2008).

Until 1980s, *S. typhimurium* was the serovar most commonly isolated worldwide, but by the late 1980s, S. *enteritidis* emerged as the most common cause of salmonellosis in Europe and during

the 1990s, it became the most prevalent serovar in many countries worldwide (Mishu *et al*., 1994; Poppe, 1999; Cogan and Humphrey, 2003; Schroeder *et al*., 2005; Betancor *et al*., 2010). Chickens can be infected with many different serovars of *Salmonella*. *S. enteritidis* frequently colonizes the alimentary tracts of chickens without causing disease. However, it can produce a systemic infection in young chicks that may further lead to the infection of egg contents (Zhang-Barber *et al*., 1999; De Buck *et al*., 2004).

The contamination of poultry meat products originates primarily from chickens infected with Salmonella during processing. In this respect, S. *enteritidis* is of particular importance, since this pathogen can colonize the chicken host without causing discernible illness in the infected chickens (Barrow *et al*., 1987; Ziprin *et al*., 1989; Schaar *et al*., 1997; Jarquin *et al*., 2009). So, the detection of *Salmonella* in primary poultry production is an issue of great concern in the European Union (EU), since control of this zoonotic disease is in part based on the reduction of the prevalence at the farm level (Carrique-Mas and Davies, 2008).

This study was planned to identify serologically and molecularly the prevalent *Salmonella* species in broiler chicken flocks in Kalubia governorate, Egypt.

MATERIALS AND METHODS

Samples: From four broilers chicken flocks at Kalubia governorate, a total of 1138 samples were collected from 293 chickens (127 apparently healthy, 62 diseased with whitish watery diarrhea and 104 dead broiler chickens) (Table 1). Complete history of the collected chicken samples including the number of birds in each flock, age, morbidity and mortality rates and the type and the numbers of samples were illustrated in Table 2. From each flock, cloacal swabs, gall bladders, yolk sacs, spleens and livers were collected as samples from apparently healthy, diseased and dead broiler chickens. The number of the collected samples from birds in each flock was demonstrated in Table 3.

Isolation of *Salmonella* (OIE, 2004): The examined samples were collected separately under hygienic measure as possible from the flocks and then transferred directly in ice box to the laboratory for bacteriological examination. One gram of each sample was inoculated in 9 mL buffered peptone water and incubated over night at 37°C for 18 h as pre-enrichment for *Salmonella*. About 0.1 mL of pre-enrichment culture was transferred to Rappaport Vassiliadis (RV) broth and incubated for 24 h at 41.5°C (as selective enrichment). In parallel 0.1 mL of the same pre-enriched culture was transferred to a tube containing 10 mL of Tetrathionate Broth (TB) and incubated at 37°C for 24 h (as selective enrichment). A loopfull from both enrichment incubated broth (RV and TB) was streaked on both *Salmonella*, *Shigella* (S.S) and Xylose Lactose Deoxycholate (XLD) agar media and incubated for 24 h at 37°C. Separate suspected colonies were picked up on Trypticase Soya (TS) agar slant for further microscopical examination, biochemical, serological and molecular identification.

Table 1: The number and condition of chickens collected for sampling

Flock	Total No. of birds/flock	Apparently healthy chickens	Diseased chickens	Dead chickens	Total
1	6984	22	15	16	53
2	5461	40	24	39	103
3	8982	36	13	28	77
4	7079	29	10	21	60
Total	28506	127	62	104	293

Table 2: Complete history of the collected broiler chickens samples for isolation

						Type and No. of collected samples				
Flocks	Visits	No. of birds/flock	Age/day	Morbidity%*	Mortality%*	Yolk sac	Cloacal swab	Gall bladder	Spleen	Liver
1	1st		3	2.01	1.00	30	20	30	30	30
	2nd		7	1.50	0.89	0	18	25	25	25
	3rd		21	1.10	0.56	0	10	20	20	20
	4th		37	0.90	0.12	0	5	10	10	10
Total No.		6984				30.	53	85	85	85
2	1st		3	1.98	1.41	15	35	15	15	15
	2nd		7	1.39	1.25	0	20	15	15	15
	3rd		21	1.20	1.03	0	30	25	25	25
	4th		37	0.65	0.24	0	18	10	10	10
Total No.		5461				15	103	65	65	65
3	1st		3	2.43	0.78	20	20	10	10	10
	2nd		7	1.76	0.50	0	18	15	15	15
	3rd		21	0.66	0.30	0	20	10	10	10
	4th		37	0.40	0.14	0	10	8	8	8
Total No.		8982				20	68	43	43	43
4	1st		3	2.09	1.23	25	25	15	15	15
	2nd		7	1.50	0.78	0	20	20	20	20
	3rd		21	0.65	0.31	0	20	15	15	15
	4th		37	0.35	0.19	0	15	5	5	5
Total No.		7079				25	80	55	55	55
Total No./all flocks		28506								

*The percentage of morbidity and mortality were calculated according to the total number of the flock

Identification of *Salmonella* isolates

Microscopical examination: Suspected colonies were stained by Gram's stain according to Quinn *et al*. (2002) and examined microscopically.

Biochemical identification: The purified isolates of *Salmonella* were examined by different biochemical reactions (Cruickshank, 1975) either by oxidase, indole, urea hydrolysis, H_2S production on triple sugar iron agar, citrate utilization, methyl red, Voges Proskauer, sugar fermentation (glucose, lactose, maltose, sorbitol and arabinose) and motility test.

Serological identification: The preliminary identified isolates by biochemical tests as *Salmonella* were subject to serological identification according to Hitchner (1975) using slide agglutination test. The polyvalent somatic (O), flagellar (H) *Salmonella* antisera and the polyvalent one were obtained from Difco Company. Suspected *Salmonella* were cultured on TS agar slants for 24 h at 73°C. A loopfull from the culture was suspended in drop of phosphate buffer saline (pH 7.4) on a slide to make a homogenous suspension and then a drop of *Salmonella* anti-sera (separate "O" and "H" factor) was added to the suspension with standard loop and thoroughly mixed to bring the organisms in close contact with anti-sera. Positive agglutination occurred within a minute and could be easily seen with the naked eye as a form of granules or follicles. A delayed or partial agglutination was considered as negative or false reaction. Polyvalent "O" and "H" anti-sera were first tried to assure that the suspected isolates were *Salmonella*.

Table 3: The types of samples according to chicken's conditions

		No. of collected samples			
Flock	Type of samples	Healthy	Diseased	Dead	Total
1	Cloacal swabs	22	15	16	53
	Gall bladder	25	10	50	85
	Yolk sac	10	10	10	30
	Spleen	30	20	35	85
	Liver	30	20	35	85
Total		117	75	146	338
2	Cloacal swabs	45	23	35	103
	Gall bladder	15	17	33	65
	Yolk	5	5	5	15
	Spleen	15	20	30	65
	Liver	15	20	30	65
Total		95	85	133	313
3	Cloacal swabs	35	10	23	68
	Gall bladder	20	10	13	43
	Yolk sac	10	5	5	20
	Spleen	10	8	25	43
	Liver	10	8	25	43
Total		85	41	91	217
4	Cloacal swabs	30	10	40	80
	Gall bladder	15	15	25	55
	Yolk sac	10	10	5	25
	Spleen	20	10	25	55
	Liver	20	10	25	55
Total		95	55	120	270
Total/all flocks		392	256	490	1138

Positive cultures were then tested with each of the "O" grouping sera followed by the respective mono-specific "O" and "H" anti-sera factors followed by phase 1 and phase 2 in order to determine the complete antigenic formula.

Molecular identification

Extraction of DNA: Positive strains of *Salmonella* (representing *S. enteritidis*, *S. infantis*, *S. chiredzi*, *S.* kentucky, *S.* typhimurium and *S. tsevie*) were grown in 10 mL TS broth at 37°C for 24 h. The overnight cultures were centrifuged at 3000 rpm for 5 min. and the supernatant were decanted carefully. The bacterial pellets were washed three times with phosphate buffer saline at pH 7.2 and re-suspended in 400 μL Tris-EDTA buffer (pH 8.0) and then heated in water bath at 100°C for 20 min. There were left to cool at room temperature and centrifuged at 14000 rpm for 10 min. An aliquot of 5 mL of the supernatant was used as template DNA in polymerase chain reaction.

Polymerase chain reaction (PCR)

Oligonucleotide primers: Oligonucleotide primers (139-141) specific for the *invA* gene located on the *Salmonella* pathogenicity island 1 which is highly conserved in *Salmonella* species and encodes a type 3 secretion system that exports proteins in response to bacterial contact with

Table 4: Oligonucleotides primers sequences

Primer	Target gene	Primer sequence (5'-3')	G+C content (%)	Annealing temperature (°C)	Size of amplified product/bp
139 (Forward)	*invA*	GTG AAA TTA TCG CCA CGT TCG GGC AA	50	55	284
141 (Reverse)	*invA*	TCA TCG CAC CGT CAA AGG AAC C	55	55	

epithelial cells were used (Galan *et al*., 1992; Rahn *et al*., 1992). The oligonucleotide primers sequences used for amplification of DNA for the detection of *Salmonella* species was designed according to Oliveira *et al*. (2002) as the following (Table 4).

DNA amplification: It was performed as the method of Oliveira *et al*. (2003). PCR amplifications were performed in a final volume of 50 mL in micro-amplification tubes (PCR tubes). The reaction mixtures consisted of 5 mL of the DNA template, 5 mL 10× PCR buffer [75 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 20 mM (NH4) $2SO_4$], 1 mL dNTPs (40 mM), 1 mL (1U Ampli Taq DNA polymerase), 1 mL (25 pmol) from the forward and reverse primers of both primer pairs and the volume of the reaction mixture was completed to 50 mL using distilled water. The thermal cycler was adjusted as follows; initial denaturation at 94°C for 5 min, followed by 35 cycles of (denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min). Final extension was carried out at 72°C for 10 min and the PCR products were stored in the thermal cycler at 4°C until they were collected.

Agarose gel electrophoresis: The PCR products were tested for positive amplification by agarose gel electrophoresis previously reported by Sambrook *et al*. (1989) using suitable molecular weight markers.

RESULTS AND DISCUSSION

Poultry are commonly infected with a wide variety of Salmonella serovars. Infections are generally sub-clinical and one serovar may be a predominant isolate in a country for several years before it is replaced by another serovar (Wray *et al*., 1996). The two serovars that have been of most concern in recent years are *S. enteritidis* and *S. typhimurium*.

Bacteriological examination is the traditional mean to obtain accurate data about the prevalence of infected host of salmonellae (Commission of The European Communities, 1992).
In the present study, from Kalubia governorate, a total number of 1138 broiler chicken samples representing 392 apparently healthy, 256 diseased and 490 dead birds were bacteriologically, serologically and molecularly examined to detect the presence of *Salmonella* species.

All *Salmonella* suspected isolates showed red colonies with black center on XLD media and white colonies with black center on S.S agar media. Gram stained colonies revealed Gram negative straight non spore forming rods (Carsiotis *et al*., 1984). The isolated suspected *Salmonella* organisms were highly motile by motility test. Biochemically, all isolates were non lactose fermenting colonies and negative oxidase, Voges Proskauer and urea hydrolysis tests. Meanwhile, most isolates produced H_2S and positive methyl red, citrate and indol tests. All of isolates were able to ferment glucose, maltose, sorbitol and arabinose (Topley *et al*., 1998).

As shown in Table 5, the number of positive *Salmonella* samples that collected from four flocks containing 392 apparently broiler healthy chickens were 10 isolates (2.55%). Table 6 revealed that the total *Salmonella* isolates were 18 (7.03%) that isolated from 256 samples representing four diseased broiler chickens and manifested whitish watery diarrhea. Moreover, positive *Salmonella*

Table 5: Incidence of *Salmonella* spp. isolated from apparently healthy broiler chicken flocks

		Salmonella positive samples		
Flocks	Type of samples	No. of total samples	No. of positive samples	%*
1	Cloacal swabs	22	1	4.54
	Gall bladder	25	0	0.00
	Yolk sac	10	0	0.00
	Spleen	30	1	3.30
	Liver	30	0	0.00
	Total	117	2	1.70
2	Cloacal swabs	45	2	5.71
	Gall bladder	15	0	0.00
	Yolk	5	0	0.00
	Spleen	15	0	0.00
	Liver	15	0	0.00
	Total	95	2	2.10
3	Cloacal swabs	35	1	2.85
	Gall bladder	20	1	5.00
	Yolk sac	10	0	0.00
	Spleen	10	0	0.00
	Liver	10	0	0.00
	Total	85	2	2.35
4	Cloacal swabs	30	3	10.00
	Gall bladder	15	0	0.00
	Yolk sac	10	1	10.00
	Spleen	20	0	0.00
	Liver	20	0	0.00
	Total	95	4	4.20
Total/all flocks		392	10	2.55

*Calculated according to the total No. of each type of sample.

Table 6: Incidence of *Salmonella* spp. isolated from diseased broiler chicken flocks

		Salmonella positive samples		
Flock	Type of samples	No. of total samples	No. of positive samples	%*
1	Cloacal swabs	15	3	20.00
	Gall bladder	10	1	10.00
	Yolk sac	10	0	0.00
	Spleen	20	0	0.00
	Liver	20	1	5.00
	Total	75	5	6.67
2	Cloacal swabs	23	2	8.69
	Gall bladder	17	0	0.00
	Yolk sac	5	0	0.00
	Spleen	20	0	0.00
	Liver	20	3	15.00
	Total	85	5	50.88
3	Cloacal swabs	10	1	10.00
	Gall bladder	10	0	20.00
	Yolk sac	5	1	00.00
	Spleen	8	2	25.00

Table 6: Continued

		Salmonella positive samples		
Flock	Type of samples	No. of total samples	No. of positive samples	%*
	Liver	8	0	0.00
	Total	41	4	9.75
4	Cloacal swabs	15	3	20.00
	Gall bladder	10	0	0.00
	Yolk sac	10	0	0.00
	Spleen	10	1	10.00
	Liver	10	0	0.00
	Total	55	4	7.20
Total		256	18	7.03

*Calculated according to the total No. of each type of samples

Table 7: Incidence of *Salmonella* sp. isolated from dead broiler chicken flocks

		Salmonella positive samples		
Flock	Type of samples	No. of total samples	No. of positive samples	*Percentage
1	Cloacal swabs	16	3	18.75
	Gallbladder	50	1	2.00
	Yolk sac	10	0	0.00
	Spleen	35	0	0.00
	Liver	35	2	5.70
	Total	146	6	2.73
2	Cloacal swabs	35	0	0.00
	Gall bladder	33	2	6.06
	Yolk sac	5	0	0.00
	Spleen	30	1	3.33
	Liver	30	3	10.00
	Total	133	6	4.51
3	Cloacal swabs	23	0	0.00
	Gall bladder	13	1	7.69
	Yolk sac	5	0	0.00
	Spleen	25	3	12.00
	Liver	25	1	4.00
	Total	91	5	5.49
4	Cloacal swabs	40	0	0.00
	Gall bladder	25	2	8.00
	Yolk sac	5	0	0.00
	Spleen	25	0	0.00
	Liver	25	4	16.00
	Total	120	6	5.00
Total		490	23	4.69

*Calculated according to the total No. of each type of samples

isolates were 23 (4.69%) that collected from four flocks representing 490 dead broiler chicken samples (Table 7).

Collectively, the incidence of *Salmonella* from apparently healthy, diseased or broiler chickens in each of the examined flocks was seen in Table 8. There were 51 positive *Salmonella* strains that isolated from all examined flocks with percentages of 3.84, 4.15, 5.06 and 5.18% from flocks 1, 2,

Table 8: Incidence of *Salmonella* spp. from different examined bird in each flock

Flock	*Positive samples (%)	No. of positive samples	Total No. of samples
1	3.84	13	338
2	4.15	13	313
3	5.06	11	217
4	5.18	14	270
Total	4.48	51	1138

*Calculated according to the total No. of each type of samples

Table 9: Serotypes of *Salmonella* strains

				Antigenic structure		
					Flagellar (H)	
Serovar	No. of strains	Percentage*	Group	Somatic (O)	Phase 1	Phase 2
S. enteritidis	19	37.25	D_1	9	g, m	-
S. infantis	10	19.60	C_1	6,7	r	1,5
S. chiredzi	1	1.96	F	11	c	1,5
S. kentucky	4	7.84	C_2	8, 20	i	z_6
S. typhimurium	15	29.41	B	4, 5, 12	i	1.2
S. tsevie	2	3.92	B	4, 5	i	e.n.z_{15}
Total	51					

*Calculated according to the total No. of each type of samples

3 and 4, respectively. These results nearly agreed with Molbak and Neimann (2002), Kimura *et al.* (2004) and Trawinska *et al.* (2008). Snow *et al.* (2008) isolated *Salmonella* in a rate of 10.7% in the United Kingdom, while, Van Overbeke *et al.* (2006) and Pieskus *et al.* (2008) reported that the incidence of *Salmonella* in broiler farms was 29% in Lithuania, 20% in Italy and 11% in Netherlands. El-Amin (2006) found that the infection rate of *Salmonella* in broiler chickens was 17.5% in Germany which was comparatively high and put Germany in the upper range in comparison to other European Union members.

The results of serotyping of *Salmonella* strains were observed in Table 9. A total of 51 strains were serotyped according somatic (O) and flagellar (H) antigens as 19 *S. enteritidis* (37.25%); 10 *S. infantis* (19.60%); 1 *S. chiredzi* (1.96%); 4 *S. kentucky* (7.84%); 15 *S. typhimurium* (29.41%) and 2 *S. tsevie* (3.92%). Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann-White Scheme; currently approximately 2500 serovars are recognized (Popoff *et al.*, 1994; Popoff, 2001).

Regarding the incidence of *Salmonella* serovars that isolated from each of the examined flock, Table 10 showed this result. In the present study, 19 *S. enteritidis* (37.25%) were isolated from 3 flocks (2.36, 1.91 and 2.3%, respectively). The serovar *S. enteritidis* was diagnosed more frequently as recorded by Abd-Allah (1995) who detected 10 (40%) serotypes of *S. enteritidis* out of 25 isolated *Salmonella* strains. Herikstad *et al.* (2002) considered *S. enteritidis* is the most common species of *Salmonella* that isolated from human worldwide. Strzalkowski *et al.* (2000) recovered *Salmonella* rods from dead broiler chicks and indicated that serovar *S. enteritidis* had the highest rate (90.88%). Also, Hui and Das (2001) recovered *S. enteritidis* and *S. typhimurium* constituting 12 (80%) and 3 (205), respectively out of 15 *Salmonella* strains. Similarly, Marin and Lainez (2009) recorded that *S. enteritidis* was the most prevalent serotype isolated during broiler

Table 10: The incidence of *Salmonella* serovars in each of the examined flock

		Salmonella serovars		
Flock	No. of examined samples	Serovars	No.	Percentage*
1	338	*S. enteritidis*	8	2.36
		S. typhimurium	5	1.47
		S. chiredzi	1	0.29
2	313	*S. enteritidis*	6	1.91
		S. infantis	4	1.27
		S. kentucky	2	0.63
		S. tsevie	1	0.319
3	217	*S. enteritidis*	5	2.30
		S. typhimurium	7	3.22
		S. kentucky	1	0.46
		S. tsevie	1	0.46
4	270	*S. infantis*	6	2.22
		S. kentucky	1	0.37
		S. typhimurium	3	1.11
Total	1138		51	4.48

*Calculated according to the total No. of each type of samples

rearing (66.7%) and Akhtar *et al.* (2010) revealed that overall serovar *enteritidis* prevalence rate in 206 *Salmonella* positive samples was 75.24% (155). Moreover, Hazem (2010) isolated from broiler chickens 6 *S. enteritidis* serotype out of a total 19 *Salmonella* serotypes. It was estimated that the national prevalence of *S. enteritidis* was 6.3% (Betancor *et al.*, 2010).

A total of 10 (19.60%) *S. infantis* serovars were represented as 4 (1.27%) from flock 2 and 6 (2.22%) from flock 4. Contrary finding was obtained by Snow *et al.* (2008) who found that *S. infantis* was not detected in broiler flock holding.

Four (7.84%) *S. kentucky* serovars were detected as 2 (0.63%) from flock 2; 1 (0.46%) from flock 3 and 1 (0.37%) from flock 4. The United States Department of Agriculture (USDA, 1999) found that although, *S. kentucky* was not among the most common serovars isolated from human sources however, approximately 50% of serovars from chicken and turkey sources were *S. kentucky*.

A total of 15 (29.41%) *S. typhimurium* serovars were isolated [5 from flock 1 (1.47%); 7 from flock 3 (3.22%) and 3 (1.11%) from flock 4]. These results were nearly relative to that observed by Oh and Choi (1996) and Chiu *et al.* (2010) while they were opposite to Snow *et al.* (2008) who isolated *S. typhimurium* in a rate 0.2%. Additionally, Sleim (2003) examined 185 chicken flocks and found 6 serotypes *S. typhimurium* out of 14 *Salmonella*.

European Food Safety Authority (EFSA, 2010) reported that the most frequently isolated *Salmonella* serovars in broiler chickens were, respectively in decreasing order, *S. infantis* (29.2% of the *Salmonella* positive broiler carcass samples), *S. enteritidis* (13.6%), *S. kentucky* (6.2%) and *S. typhimurium* (4.4%).

Figure 1 shows the results of agarose gel electrophoresis of *Salmonella* strains. The PCR produced positive amplification of 284 bp fragments of *invA* genes (100%), specific for all members of *Salmonella* species. These results were parallel to those obtained by Oliveira *et al.* (2002), Malorny *et al.* (2003) and Moussa *et al.* (2010) who reported that 139-141 primers, which target the *invA* gene were able to identify all the examined *Salmonella* serovars. The combination of a

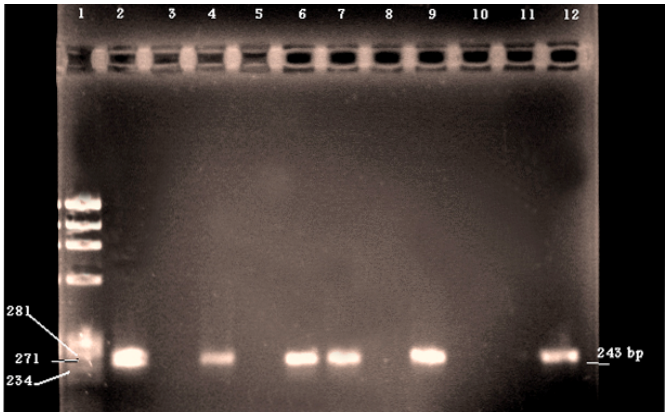

Fig. 1: Agarose gel electrophoresis showing amplification of 284 bp fragments of *invA* genes. Lane 1: DNA marker. lanes, 2, 4, 6, 7, 9 and 12 showing positive amplification of 284 bp fragments of *Salmonella* species, while lanes 3, 5, 8, 10 and 11 showing no amplification

routine PCR test in conjunction with traditional identification methods could be effective in providing a more accurate profile of the prevalence of *Salmonella* in poultry flocks.

It could be concluded that there are different *Salmonella* serotypes including *S. enteritidis*, *S. infantis*, *S. chiredzi*, *S. kentucky*, *S. typhimurium* and *S. tsevie* circulating in broiler chicken farms in Kalubia governorate, Egypt and the most prevalent ones are *S. enteritidis* and *S. typhimurium*. The prevalence of both *Salmonella* serotypes in our broiler chicken farms constitutes an important problem due to their zoonotic importance and consequently the adverse effect on the human health.

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