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Seroprevalence of Salmonellosis among Pigeon and its Surrounding Environment and Isolation of *Salmonella* Species

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Abstract: ***Aim:*** ***To determine seroprevalence of Salmonellosis among Pigeon and its surrounding environment in Egypt, isolation of Salmonella species from collected samples and serotyping of isolated stains.*** ***Materials and Methods:*** ***In this study, a total of 400 samples were collected from squabs and adult pigeon represented in cloacal swabs from diseases, liver, intestine and lymph node from apparent healthy, slaughtered and freshly dead one. Screening of pigeon environment was done by collection of 150 environmental samples. Bacteriological and serological examination of isolated salmonella species from positive samples were carried out.*** ***Results:****The prevalence rate of Salmonella isolates for squabs, pigeon and environmental samples was 5%, 3. 5% and 4. 6% respectively. The isolated serotypes recovered from squabs were 4 isolates of S. Typhimurium, and 2 isolates from each of S. Enteritidis, S. Agona and S. Montevideo. While in adults were S. Typhimurium and S. Enteritidis(3 isolates from each) and one isolates from S. agona. serotyping result of environmental samples revealed 3 serotypes of S. Typhimurium, S. Agona and S. Virginia.* ***Conclusion:*** ***Seroprevalence of Salmonella species is higher in squabs than that occur in adult with a higher rate in diseased followed by freshly dead and finally by apparently healthy slaughtered birds. Pigeon surrounding environment was screened for Salmonella species and isolated with a percent 4. 7% and serotyped as S. Typhimurium, S. Agona and S. Virginia.***

Keywords: Salmonellosis, Pigeon, Seroprevalence, Environment, Seroprevalence.

1. Introduction

Pigeons are common carriers of *Salmonella* as susceptible reservoirs to the bacteria, which is generally passed through drinking water or dust, food particles and fecal matter in the air. Pigeons dropping isa source of several zoonotic agents for birds, animals and humans, especially *Salmonella*, *E.* Coli as well as *Mycobacterium spp*. *Salmonella* has one of the highest mortality rates of infectious bacterial diseases in pigeons [1]. They are most susceptible to infection during the breeding period, as their disease resistance is compromised when they are stressed. In addition to the common avian symptoms, pigeons infected with *Salmonella* may contract arthritis, which is evident in their hesitation to move, unsteadiness on their feet and sometimes a complete loss of use of their legs. In the most severe infections, pigeons also can contract conjunctivitis, an eye infection, and excessive thirst. Damage to the heart, kidneys, liver and spleen also occurs, but there are often no outward symptoms of this except in the pigeon's death. This is why pigeons are one of the most dangerous carriers of *Salmonella*; they often exhibit no outward signs of Paratyphus until most of the flock has been infected [2]. Salmonellosis is the most common pigeon disease, caused by *S.* Typhimurium and *S.* Enteritidis. The disease is transmitted from parents to young pigeons and it can also be transmitted from sick pigeons by contamination of water or food with pigeon's excrements. Rodents, cockroaches and also humans can transmit *Salmonella* but these situations rarely occur. High rate of mortality in the first day of pigeon squab's life is a sign that pigeons have been infected with *Salmonella*. *Salmonella* progresses very slowly at adult pigeons with symptoms of diarrhea, anorexia, and polydipsia. Pigeons start losing weight and inflammations of joints start appear. These inflammations, untreated, can lead to arthritis or even paralysis. In some cases, pigeons avoid flying, get tired very fast. Spleen and liver grow in size and nodules in the pigeons internal organs can occur [3]. Severe losses due to this organism are seen in young domestic birds. In pigeons lofts *S.* Typhimuriu*m* causes heavy losses in squabs, squabs either die soon after hatching or develop swollen wing joints which render them unable to fly. Infection by this organism is manifested by enteritis, diarrhea and septicemia in fetal cases. Another important symptom is the neuromotor defects caused by encephalitis or infection of the inner ear. One hundred fifty samples were collected 150 samples from adult pigeons died suddenly from different pigeon's farms at different localities in Egypt. All cases were subjected to post-mortem and bacteriological examination, *S.* Typhimurium was isolated from the examined cases in ratios of 50% [4]. Pigeons in Cairo, Egyptwere screened for presence and antimicrobial susceptibility of *Salmonella*species. Multiresistant serotypes which were isolated from pigeon fecal samples are, *Salmonella* serotype Typhimurium, Braenderup, and Lomita, all strains were multiresistant [5]. Fivty fresh diarrheic faecal samples were collected from pigeon and 100 fresh samples of liver and intestine were collected from fresh pigeon carcasses. Out of

150 samples examined for *Salmonell*a species from pigeons, 12 (8 %) Salmonella isolates were detected. All the strains were identified as *S.* Typhimurium [6]. [7] study surveillance, identification and genetic characterization of *Salmonella species* which were isolated from poultry farm environment. Fifty nine isolates were collected from two farms (N=164 pens, feed, water and insects traps), all of these isolates were serotyped. The prevalence of serotypes detected were *S.* Enteritidis (24%) and *S.* Montevideo (5%). [8] focused on the possible health risks of workers during cleaning places contaminated with pigeon's faeces, *Salmonella spp*. are rarely isolated. [9] examined a total of 192 samples included fecal material on the floor, utensils, water, and carcasses and livers samples at different stages of processing. Incidence rates of *Salmonella* was increased from 30% in fecal material which were collected from incoming birds to 60% in air-chilled carcasses and 80% in cold-stored livers, the obtained data indicate occurrence of cross-contamination. Out of 112 strains isolated, 87 (77. 6%) were *S.* Enteritidis at the post-spray wash site, while 7 (6. 2%) were *Salmonella* serotype 4, 5, 12:b:-(II), and 6 (5. 4%) were *Salmonella* serotype 4, 12:b:-(II), and 12 strains were equally distributed among *S.* Typhimurium, *S.* Virchow, *and S.* Blockley (3. 6% each). [10] Surveyed the contamination rate by using*Salmonella* species of poultry feeds and feed components. Out of 360 samples 10% were founded to be contaminated. Mash feeds were more contaminated (21%) than pelleted feed (1. 4%). Twenty-eight serotypes of *Salmonella* were detected, while *S.* Enteritidis was not founded, despite the incidence of an epidemic infection caused in poultry by this serotype since 1987. The most frequently isolated serotypes were not the same as those encountered in poultry flocks. Therefore this work was aimed to determine seroprevalence of salmonellosis among pigeon and its surrounding in Egypt, bacteriological identification of isolated strains and serotyping of isolated strains.

2. Material and Methods

2.1 Samples

Samples were collected from diseased, apparently health slaughtered and freshly dead pigeon and squabs, where they obtained from different private pigeon farmer houses, according to Table (1), where located in Al-Giza Governorate, Egypt , during period from July 2010 till July 2013.

Table 1: Specimens for *Salmonella* isolation in squabs and pigeons

Health status of examined birds	*Type of samples*	*Squaps*		*Pigeons*	
		No. of examined squabs	*No. of samples*	*No. of examined pigeons*	*No. of samples*
Diseased	Cloacal swabs	95	95	60	60
Freshly dead	Liver, Intestine and lymphnode	50	50 50 50	40	40 40 40
Apparently Healthy slaughtered	Liver, Intestine and Lymphnode	55	55 55 55	100	100 100 100
Total		200	410	200	480

a) Environment of the diseased and freshly dead pigeons
A total of 90 samples were collected from different private pigeon farmer houses as follow, fifteen swabs from workers hands, 25 land filter paper, 25 samples from feedstuffs and 25 water samples.

b) Environment of the apparently healthy slaughtered pigeons
Sixty samples were collected from various pigeon slaughter shops as follow, fifteen swabs from workers hands, 15 swabs from trays and 30 samples from washing water.

c) Preparation of collected samples
Obtained samples were collected under aseptic condition. Twenty five gm of each sample were minced and homogenized in a separate sterile blender, according to [11].

d) Pre-enrichment
The prepared samples were placed in a sterile flask containing 225 ml of 1% pepton water and incubated at 37°& for 24 hrs according to [11].

e) Selective enrichment
One ml of the pre-enrichment culture was inoculated into tube containing 10 ml of Rappaport-Vassiliadis soy (RVS) broth at 41. 5ºC for 24hrs.

f) **Selective agar plates**
A loopfull from the inoculated and incubated RVS broth was streaked on XLD, MacConkey and S. S agar plates and incubate at 37ºC for 24 hrs.

g) Stock culture
Suspected colonies were picked up and streaked onto slope agar and incubated at 37ºC for 24 hrs. Then were used as a stock culture for further identification.

2. 2 Identification of bacterial isolates:

Purified bacterial isolates were subjected to cultural, morphological and biochemical identification by using the following tests:

a) Morphological identification:
A film from suspected colonies was stained with Grams stain and examined microscopically for morphological characters as described by [12**].** Colonies showing the morphological character of Salmonella were preserved on semisolid agar for biochemical identification.

b) **Biochemical identification**
Isolates were identified biochemically using the criteria of [13] and [14].

2. 3 API 20 Kits and diagnostic antisera

API-20E test kit used for the identification of Enteric bacteria (bioMerieux, Inc., France) provides an easy way to inoculate and read tests relevant to members of the Family *Enterobacteriaceae* and associated organisms. A plastic strip holding twenty mini-test tubes is inoculated with a saline suspension of a pure culture (as per manufacturer's directions). This process also rehydrates the desiccated medium in each tube. Few tubes are completely filled (CIT, VP and GEL), and some tubes are overlaid with mineral oil so that anaerobic reactions can be carried out (ADH, LDC, ODC, H2S, URE). After incubation in a humid chamber for 24 hours at 37ºC, the color reactions are read (some with the aid of added reagents), and the reactions (plus the oxidase reaction done separately) are converted to a seven-digit code. The code is fed into the manufacturer's database gives back the identification, usually as genus and species.

24 Serological identification

Isolates that were preliminary identified biochemically as *Salmonella* were subjected to serological identification according Kauffmann-White scheme [15] as follow: Suspected *Salmonella* isolates were cultured onto nutrient agar slop for 24 hours at 37ºC. Serological agglutination technique was applied by taking a loopful from suspected colonies and suspended in a drop of phosphate buffer saline (PBS) on a slide, so as to make a homogenous suspension. Only smooth isolates were examined serologically and rough autoagglutinable isolates were discarded. A drop of *Salmonella* antisera was added to the suspension with a standard loop and thoroughly mixed to make the organism in close contact with antisera. Positive agglutination occurred within one minute and could be easily seen with the naked eye. A delayed or partial agglutination was considered as negative or false.

2. 5 Determination of O (somatic) and H (flagellar) antigen [12].

Polyvalent somatic "O" and flagellar "H" antigens were first tried to assure that the suspected isolates were *Salmonella*. Positive culture were then tested with each of the O-grouping sera followed by the respective monospecific O and H antisera factors in order to determine the complete antigenic formula.

3. Results

3.1 Prevalence of *Salmonella*species in squabs and adult pigeon:

The prevalence rate of *Salmonella* species was 5% in squabs and 3. 5% in adult pigeon, as shown in Table (2) and Table (3) respectively. The incidence rate of *Salmonella* differ according to health status as it was high in diseased squabs and apparently healthy pigeon (1. 5%) followed by apparently healthy and freshly dead squabs (1. 5%).

Table 2: Prevalence of *Salmonella* in squabs (n=200).

Health status of squabs	No. of examined squabs	*Salmonella* positive samples in squabs	
		No.	%
Diseased	95	4	2
Freshly dead	50	3	1.5
Apparent healthy Slaughtered	55	3	1.5
Total	200	10	5

Table 3: Prevalence of *Salmonella* in adults (n=200).

Health status of squabs	No. of examined sample	*Salmonella* positive	
		No.	%
Diseased	60	2	1
Freshly dead	40	2	1
Apparent healthy Slaughtered	100	3	1.5
Total	200	7	3.5

3. 2Prevalence of Salmonella in Environments

The results presented in Table (4) indicated that out of 150 samples, 7 *Salmonella* species were isolated. The highest prevalence occurred in land filter paper from different private pigeon farmer houses (8%).

Table 4: Prevalence of *Salmonella* isolates in the environment.

	Type of examined sample	No. of examined sample	Salmonella positive	
			No.	%
Environment of diseased and freshly dead pigeons	Feed stuffs	25	1	4
	Water	25	0	0
	Land filterpaper	25	2	8
	Swabs from worker's hand	15	1	6.6
Environment of apparent healthy pigeons	Wash water after washing	30	1	3.3
	Swabs from trays	15	1	6.6
	Swabs from worker's hands	15	1	6.6
Total		150	7	4.66

3. 3 Identification of the Isolated Organism

a) Morphological identification:
On MacConkey agar, *Salmonella* colonies appeared colorless or pale (non-lactose fermenter). On XLD agar, *Salmonella* colonies appeared as red colonies with a black center. On S. S agar, *Salmonell*a colonies appeared as white colonies with a black center. Gram's stain smears from suspected colonies showed Gram-negative rod-shaped *Bacilli*Bacillus.

b) Biochemical identification:
The isolated *Salmonella* species were subjected to further biochemical tests and API 20 E test. Results were recorded in Table (5) and (6).

Table 5: Biochemical identification of *Salmonella* isolates.

Medium	*Reactions/enzymes*	*Results*		*Salmonella reaction*
		Negative	**positive**	
TSI	Acid production (if the butt is yellow, and the slope is red, acid production is only from glucose)	Butt red	Butt yellow	+
	Acid production	Surface	Surface	+
	H_2S production	No black	Black	+
Urea broth	Urease	Remain	Purple	_
LDC test	Lysine	A yellow	A	+
Nitrate ager	Nitrate reductase	No colour	Red	+
VogesProskauer	Acetoin production	Remain colourless	A pink/red colour	_
Methyle red	Pyruvic acid	Diffuse yellow colour	Diffuse red colour	+
Indole	Indole production	Yellow	Red /	_
Citrate	Sodium Carbonate	Remain	blue	+
Oxidase test	Oxidase production	No change in colour	Deep purple colour	_

Table 6: Result of API 20 test.

ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	**Identification**
-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	***Salmonella* spp.**

3. 4 Serological identification of the isolated *Salmonella*

The biochemically identified *Salmonella* culture were subjected to serological identification using polyvalent and monovalent "O" and: H" *Salmonella* antisera.

Serotyping of *Salmonella* isolates from pigeon:
Serotyping of *Salmonella* isolates from squabs:

According to Table (7), serotyping of *Salmonella*species isolated from squabs revealed 4 serotypes represented in *S. Typhimurium*(4 isolates), *S.* Enteritidis, *S.* Agona and*S.* Montevideo (2 isolates from each). *Salmonella* examination of the internal organs of freshly dead and apparently healthy slaughtered squabs for signs of *Salmonella* infection revealed that the lowest signs occurred in liver of apparently healthy slaughtered and the highest prevalence occurred in intestine of freshly dead squabs, As shown Table (8).

Table 7: Results of serotyping of isolated *Salmonella* in squabs.

Health status of examined squabs	No. of examined pigeons	*Salmonella* positive		
		No.	**%**	Serovars
Diseased	95	4	4. 2	*S.* Enteritidis *S.* Montevideo (2) *S.* Typhimurium
Freshly dead	50	3	6	*S.* Agona *S.* Enteritidis *S.* Typhimurium
Slaughtered	55	3	5. 45	*S.* Agona *S.* Typhimurium (2)
Total	200	10	5	

Table 8: Prevalence of *Salmonella* in internal organs of freshly dead and apparent healthy slaughtered squabs

Health status of the birds	Examined internal organ	Examined No.	*Salmonella* +ve No.	*Salmonella* +ve %	*Salmonella* spp.
Apparent healthy slaughtered	Lymph node	55	2	3. 6	*S.* Typhimurium (2)
	Intestine	55	3	5. 5	*S.* Agona (1) *S.* Typhimurium (2)
	Liver	55	1	1. 8	*S.* Typhimurium (1)
Freshly dead pigeon	Lymph node	50	2	4	*S.* Enteritidis (1) *S.* Typhimurium (1)
	Intestine	50	3	6	*S.* Agona (1) *S.* Enteritidis (1) *S.* Typhimurium (1)
	Liver	50	1	2	*S.* Typhimurium (1)

3. 5 Serotyping of *Salmonella* isolates from adult pigeons

Serotyping of *Salmonella* species isolated from adult pigeons revealed 3 serotypes represented in *S.* Typhimurium, *S.* Enteritidis (3 isolates from each) and one isolate from *S.* Agona, as shown in Table (9), *Salmonella* examination of the internal organs of freshly dead and apparent healthy slaughtered adults revealed that the lowest prevalence occurred in liver of apparent healthy slaughtered and the highest prevalence occurred in intestine of freshly dead adult pigeons, as shown in Table (10). \

Table 9: Results of serotyping of isolated Salmonella in adult pigeons

Health status of examined adult pigeons	No. of examined adult pigeons	*Salmonella* positive		
		No.	%	Serovars
Diseased	60	2	3.3	*S.* Typhimurium (2)
Freshly dead	40	2	5	*S.* Typhimurium (1) *S.*Enteritidis (1)
slaughtered pigeon	100	3	3	*S.* Agona (1) *S.*Enteritidis (2)
Total	200	7	3.5	

Table 10: Prevalence of *Salmonella* in internal organs of freshly dead and apparent healthy slaughtered adult pigeons

Health status of the birds	Examined internal organ	Examined No.	*Salmonella* +ve No.	*Salmonella* +ve %	*Salmonella* spp.
Apparent healthy slaughtered	Lymph node	100	2	2	*S.* Agona (1) *S.* Enteritidis (1)
	Intestine	100	3	3	*S.* Agona (1) *S.* Enteritidis (2)
	Liver	100	1	1	*S.* Enteritidis (1)
Freshly dead pigeon	Lymph node	40	1	2.5	*S.* Enteritidis (1)
	Intestine	40	2	5	*S.* Enteritidis (1) *S.* Typhimurium (1)
	Liver	40	1	1	*S.* Enteritidis (1)
	Intestine	40	2	5	*S.* Enteritidis (1) *S.* Typhimurium (1)
	Liver	40	1	1	*S.* Enteritidis (1)

3. 6 Serotyping of the *Salmonella* isolates from environments:

The results of serotyping of *Salmonella* isolates in environments are presented in Table (11), the isolated *Salmonella* revealed 5 isolates identified as *S.* Typhimurium, one isolate from *S.* Agona and *S.* Virginia.

Table 11: Results of serotyping of isolated *Salmonella* in environments

Type of examined sample	No. of examined sample	*Salmonella* positive		
		No.	%	Serovars
Feed stuffs	25	1	4	*S.* Typhimurium
Water	25	0	0	
Land filterpaper	25	2	8	*S.* Typhimurium *S.*Virginia
Swabs from worker's hand	15	1	6.6	*S.*Typhimurium
Wash water after washing	30	1	3.3	*S.*Typhimurium
Swabs from trays	15	1	6.6	*S.*Agona
Swabs from worker's hands	15	1	6.6	*S.*Typhimurium

4. Discussion

In this study200 squabs and 200 adult pigeons, were examined for isolation and identification of *Salmonella*. Seventeen *Salmonella* isolates (4. 75%) , 10 in squabs (5%) and 7 in adult pigeons (3. 5%), were isolated and serotyped in squabs as *S.* Typhimurium, *S.* Enteritidis, *S.* Agona and *S.* Montevideo (40, 20, 20 and 20%) respectively, and serotyped in adults as *S.* Typhimurium, *S.* Enteritidis, and *S.* Agona (42. 9, 42, 9 and 14. 8%) respectively, this result differ than result obtained by [16] who isolated 9 *Salmonella* (1. 3%) out of 700 feral pigeons captured in public parks and storehouses of animal feeds. In this study, prevalence of *Salmonella* in slaughtered squabs liver, intestine and intestinal lymph node was at a percentage of (1. 8%, 5. 5% and 3. 6%) and in adults pigeon liver, intestine and intestinal lymph node was with a percentage of (1%, 3% and 2%) while [17] recorded only 2% *Salmonella* positive in slaughtered pigeons but [18] detected (12%) *S.* Typhimurium from wooden pigeon carcasses and liver was highly contaminated with *Salmonella* (8%) but no *S.* Typhimurium was detected in squabscarcasses. On the other hand, [19] recorded 1. 4% *Salmonella* from 18 farms (1110 squab) , 4. 3% *Salmonella* from 1 farm (250 squab) and 4. 1% *Salmonella* positive from 23 farms (2900 squab) but [20] revealed no positive samples for *Salmonella* from 50 squabs carcasses from different markets in Cairo and Giza governorates.

In this study, we isolated 6 strains of *Salmonella* from cloacal swabs of diseased squabs and adults pigeon at a percentage of (4. 2% &3. 3%) respectively. [21] isolated six Salmonella strains from faecal samples of pigeons from lofts suffering from salmonellosis but [22] isolated one hundred-eleven *Salmonella*samples from domestic pigeons suspected to salmonellosis. Salmonellae isolates belonged to serogroups D1(84. 26%), B(8. 33%) and C1(7. 41%). In this study, 5 strains were isolated of *Salmonella* from freshly dead squabs and pigeons with a percentage of (6% &5%) respectively**.** [23] collected 150 samples from adult pigeons died suddenly from different pigeon's farms at different localities in Egypt. *S.* Typhimurium was isolated in ratios of 50%. In our study, 7 samples out of 150 pigeon environment samples were founded to be positive to *Salmonella* species(4. 7%) and serotyped as *S.* Typhimurium, *S.* Agona and *S.* Virginia (71. 4%, 14. 3% and 14. 3%) respectively and this result differ from [24] who surveyed the rate of contamination with *Salmonella* species of poultry feeds and feed components. Ten percent of 360 samples were founded to be contaminated. Twenty-eight serotypes of Salmonella

were isolated, but no *S. Enteritidis* wasn't found while [25] examined 192 samples included fecal material on the floor, utensils, water, and carcasses and livers at several stages of processing. From a total of 112 isolated strains, 87 (77. 6%) were *S. Enteritidis* at the post-spray wash site, 7 (6. 2%) *Salmonella* serotype 4, 5, 12:b:-(II) and 6 (5. 4%) *Salmonella* serotype 4, 12:b:-(II), and the remaining 12 strains were equally distributed among *S.* Typhimurium, *S.* Virchow and *S.* Blockley (3. 6% each). On the other hand [26] who focused on the possible health risks of workers during cleaning places contaminated with pigeon's faeces*Salmonella spp*. are rarely isolated while [27] isolated *Salmonella* from four (80%) of five farms with window less poultry houses in Japan. The isolation rate of *S.* Enteritidis as compared with the other serotypes were 90. 9% of environments. [28] detected 59 *Salmonella* isolates from two farms (N=164; pens, feed, water and insects traps). The prevalence of serotypes detected were *S.* Enteritidis (24%) and *S.* Montevideo (5%).

5. Conclusion

In conclusion, in this study *Salmonella* species were isolated from freshly dead, diseased and apparently healthy slaughtered squabs at a percent 5% and from freshly dead, diseased and apparent healthy slaughtered adult pigeons with a percent 3. 5%, these isolates were serotyped in squabs as *S.* Typhimurium, *S.* Enteritidis, *S.* Agona and S. Montevideo (40, 20, 20 and 20%) respectively, and serotyped in adults as *S.* Typhimurium, *S.* Enteritidis, and *S.* Agona (42. 9, 42, 9 and 14. 8%) respectively. So, the prevalence of *Salmonella* is higher in squabs than that occur in adult with a higher rate in diseased followed by freshly dead and finally by apparently healthy slaughtered pigeons. The most predilection site for *Salmonella* isolation was intestine followed by intestinal Lymph node then liver. Also, pigeon environment were screened for *Salmonella* species and was isolated at a percent 4. 7% and serotyped as *S.* Typhimurium, *S.* Agona and *S.* Virginia (71. 4% , 14. 3% and 14. 3%) respectively.

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