

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 37°C, 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.

2.4 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 37°C. 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	<i>Salmonella</i> 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	<i>Salmonella</i> 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	<i>Salmonella</i> 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, *Salmonella* 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	+
		Surface	Surface	
		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
-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	<i>Salmonella</i> 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	<i>Salmonella</i> positive		
		No.	%	Serovars
Diseased	95	4	4.2	<i>S. Enteritidis</i> <i>S. Montevideo</i> (2) <i>S. Typhimurium</i>
Freshly dead	50	3	6	<i>S. Agona</i> <i>S. Enteritidis</i> <i>S. Typhimurium</i>
Slaughtered	55	3	5.45	<i>S. Agona</i> <i>S. Typhimurium</i> (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.	<i>Salmonella</i> +ve No.	<i>Salmonella</i> +ve %	<i>Salmonella</i> spp.
Apparent healthy slaughtered	Lymph node	55	2	3.6	<i>S. Typhimurium</i> (2)
	Intestine	55	3	5.5	<i>S. Agona</i> (1) <i>S. Typhimurium</i> (2)
	Liver	55	1	1.8	<i>S. Typhimurium</i> (1)
Freshly dead pigeon	Lymph node	50	2	4	<i>S. Enteritidis</i> (1) <i>S. Typhimurium</i> (1)
	Intestine	50	3	6	<i>S. Agona</i> (1) <i>S. Enteritidis</i> (1) <i>S. Typhimurium</i> (1)
	Liver	50	1	2	<i>S. Typhimurium</i> (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	<i>Salmonella</i> positive		
		No.	%	Serovars
Diseased	60	2	3.3	<i>S. Typhimurium</i> (2)
Freshly dead	40	2	5	<i>S. Typhimurium</i> (1) <i>S. Enteritidis</i> (1)
slaughtered pigeon	100	3	3	<i>S. Agona</i> (1) <i>S. Enteritidis</i> (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.	<i>Salmonella</i> +ve No.	<i>Salmonella</i> +ve %	<i>Salmonella</i> spp.
Apparent healthy slaughtered	Lymph node	100	2	2	<i>S. Agona</i> (1) <i>S. Enteritidis</i> (1)
	Intestine	100	3	3	<i>S. Agona</i> (1) <i>S. Enteritidis</i> (2)
	Liver	100	1	1	<i>S. Enteritidis</i> (1)
Freshly dead pigeon	Lymph node	40	1	2.5	<i>S. Enteritidis</i> (1)
	Intestine	40	2	5	<i>S. Enteritidis</i> (1) <i>S. Typhimurium</i> (1)
	Liver	40	1	1	<i>S. Enteritidis</i> (1)
	Intestine	40	2	5	<i>S. Enteritidis</i> (1) <i>S. Typhimurium</i> (1)
	Liver	40	1	1	<i>S. Enteritidis</i> (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	<i>Salmonella</i> positive		
		No.	%	Serovars
Feed stuffs	25	1	4	<i>S. Typhimurium</i>
Water	25	0	0	
Land filterpaper	25	2	8	<i>S. Typhimurium</i> <i>S. Virginia</i>
Swabs from worker's hand	15	1	6.6	<i>S. Typhimurium</i>
Wash water after washing	30	1	3.3	<i>S. Typhimurium</i>
Swabs from trays	15	1	6.6	<i>S. Agona</i>
Swabs from worker's hands	15	1	6.6	<i>S. Typhimurium</i>

4. Discussion

In this study 200 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. *Salmonella* 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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