Studies on *Salmonella* species from migratory and native wild birds

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Abstract

298 samples from 37 species of wild birds from different sources were examined bacteriologically for *Salmonella*. 32 samples were positive for *Salmonella* spp., with total incidence of 13.5% .The incidence in migratory, free living and Zoo birds were 28.26%, 18.6% and 4.9%, respectively. Serotyping of the isolates revealed *Salmonella* Typhimurium, *Salmonella* Rissen, *Salmonella* Regent, *Salmonella* Doncaster, *Salmonella* Curacao, *Salmonella* IIIb group (O65), and untyped *Salmonella* .The most isolated strain was *Salmonella* Typhimurium with percent of 40.63%. Antibiogram test was performed on the 2 strains from Zoo birds, the first strain was sensitive to chloramphenicol, colistin, streptomycin, tetracycline, nalidixic acid, erythromycin and danofloxacin, this strain was intermediately sensitive to doxycycline and gentamycin, while, it was resistant to ampicillin, neomycin and pencillin G. On the other hand *Salmonella* IIIb (group O65), was only sensitive to danofloxacin and resistant to the other antimicrobial agents. 26 serum samples were examined using *S.* Typhimurium antigen from which 15 samples were positive. PCR technique was done on 30 samples to compare the sensitivity of two different isolation enrichments (RV and BP) with that of SMT; it was reported that RV-PCR was more sensitive than BP-PCR and SMT with percent of 66.7%, 46.7%, and 13.3%, respectively.

Key words: *Salmonella* Typhimurium*, Salmonella* Rissen*, Salmonella* Regent, *Salmonella* Doncaster*, Antibiotic resistance.*

Introduction

Wild birds are important to public heath because they can be infected by different disease agents especially salmonellosis. They have been infected with different *Salmonella* serovars such as *Salmonella* pullorum which cause pullorum disease and *Salmonella gallinarum* which cause fowl typhoid. But, wild birds are more commonly infected by the variant of *Salmonellae* that are collectively referred to as paratyphoid forms, of which *Salmonella* Typhimurium is a predomenent representative **(Friend and Franson, 1988).**

Due to the need to constantly monitor the health of the birds and its relation to human, isolation and identification of *Salmonella* are very important as well as detection of the common antimicrobial drugs which are required for treatment of birds suffering from salmonellosis **(Olivera *et al*., 2006).**

There was an alarming increase in wild birds' mortality; the species affected were primarily Pine siskins, Purple finches, House sparrows and all of the examined birds died due to infection with *Salmonella* Typhimurium **(Bowes, 1993).**

The agglutination tests have been used for detecting antibodies to various paratyphoid *Salmonellae* especially *S.* Typhimurium **(Swayne *et al.*,1998).**

Serological tests are developed for the diagnosis of *Salmonella* infection in animals and birds. These tests are normally designed to detect a limited range of *Salmonella* serovars. Serum agglutination test (SAT) is used successfully for over 50 years for identification of infected flock; tube agglutination test (TAT) is the method of choice for diagnostic purposes for samples from all species of animals and

birds **(OIE, 2004).**
PCR represents a major advance in diagnostic methods in terms of speed and sensitivity **(Freschi *et al.*, 2005)**.
The aim of the work was to estimate the incidence of *Salmonellae* in wild birds of different species (migratory birds, Zoo birds and free living birds), serotyping of the isolates of *Salmonella* by slide agglutination test using poly and monovalent antisera, application of antibiotic sensitivity test against the *Salmonella* isolates from Zoo birds species. Detection of *Salmonella* Typhimurium antibodies among Zoo birds using tube agglutination test was applied to differentiate the infected from carrier birds. Amplification of the (*invA*) gene P CR to confirm the identification of *Salmonella* spp. and finally to evaluate PCR under different enrichments for *Salmonella* isolates.

Materials and Methods:
A total of 298 different samples from different wild birds were collected to be examined. These were 209 cloacal swabs, 28 intestinal contents, thirty five drag swabs and 26 serum samples.
(ISO 6975, 2002) was used for isolation and identification of *Salmonella*. The organism were serotyped according to **(Kauffmann and Das-Kauffmann, 2001)** using O and GHHH H antisera. Antibiotic sensitivity test using the disk diffusion technique was applied according to **(Cruickshank *et al*., 1995).**
Tube agglutination test was done for detection of *Salmonella* Typhimurium antibodies among the examined Zoo birds **(Swayne *et al.*, 1998).** These antigens which were used in the tube agglutination test were kindly obtained from Institute of Serum and Vaccine Production, Abassia, Egypt.
Extraction of *Salmonella* DNA was done by boiling method **(Croci, 2004)**, and amplification of *invA* gene was done according to **(Olivera, 2003).** Initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 1 s, annealing at 55˚C for 1 s, extension at 72˚C for 21 s and a final extension at 72˚C for 7 min was applied in **Biometra T3000** thermocycler.

Results and discussion:
Salmonella spp. was identified by culture characters as well as the identical biochemical tests.
Most isolated *Salmonella* spp. were from migratory birds followed by free living birds then Zoo birds. The results revealed that on examination of 237 samples collected from 37 wild bird species, *Salmonella* species were isolated with an incidence of 13.5 %.
Incidence of *Salmonella* from different bird groups.
The incidence of *Salmonella* species from migratory birds was 28.26% (13/46), While, it was 4.9% (6/121) from Zoo birds and by examination of free living birds (feral birds), the incidence of *Salmonella* was 18.6% (13/70).

Table (1). Incidence of *Salmonella* species isolated from wild birds of different sources.

Different wild Birds	No. of examined samples	No. of wild bird species	Incidence of *Salmonella* isolation			
			Negative		Positive	
			No.	%	No.	%
Migratory	46	8	33	71.74	13	28.26
Zoo birds	121	22	115	95.04	6	4.96
Feral	70	7	57	81.42	13	18.6
Total	237	37	205	86.5	32	13.5

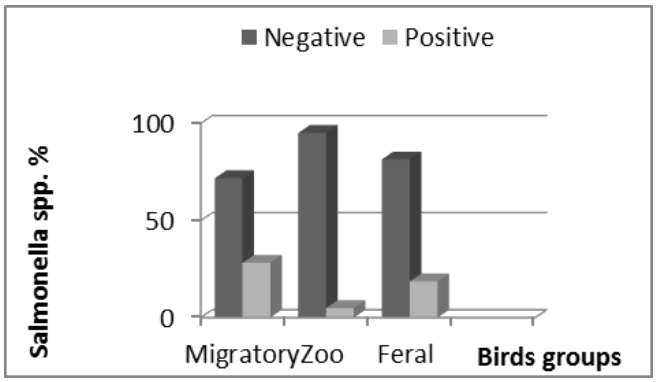

Fig. (1). Rate of *Salmonella* incidence from different bird species.

Incidence of *Salmonella* from live and dead Migratory birds.

Table (2). Incidence of *Salmonella* species isolated from the different examined migratory birds.

Species	No. of examined samples	Incidence of *Salmonella*	
		No.	%
Guinea fowl	9	0	0.00
Common pin tail	4	3	75.00
Common coot	5	1	20.00
Shoveler	5	0	0.00
Little stint	2	0	0.00
Green winged duck	9	7	77.80
Water fowl	10	1	10.00
Sheldrake	2	1	50.00
Total	46	13	28.26

Incidence of *Salmonella* from different live migratory birds.

On examination of 25 samples from 5 species of migratory birds, 4 positive samples (from Common pin tail and Shoveler) were detected with a percentage of 16%.

Incidence of *Salmonella* from different dead migratory birds.

Out of 21 samples that were examined from dead migratory bird related to 3 species (Green winged duck ,water fowl and Sheldrake), 9 samples were positive with a percentage of 43% .

Incidence of *Salmonella* from Zoo birds.

6 positive cases of *Salmonella* species were recorded with an incidence of 4.96% (6/121) from 22 examined species of Zoo birds. **Table (3).**

Table (3). Incidence of *Salmonella* from Zoo birds.

Species of Zoo birds	No. of examined sample	Incidence of *Salmonella* species			
		Negative		Positive	
		No.	%	No.	%
Galliformes:					
Common pea fowl	10	10	100.0	0	0.0
White pea fowl	10	10	100.0	0	0.0
Helmeted guinea fowl	10	10	100.0	0	0.0
Golden pheasant	2	2	100.0	0	0.0
Mongolian pheasant	2	2	100.0	0	0.0
Silver pheasant	2	2	100.0	0	0.0
Spotted sand grouse	3	3	100.0	0	0.0
Anseriformes:					
Mallard duck	10	10	100.0	0	0.0
Grey Chinese goose	9	7	77.7	2	22.3
Egyptian goose	3	3	100.0	0	0.0
Pick duck	3	3	100.0	0	0.0
Wild turkey	5	5	100.0	0.	0.0
Passeriformes:					
Grey and white Zebra finches	4	4	100.0	0	0.0
Psittaciformes:					
Peach faced rosy	6	6	100.0	0	0.0
African grey parrot	4	2	50.0	2	50.0
Ornate lorry	2	2	100.0	0	0.0

Incidence of *Salmonella* from live and dead feral birds.

Table (4). Incidence of *Salmonella* species isolated from the different examined feral birds.

Species	No. of examined sample	Incidence of *Salmonella*	
		No.	%
Quail	14	0	0.00
Rook	5	0	0.00
Kestrel	14	8	57.00
Falcon	2	0	0.00
Pigeon	23	4	20.00
Sparrows	5	0	0.00
Parrots	7	1	14.30
Total	70	13	18.60

Incidence of *Salmonella* from different Live feral birds.

Among 63 samples related to 7 species of live feral birds, 12 positive samples were detected with the total percentage of 19.04%. The positive result was obtained from Kestrel and Pigeon only.

Incidence of *Salmonella* from different Dead feral birds.

Out of 7 samples from dead feral birds related to 3 species, only 1 sample was positive for *Salmonella* from Parrots with a percentage of 14.3%, while 6 samples were negative with a percentage of 85.7%.

***Salmonella* Serotyping**:

Salmonella Typhimurium was the most isolated strain (13/32) with a percentage of 40.6%

followed by *Salmonella* Doncaster (5/32) with a percentage of 15.63%, Both *Salmonella* Curacao *and Salmonella* Rissen (4/32) with percentage of 12.5% each, *Salmonella* Regent (3/32) with a percentage of 9.37%, *Salmonella* IIIb (2/32) with a percentage of 6.25% and untyped one (1/32) with a percentage of 3.125%.

Antibiotic sensitivity testing was done for the 2 isolated strains from the Zoo birds [*Salmonella* Curacao and *Salmonella* IIIb (group O65)]. *Salmonella* Curacao was sensitive to chloramphenicol, colistin, streptomycin, tetracycline, nalidixic acid erythromycin, danofloxacin, this strain was intermediately sensitive to doxycycline and gentamycin, while, it was resistant to ampicillin, neomycin and pencillin G as shown in **Table (5).**

On the other hand, *Salmonella* IIIb (group O65), was only sensitive to danofloxacin and resistant to the rest of the antimicrobial agents (multidrug resistant strain).

Table (5). Results of antibiotic sensitivity testing for the two isolates of *Salmonella* from Zoo birds.

Antimicrobial agent	*S.*Curacao		*S.* IIIb (group O65)	
	Value	Analysis	value	Analysis
Ampicillin 10µg	9	R	9	R
Chloramphenicol 30 µg	20	S	7	R
Colistin 10 µg	13	S	6	R
Danofloxacin 5 mg	21	S	27	S
Doxycycline 30 µg	14	I	6	R
Erythromycin 15 µg	18	S	9	R
Gentamycin 10 µg	13	I	12	R
Nalidixic acid 30 µg	20	S	13	R
Neomycin 30 µg	9	R	4	R
Pencillin G 10 µg	17	R	12	R
Streptomycin 10 µg	20	S	8	R
Tetracycline 30 µg	20	S	6	R

S: sensitive I: intermediate R: resistant

Result of *Salmonella* isolation from drag swabs.

All the samples were negative for *Salmonella* spp. either from the (13) feral Pigeon houses or the 22 Zoo bird houses.

Tube agglutination test. From the 26 serum samples examined from the 6 Zoo bird species, 15 sera were positive (suspected to be positive carriers) with 57.6%.

Table (6). Serological identification of *Salmonella* Typhimurium by tube agglutination test .

1/25 or 1/50 was considered as +ve carrier bird for *S.* Typhimurium.

Species	No of examined samples	Results of *S.* Typhimurium antigen (TAT) titer
Helmented Guinea fowl	4	2 (–ve), 2 (+ve)
White Guinea fowl	2	1 (-ve), 1 (+ve)
Mallard duck	4	4 (+ve)
Fantail pigeon	4	2 (–ve), 2 (+ve)
White pea fowl	4	3 (–ve), 1 (+ve)
Common pea fowl	5	3 (–ve), 2 (+ve)
Grey Chinese goose	3	3 (+ve)

PCR for detection of *Salmonella* isolated from Migratory birds.

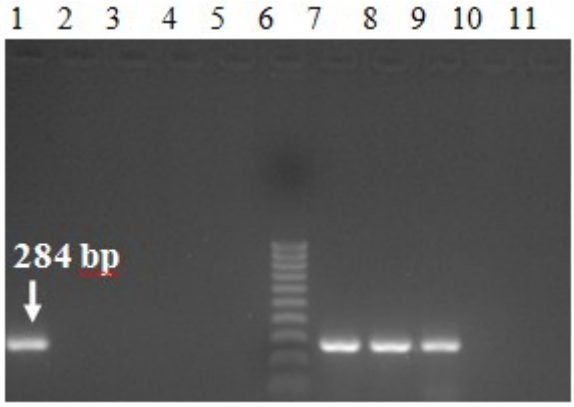

Photo. (1). Agarose gel electrophoresis showing the result of PCR for detection of *Salmonella* isolated from migratory birds. Positive amplification of 284 bp for *invA* gene of *Salmonella* from the Buffer peptone enrichment sample obtained from Green winged duck 1. (lane, 1).
Positive amplification of 284 bp for *invA* gene (lane 7, 8 and 9) represent RV enriched *Salmonella* samples obtained from Green winged duck 1, Green winged duck 2 and Sheldrake.

PCR for detection of *Salmonella* isolated from Zoo birds

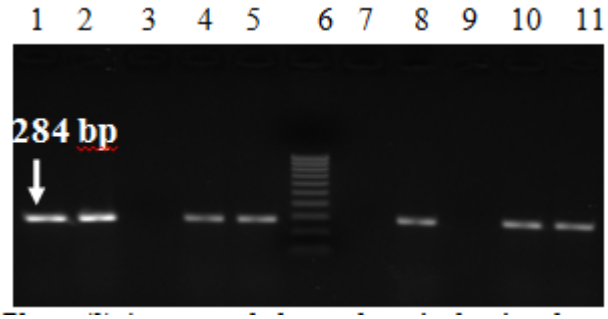

Photo. (2) Agarose gel electrophoresis showing the result of PCR for detection of *Salmonella* isolated from Zoo birds.
(Lane 1, 2 and 4): Positive amplification for 284 bp of *invA* gene, representing BP enriched *Salmonella* samples of Helmented Guniea fowl, Mallard duck and Grey Chinese goose, respectively. **(**Lane 7, 8 and 10): positive amplification of the same gene represent the RV enriched *Salmonella* samples for the same spp., respectively, (Lane 10) represent positive RV enriched sample of Blue and yellow marcow.

Results of PCR for *Salmonella* isolated from Feral birds.

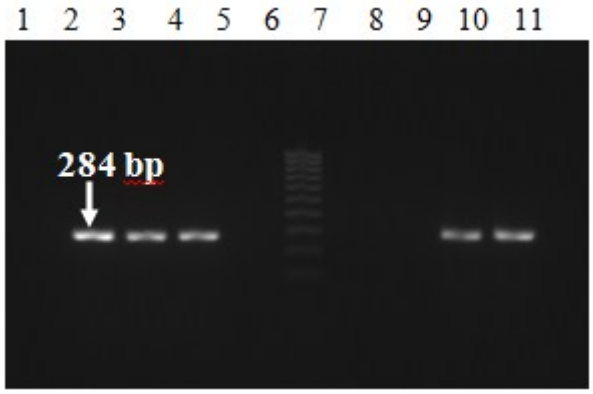

Photo. (3). Agarose gel electrophoresis showing the result of PCR for *Salmonella* isolated from feral birds. Positive amplification of 284 bp of *invA* gene of *Salmonella* represent samples enriched with BP obtained from Sparrow (lane 2), Pigeon 1 (lane 3). Positive amplification of 284 bp for *invA* gene of *Salmonella* represnt samples enriched with RV obtained from Pigeon (lane, 8 and 9), and Sparrow (lane 10).

After a comparison between SMT and PCR using enriched samples with BP and RV, it was revealed that *Salmonella* species were detected in the percentages of 13.33%, while they were detected by BP-PCR and RV-PCR with the percentages of 46.7% and 66.7%, respectively.

The potential for spread of infectious agents from wild birds and animals to human and domestic livestock is great, and this prospect is even more pronounced for wild birds. Many birds' species play an important rule in faecal contamination of drinking water sources and agricultural crops and may also come into close contact with domestic birds enabling direct transfer of infectious agents to take place **(Lillehaug *et al*., 2005).**

Myint *et al*. (2006) used the Buffer peptone water for *Salmonella* isolation followed by rappaport Vassiliadis medium and tetrathionate broth.

The incidence of *Salmonella* species isolation from migratory birds was 28.26% (13/46). While, it was 4.9% (6/121) from Zoo birds, and by examination of free living birds (feral birds)**,** the incidence of *Salmonella* was 18.6% (13/70)**.**

These results are nearly in agreement with that obtained by **Faddoul *et al.* (1965)** who surveyed wild birds and isolated *Salmonella* from 12 out of 100 samples. Eight of this isolates were from Cowbird, 2 from House sparrow, 1 from each of White throated sparrows and Herring gull with an incidence of 12%. **Cizek *et al.* (1995)** isolated *Salmonella* from 8 birds out of the 31 examined birds with an incidence of 25.8% .On various agricultural farms, *Salmonella* were found in 2 birds out of 2186 birds examined. Out of 35 birds caught at a municipal waste-dump site, Salmonellae were isolated from one specimen. While, none of *Salmonella* were found in birds living in reed growths.

Also **MIRZAIE *et al.* (2010)** reported that from 470 house sparrows that were subjected to culture, the results showed that 18 samples (3.8%) were positive for *Salmonella*. The 18 *Salmonella* isolates that were characterized showed that the most predominant serovars were *Salmonella* Typhimurium and *S*. enteritidis (9 and 8 cases each, respectively), whereas only 1 serovar belonged to *S*. Montevideo.

Different species of migratory birds were examined in this search for *Salmonella* in our Reference laboratory for veterinary quality control on poultry production. On examination of 46 samples related to 8 species of live and dead migratory birds, only 13 samples were positive with a percentage of 28.26%. While 33 samples were negative with a percentage of 71.73% (Table, 2). It was revealed that Green winged duck had the highest percentage of isolation (77.8%), and then Common pintail (75%) and Sheldrake (50%). On the other hand, Water fowl had the lowest percentage (10%).

Nielsen (1960) detected an outbreak of salmonellosis in Mallard duck raised for hunting and concluded that they acquired infection from other wild birds. While, in **1999, Pennycott and Duncan** reported an outbreak of salmonellosis in wild ducks and gulls in Northern Hemisphere.

Also different species of migratory ducks were examined in our search as Green winged duck, Common pin tail, Sheldrake, and common coot and *Salmonella* was isolated with an incidence of 77.8%, 75%, 50%, and 20%, respectively.

On examination of 477 wild ducks by **Mitchell and Ridewell (1971),** 20 ones were positive for *Salmonella* with a percentage of 4.11%. While, **Muller (1965)** detected *Salmonella* in an incidence of 16 % of wild duck feces. In Egypt, **Abd El Aziz *et al*. (2002)** reported that after bacteriological examination of migratory ducks different bacteria were isolated and the numbers of positive samples for *Salmonella* were 6 from 120 with the percent of 5%.

Literak and Kraml (1985) suggested that laughing Gull can be considered as a possible source of *Salmonella* for farm animal stocks particularly Water fowl. **Refusm *et al*. (2002)** revealed postmortem lesions of *Salmonella* in wild-living birds in Norway with the laboratory-confirmed findings of *Salmonella* which was isolated from 470 birds belonging to 26 species. The *Salmonella*-positive birds included 441 small passerines, 15 Gulls, 5 Water

fowl, 4 birds of prey, 3 Doves, and 2 Crows. Many authors examined the same species of Zoo wild birds and isolated *Salmonella* from them as **Macdonald (1965)** who isolated *Salmonella* serotype Typhimurium 19 times. **Friend and Franson (1988)** reported salmonellosis in different species, such as Grouse, Pheasants and several species of Ducks.

Many researchers examined free-ranging birds and isolated *Salmonella* as **Brittingham and Temple (1986); Hilton *et al.* (1997)**, and **Pennycott (1999)** who isolated *Salmonella* from wild free living Pigeons and Sparrows in a garden, and **Hudson *et al.* (2000)** who isolated *Salmonella* from free living Pigeons.

Also the present study supported the results obtained by **Daoust *et al.* (2000)** who investigated 73 cases of *Salmonella* from the dead several species of Song birds.

Salmonella Typhimurium was the most isolated strain (13/32) with a percentage of 40.6%. These results are nearly close to **Kirkpatrick and Clovin (1986),** and **Kirkpatrick and Trexler- Myren (1986)** who reported that the most isolated serotype was *Salmonella* Typhimurium which was isolated from Kestrel, as well as **Bowes (1993), Kirk *et al.* (2002)** and **Refsum *et al.* (2002**) who recorded that *S.* Typhimurium was recovered from all cases of wild birds examined from the period from 1969 to 2000.

Multiple antimicrobial resistant serotypes of *Salmonella* were usually isolated from both humans and animals at an increasing and alarming rate **(Kirkpatrick and Colvin, 1986).**

The control of *Salmonella* antibiotic therapy may aid in overcoming an outbreak **(Stroud and Friend, 1987)**. And antibiotic therapy should be based on results of susceptibility testing **(Quinn *et al.*, 2002).** The same result was reported by **NCCIS (2000)** who recommended that only ampicillin, quinolone and trimethoprim+ sulfamethoxazole should be tested and reported for the *Salmonella*. Monitoring programs are needed to detect these resistant strains before they become widely distributed.

Nagaraja *et al.* (1991) mentioned several serological tests for detecting antibodies for *Salmonellae.*

Many researchers proved that the standard methods for isolation of *Salmonella* and other bacteria require several days up to 7 as **Wilde *et al.* (1990), Andrew and Hannack (2003).** They reported that PCR method targets specific segment of DNA that could be detected with minute quantities of DNA. [(**Hasan *et al.*, 1991)** and **(Cohen *et al.*, 1994)].** And with different or contaminated samples as **Naguyen *et al.* (1994).**

Soumet *et al.* (1997), Li *et al.* (2000), Scholz *et al.* (2001) and **Myint *et al.* (2006)** proved that the high sensitivity and specificity of PCR needs about 16- 24 hr.

Many authors as **Tuchili *et al.* (1995), Darwin and Miller (1999)** selected the *invA* and explained that this gene was necessary for the invasion to the cell**.** Although, **Lampel *et al.* (2000), Ferretti *et al.* (2001), Liu *et al.* (2002),** and **Salehi *et al.* (2005)** supported the use of *invA* primer due to its accuracy and uniform distribution.

RV enrichment resulted in great PCR sensitivity than non selective enrichment BP. These results were corborated with **Carli *et al.* (2001), Olivera *et al.* (2002), Olivera *et al.* (2003), Freschi *et al.* (2005),** and **Myint *et al.* (2006).** The present results didn't corborate the finding that RV medium was inhibitory to PCR than SC medium and MKTT as **Stone *et al.* (1994)**, **Soumet *et al.* (1994).** But, other researchers as **Schrank *et al.* (2001)** who combined PCR with MKTT and found that this medium was more sensitive than SC medium. But, **Gunaydin *et al.* (2007)** proved that MKTT was more superior to RV. These results might be due to the using of capillary PCR. While, in the present study, single conventional PCR was used. Also, **Olivera *et al.* (2003)** reported that RV- PCR was more superior to SMT.

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دراسات عن السالمونيلا فى الطيور البرية المهاجرة و المحلية "

د/ أزهار جابر على شلبى باحث بالمعمل المرجعى للرقابة البيطرية على الإنتاج الداجنى
أ. د/ منى ابراهيم الانبعاوى استاذ الميكروبيولوجيا – كلية الطب البيطرى جامعة القاهرة
أ. د/ سعاد عبد العزيز عبد الونيس رئيس بحوث أمراض الدواجن ورئيس المعمل المرجعى للرقابة البيطرية على الانتاج الداجنى).
د/ أحمد محمد عبد الرحمن عرفان باحث بالمعمل المرجعى للرقابة البيطرية على الإنتاج الداجنى

الملخص العربى

فى هذا البحث تم فحص 298 عينة ينتموا إلى 37 نوع من الطيور البرية المختلفة بكتريولوجيا. وقد وجد أن 32 عينة كانت اجابية للسالمونيلا بنسبة 13.5%0 وكانت نسبة العزل من الطيور المهاجرة و الطيور البرية الحرة وطيور حديقة الحيوان هى 28.26%, 18.6% و 4.9% على التوالى. تم التصنيف السيرولوجى ل32 عترة من السالمونيلا وكانت انواع العترات هى سالمونيلا تيفيميوريم و سالمونيلا ريسسين و سالمونيلا ريجنت و سالمونيلا دونكاستر و سالمونيلا كيوريسو و سالمونيلا IIIb مجموعة O65 وعترة غير مصنفة. وكانت اكبر نسبة من السالمونيلا هى التيفيميوريم 40.63%0عند اجراء اختبار الحساسية للمعزولتين من حديقة الحيوان وكانت العترة الاولى سالمونيلا كيوريسو ذات حساسية لكلا من (كلورمفنيكول- الكولستين- ستريبتومايسين- تتراسيكلين- حمض الناليديكسك- الايرثرومايسين والدانوفلوكاسين) ومتوسطة الحساسية للدوكساسيكلين و الجينتامايسين ومقاومة للأمبسيلين و النيوميسين و البنسيلين. كما وجد أن العترة الاخرى ذات حساسية للدانوفلوكساسين ومقاومة لجميع المضادات الحيوية الاخرى. تم عمل اختبار التلازن فى الانابيب للسامونيلا بللورم ل 26 عينة مصل الدم و ظهر 15 عينة منهم ايجابية للسالمونيلا تايفيميوريم. تم ايضا اجراء اختبار PCR ل30 عينة تم تحضينهم فى مستنبتات غنية هما (BP and RV) ومقارنة حساسية هذا الاختبار بالعزل الميكروبيالوجى للسالمونيلا وقد وجد ان RV PCR اكثر حساسية من BP PCR و طريقة العزل الميكروبيولوجى بنسبة 66.7%, 46.7% و 13.3% على التوالى.