Surveillance for *Salmonella* in migratory, feral and zoo birds

Azhar G. Shalaby; Erfan A. M. and Soad A. Nasef
Reference lab for Veterinary Quality Control on Poultry Production (Animal Health research institute)

Abstract

In a surveillance targeting different birds communities, 237 cloacal swabs and intestinal samples from 39 different species of wild birds from different sources were examined bacteriologically for *Salmonella*. 32 samples were positive for *Salmonella* spp., with an incidence of 13.5%. Migratory birds had the highest incidence with a 28.26 percentage. The incidence in free living birds was somewhat lower (18.57%). While the Zoo birds had the lowest incidence (4.96%). Serotyping of the isolates revealed 6 different serotypes [*Salmonella* Typhimurium, *Salmonella* Rissen, *Salmonella* Regent, *Salmonella* Doncaster, *Salmonella* Curacao, *Salmonella* IIIb group (O65) and untyped *Salmonella*] . *Salmonella* Typhimurium represented 40.63% of the isolated serotypes. In vitro antibiogram test was performed for the 2 strains isolated from Zoo birds. *Salmonella* Curacao was sensitive to most of the utilized antimicrobial agents. However *Salmonella* IIIb group (O65) had surprising results as it was resistant to 10 out of the 11 applied antibiotics. 26 serum samples were examined by tube agglutination test using *S*. Typhimurium antigen where 15 samples were positive. PCR technique was done on 30 samples to assess the power of two different isolation enrichments[Rappaport vassiliadis (RV) and buffered peptone water (BPW)] in comparison to that of the standard microbioligical techniques (SMT). The detection percentages of RV-PCR, BPW-PCR and SMT was 66.7%, 46.7%, and 13.3%, respectively.

Key words. *Salmonella*, feral, migratory, zoo, PCR, agglutination.

Introduction:

Public heath can be severely affected by wild birds because they can be infected by different disease agents especially Salmonella. 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 predominant representative ***(Friend and Franson, 1988).***

Monitoring the health of the birds and its relation to human is so important through isolation and identification of *Salmonella* 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 study 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 antimicrobial susceptibility 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 PCR to confirm the identification of *Salmonella* spp. and finally to evaluate PCR under different enrichments for *Salmonella* isolates.

Material and Methods:

Sampling. A total of 237 different samples from 39 different wild birds species were collected to be examined. These were 209 cloacal swabs, 28 intestinal samples. Thirty five drag swabs (13 from feral birds houses and 22 from zoo birds) were collected. Also, 26 serum samples were collected from zoo birds only.

Isolation and identification of Salmonella. ***ISO 6579 (2002)*** was used for isolation and identification of *Salmonella*.

Salmonella Serotyping. The organisms were serotyped according to ***Kauffmann and Das-Kauffmann (2001)*** using O and H antisera (Mast assure Co.). Antimicrobial susceptibility test using the disk diffusion technique was applied according to ***(Cruickshank et al, 1975).***

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.

Salmonella PCR. Extraction of *Salmonella* DNA was done by boiling method ***(Croci, 2004)***, and amplification of *invA* gene was done according to ***Olivera et al (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:

Incidence of Salmonella from different bird groups.

Salmonella spp. was identified by culture characters as well as the biochemical and serological 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 39 wild bird species, *Salmonella* species were isolated with an incidence of 13.5 %. Table (1).

The incidence of *Salmonella* species from migratory birds was 28.26% (13/46), While, it was 4.96% (6/121) from Zoo birds and by examination of free living birds (feral birds), the incidence of *Salmonella* was 18.57% (13/70). Table (1).

Incidence of Salmonella from Zoo birds.

Positive cases of *Salmonella* species were recorded with an incidence of 4.96% (6/121) from 22 examined species of Zoo birds. Table (4).

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 susceptibility 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 and danofloxacin. This strain was intermediately sensitive to doxycycline and gentamicin, while, it was resistant to ampicillin, neomycin and penicillin G as shown in Table (7).

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).

Result of Salmonella isolation from drag swabs.
All the samples were negative for *Salmonella* spp. either from the (13) feral Pigeon houses or the 24 Zoo bird houses.

Tube agglutination test. From the26 serum samples examined from the 6 Zoo bird species, 15 sera were positive (suspected to be positive carriers) with 57.6%.

PCR results. After a comparison between SMT and PCR using enriched samples with BPW and RV, it was revealed that *Salmonella* species were detected in the percentages of 13.3%, while they were detected by BPW-PCR and RV-PCR with the percentages of 46.7% and 66.7%, respectively as shown in Table (9).

Table (1): Incidence of *Salmonella* species isolated from cloacal swabs and intestinal samples of wild birds.

Different wild Birds	No. of examined samples		No. of wild bird species	Incidence of *Salmonella* isolation	
	Live	Dead		No.	%
Migratory	25	21	8	13	28.26
Zoo birds	121	0	24	6	4.96
Feral	63	7	7	13	18.57
Total	237		39	32	13.5

Table (2) *Incidence of Salmonella species isolated from live migratory birds.*

Species	No. of examined cloacal swabs samples	Incidence of *Salmonella* isolation	
		No.	%
Guinea fowl	9	0	0.00
Common pintail	4	3	75
Common coot	5	1	20
Shoveler	5	0	0
Little stint	2	0	0
Total	25	4	16

Table (3) *Incidence of Salmonella species isolated from dead migratory birds.*

Species	No. of examined intestinal content samples	Incidence of *Salmonella* isolation	
		No.	%
Green winged duck	9	7	77.78
Water fowl	10	1	10.00
Sheldrake	2	1	50.00
Total	21	9	42.86

Table (4): *Incidence of Salmonella from Zoo birds.*

Species of Zoo birds	No. of examined cloacal swabs samples	Incidence of *Salmonella* isolation	
		No.	%
Galliformes:			
Common pea fowl	10	0	0.00
White pea fowl	10	0	0.00
Helmented guinea fowl	10	0	0.00
Golden pheasant	2	0	0.00
Mongolian pheasant	2	0	0.00
Silver pheasant	2	0	0.00
Spotted sand grouse	3	0	0.00
Anseriformes:			
Mallard duck	10	0	0.00
Grey Chinese goose	9	2	22.2
Egyptian goose	3	0	0.00
Pick duck	3	0	0.00
Wild turkey	5	0	0.00
Passeriformes:			
Grey and white Zebra finches	4	0	0.00
Psittaciformes:			
Peach faced rosy	6	0	0.00
African grey parrot	4	2	50.0
Ornate lorry	2	0	0.00
Pied cockatiel	4	2	50.0
Blue and yellow marcow	4	0	0.00
Ciconiiformes:			
White stork	2	0	0.00
Phoenicopterformes:			
Greater flamingo	3	0	0.00
Strathionformes:			
Ostrich	6	0	0.00
Emu	4	0	0.00
Pelicaniformes:			
White pelican	8	0	0.00
Columbiformes:			
Fantail pigeon	5	0	0.00
Total	121	6	4.96

Table (5). *Incidence of Salmonella species isolated from the different examined live feral birds.*

Species	No. of examined cloacal swabs samples	Incidence of *Salmonella* isolation	
		No.	%
Quail	14	0	0.0
Rook	5	8	0
Kestrel	14	8	57.14
Falcon	2	0	0
Pigeon	20	4	20
Sparrow	4	0	0
Parrots	4	0	0
Total	63	12	19.04

Table (6): *Incidence of Salmonella species isolated from the different examined dead feral birds.*

Species	No. of examined intestinal content samples	Incidence of *Salmonella* isolation	
		No.	%
Pigeon	3	0	0
Parrots	3	1	33.3
Sparrow	1	0	0
Total	7	1	14.3

Table (7): *Results of antibiotic sensitivity testing for the two isolates of Salmonella from Zoo birds.*

Antimicrobial agent	*S.* Curacao susceptibility	*S.* IIIb (group O65) susceptibility
Ampicillin (Amp) 10µg	R	R
Chloramphenicol (C) 30 µg	S	R
Colistin (CT) 10 µg	S	R
Danofloxacin (DFX) 5 mg	S	S
Doxycycline (DO) 30 µg	I	R
Erythromycin (E) 15 µg	S	R
Gentamicin (GM) 10 µg	I	R
Nalidixic acid (NA) 30 µg	S	R
Neomycin (N) 30 µg	R	R
Penicillin G (P) 10 µg	R	R
Streptomycin (Strep) 10 µg	S	R
Tetracycline 30 µg	S	R

S: sensitive I: intermediate R: resistant

Table (8): *Serological identification of Salmonella Typhimurium by tube agglutination test.*

Species	No of examined samples	Results of *S.* Typhimurium antigen (TAT) titer
Helmented Guinea fowl	4	2 (+ve)
White Guinea fowl	2	1 (+ve)
Mallard duck	4	4 (+ve)
Fantail pigeon	4	2 (+ve)
White pea fowl	4	1 (+ve)
Common pea fowl	5	2 (+ve)
Grey Chinese goose	3	3 (+ve)
Total	26	15

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

Table (9): *Comparison between standard microbiological techniques (SMT), BPW-PCR and RV-PCR for detection of Salmonella among different wild birds from different sources.*

Samples sources	SMT	BP PCR	RV PCR
Migratory birds			
Green winged duck (1)	+ve	+ve	+ve
Green winged duck (2)	-ve	-ve	+ve
Sheldrake	-ve	-ve	+ve
Shoveler	-ve	-ve	-ve
Guinea fowl	-ve	-ve	-ve
Zoo birds			
Helminted Guinea fowl	-ve	+ve	+ve
Mallard duck	-ve	+ve	+ve
Common pea fowl	-ve	-ve	-ve
Grey Chinese goose	-ve	+ve	+ve
Blue and yellow marcow	-ve	-ve	+ve
Feral birds			
Kestrel	-ve	-ve	-ve
Sparrow	-ve	+ve	+ve
Pigeon (1)	-ve	+ve	+ve
Pigeon (2)	+ve	-ve	+ve
Pigeon (3)	-ve	-ve	-ve
Total Positive	2	6	10
Percent = %	**13.3%**	**46.7%**	**66.7%**

The percent was calculated in relation to the total number of samples (15).

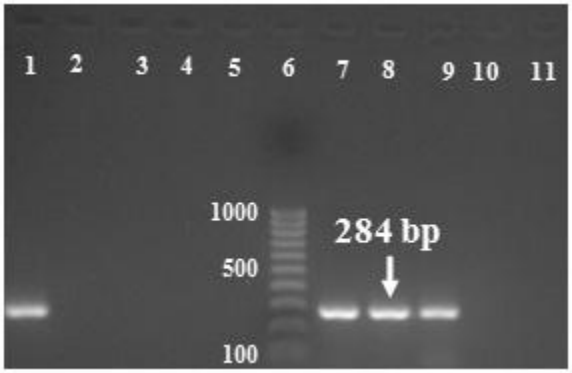

Photo. (1) *Agarose gel electrophoresis showing the result of PCR for detection of Salmonella isolated from migratory birds.*
Lanes 1-5 represent the Buffer peptone enrichment samples obtained from Green winged duck 1, Green winged duck 2, Sheldrake, Shoveler and Guinea fowl, respectively. Lane 6 represents the molecular weight marker (100 bp ladder, fermentas). Lanes 7-11 represent the RV enrichment samples obtained from the same species respectively. Positive amplification of 284 bp of the *invA* gene of *Salmonella* was recorded in lanes 1, 7, 8 and 9.

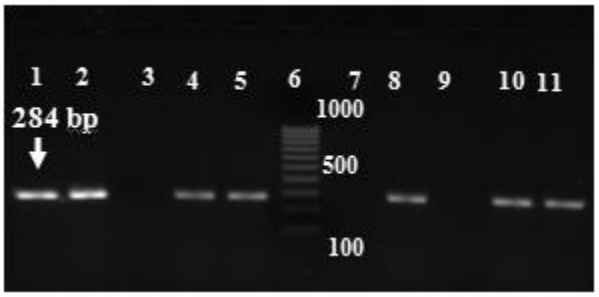

Photo. (2) *Agarose gel electrophoresis showing the result of PCR for detection of Salmonella isolated from Zoo birds.*
Lanes 1-5 represent the RV enrichment sample obtained from Helmented Guniea fowl, Mallard duck, Common pea fowl, Grey Chinese goose and Blue and yellow marcow , respectively. Lane 6 represents the molecular weight marker (100 bp ladder, fermentas). Lanes 7-11 represent the Buffer peptone enrichment sample obtained from the same species respectively. Positive amplification of 284 bp of the *invA* gene of *Salmonella* was recorded in lanes 1, 2, 4, 5, 8, 10 and 11.

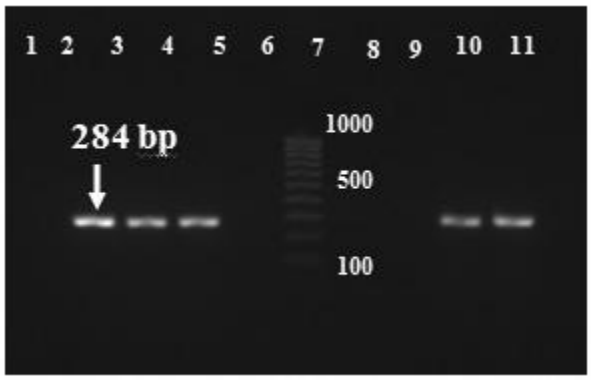

Photo. (3) *Agarose gel electrophoresis showing the result of PCR for Salmonella isolated from feral birds.*

Lanes 1-5 represent the RV enrichment samples obtained from Kestrel, Sparrow, Pigeon 1, Pigeon 2 and Pigeon 3, respectively. Lane 7 represents the molecular weight marker (100 bp ladder, fermentas). Lanes 6 represents the Buffer peptone enrichment samples obtained from Kestrel. Lanes 8 and 9 represent the same as lane 6 but for Pigeon 2 and Pigeon 3, respectively. Lanes 10 and 11 represent the same as lane 6 but for Sparrow and Pigeon 1, respectively. Positive amplification of the 284 bp for *invA* gene of *Salmonella* was recorded in lanes 2, 3, 4, 10 and 11.

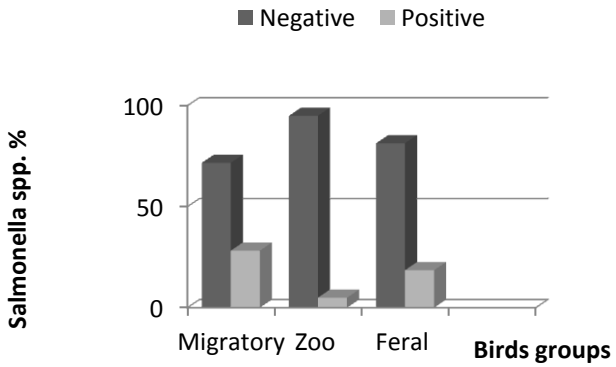

Fig. (1): *Salmonella incidence in different bird species.*

Discussion:

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 role 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 4.96% (6/121) from Zoo birds, and by examination of free living birds (feral birds)**,** the incidence of *Salmonella* was 18.57% (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 spp. were isolated from one specimen. While, none of Salmonella spp. 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 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%. **(Table 2)**. It was revealed that Green winged duck had the highest percentage of isolation (77.78%), 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.

On examination of 477 wild ducks by ***Mitchell and Ridgwell (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 incidence 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. ***Refsum 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 and Duncan (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 Colvin (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 ***NCCLS (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)*** and ***Andrew and Hannack (2003).*** While, PCR method targets specific segment of DNA that could be detected with minute quantities of DNA. ***(Hasan et al, 1991and 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)*** and ***Drawin 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 BPW. 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 as reported by ***Stone et al (1994)***, ***Soumet et al (1997).*** 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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الملخص عربى

التقصى عن السالمونيلا فى الطيورالمهاجرة و الحرة و طيور حديقة الحيوانات

أزهار جابر على شلبى ، أحمد محمد عبد الرحمن عرفان وسعاد عبد العزيز عبد الونيس
المعمل المرجعى للرقابة البيطرية على الإنتاج الداجنى

فى تقصى يستهدف مجتمعات مختلفة من الطيور تم جمع ٢٣٧ عينة من ٣٩ نوع من الطيور البرية و من مصادر مختلفة و تم فحص هذه العينات بكتريولوجيا للسالمونيلا. كانت ٣٢ عينة ايجابية للسالمونيلا بنسبة ١٣,٥ %. كانت للطيور المهاجرة أعلى نسبة ايجابية بنسبة ٢٨,٦%. بينما كانت النسبة فى الطيور الحرة أقل ١٨,٥٧%. لكن كانت طيور حديقة الحيوانات لها أقل نسبة ٤,٩٦%. التصنيف السيرولوجى للعينات نتج عنه ٦ أنواع مختلفة هى (السالمونيلا تيفيميوريم و السالمونيلا ريزن و و السالمونيلا ريجينت و السالمونيلا دونكاستر و السالمونيلا كوراكاو و السالمونيلا IIIb مجموعة O65 و سالمونيلا غير مصنفة. مثلت السالمونيلا تيفيميوريم ٤٠,٦٣% من الانماط المصلية المعزولة. تم إجراء اختبار الحساسية للمضادات الحيوية للنوعين المعزولين من طيور حديقة الحيوانات. فكانت السالمونيلا كوراكاو حساسة لمعظم المضادات الحيوية المستخدمة. لكن كانت للسالمونيلا IIIb مجموعة O65 نتيجة مثيرة حيث كانت مقاومة لعشرة أنواع من الاحدى عشر نوعا من المضادات الحيوية المستخدمة. تم فحص ٢٦ عينة سيرم باختبار التلزن فى الأنابيب باستخدام أنتيجين السالمونيلا تيفيميورييم حيث كانت ١٥ عينة إيجابية. تم إجراء اختبار PCR ل٣٠ عينة للحكم على قدرة ال RV و BP لتنمية السالمونيلا بالمقارنة مع العزل الميكروبيولوجى التقليدى. و كان نسبة الكشف لل RV و BP و العزل الميكروبيولوجى ٦٦,٧% و٤٦,٧% و ١٣,٣% على الترتيب.