Isolation of *Salmonella* Typhimurium from Some Psittaciformes Species and Detection of Antibiotic Resistant Genes In Egypt

Dalia M. Elsayad¹; Kamel, A. M. ²; Mohamed A. Gamal-Eldein², Algammal A. M. ³; Azza S. A. Gouda⁴.

¹Wildlife Dep., Animal Quarantine, Cairo international airport; ²Wildlife Management and Zoo Medicine Dep., and ³Bacteriology, Immunology and Mycology Dep. of Faculty of Veterinary Medicine, Suez Canal University, ⁴Animal Health Dept., Desert Reserch Center, Cairo, Egypt

Abstract

This study was conducted to isolate and identify *Salmonella* as one of important zoonotic microorganisms from different spp. of captive bred psittaciformes. A total of 300 psittaciformes (were collected from private wildlife farms, pet shops and households) belonging to 15 different species were clinically examined and samples, (219) fecal samples, (72) cloacal samples and internal organs (intestine, liver, lung, spleen and kidney) from 9 freshly dead birds were taken for detection of salmonella infections using traditional methods of isolation and polymerase chain reaction (PCR) based on invA gene as a confirmatory accurate technique on isolated strains, estimation of the antibiotic susceptibility and detection of resistance genes. The result revealed that, the incidence of the infection constituted 3.33% (10 isolates) of total number of investigated psittacine birds and the most common affected psittacines were (5) rosy-faced lovebirds, (4) budgerigars and (1) green rosella which were bought from illegal wildlife trafficking. All 10 isolates were *Salmonella* Typhimurium confirmed by PCR based on invA gene. The antibiotic sensitivity tests revealed that, all 10 isolates were highly susceptible (100%) in vitro to amoxicillin / clavulanic acid, ciprofloxacin and gentamycin, while 100% of the isolates exhibited complete resistance to doxycycline and sul./ trimethprim. Detection of resistance genes was tested by PCR targeting (*tet*1, *tet* 2, *sul* 1and *sul*2) antimicrobial genes. Resistant genes were detected of *Salmonella* Typhimurium isolates. (6) Against tetracycline A *tet* A gene, (7) tetracycline gene B *tet* B and (7) sulphonamide gene 1 *sul* 1, meanwhile all strains were negative for *sul* 2 resistant gene.
**Introduction**

Previous studies on microbiota of psittacine birds indicated that, normal microbiota of them are composed only of gram positive bacteria and yeasts (Lopes et al., 2014). The presence of enterobacteria is not considered normal components of unstressed parrots microflora (Ritchie et al., 1994).

Salmonella infections occur in wild birds where they can cause disease and death, or even spread from their avian hosts to domestic mammals and man. In spite of being recognized as an avian disease for over a hundred years, Salmonellosis is an emerging disease as a result of increased artificial feeding by human. Salmonella may be present in feces for a short time, as a result of environmental contamination (Tizard, 2004). Multiple serovars of Salmonella enterica originating from mammalian, reptilian and avian hosts have been reported to cause infections in human, wildlife and exotic pets harboring Salmonella are potential sources for human infections (Hoelzer et al., 2011).

Transmission of Salmonella from wildlife and exotic animals to humans occurs through multiple pathways. Evidences increasingly suggest that, there could be a bidirectional transmission of Salmonella between domesticated and wild animals. Farm animals acquiring Salmonella from wildlife, could increase the risk of human infection. Salmonella infections in humans have also been reported through direct contact with exotic pets and wildlife, especially those in captivity (Krueger et al., 2014).

Salmonellosis is well-known cause of disease and intermittently reported disease in psittacine birds (Oros et al., 1998). The risk of disease dissemination must be considered, given that captivity allows greater contact between species, favoring the transmission of infectious agents (Alves et al., 2013). On the other hand, Salmonella spp. serotype most frequently isolated from psittacines is Salmonella Typhimurium (Hidasi et al., 2013).

So this study aimed to detect the prevalence of Salmonella infection in some species of captive bred psittaciformes in Egypt from different sources, as well as using of PCR based on inv A gene as a sensitive and a specific accurate tool for confirmation and detection of Salmonella. Beside, antibiotic sensitivity and antibiotic resistant genes were performed on Salmonella Typhimurium strains.

**Material and Method**

**Birds:** 300 psittaciformes belonging to 15 different species (257 apparently healthy, 34 diseased and 9 freshly dead birds) were collected from different private farms of psittacines (234), pet shops (25) and households (41) in Egypt.
Samples: a total number of 300 samples were collected on aseptic condition from all investigated psittacine birds. All samples, (219) fecal samples, (72) cloacal samples and internal organs (intestine, liver, lung, spleen and kidney) from 9 freshly dead birds were labeled with code number, type of sample, bird species and date of collection and were submitted to bacteriological and serological examination.

Bacteriological examination of collected samples:
1- Cultivation in liquid media:
The swabs from sample (fecal, cloacal and tissue) were collected aseptically and inoculated into selenite F broth incubated at 37°C for 18-24 hours.

2- Plating out onto solid media:
A loopfull from the incubated broth was placed and streaked onto Salmonella Sigella agar "S S Agar", MacConkey agar, Xylose lysine deoxycholate agar "XLD agar" and Brilliant green agar plate (Oxoid), then incubated at 37°C for 24 hours. Semisolid nutrient agar "0.4 %"(Oxoid) was used for detection of motility as well as preservation of the isolated strains as those were carried out by Wilson and Miles (1975). Suspected colonies were subjected to morphological and biochemical identification according to Cruickshank et al., (1975).

Serological identification of the isolated Salmonella:
Serotyping of isolated Salmonella was carried out in serological unit, Animal Health Research Institute, Dokki, Giza according to Edwards and Ewing (1972).

Antimicrobial susceptibility testing by disc diffusion method:
The test was performed according to the procedures of (NCCLS, 2007) using disc diffusion technique (Table 1).

1-Preparation of Salmonella isolates for DNA extraction:
Pure colony of Salmonella from selective medium was transferred to nutrient agar medium and incubated for 24 hr at 37°C (Shanmugasamy et al., 2011) then, 3 ml phosphate buffered saline was added on the medium and harvesting the growth by pipetting them and collecting in 15 ml falcons tube (pelleting).

2-Bacterial genomic DNA extraction:(Freschi et al., 2005)
1- 100μl from pellet was transferred into eppendorff tube after vortex.
2- Eppendorff tube was put into heat block at 95°C for 10 minutes, then to freezer overnight, centrifugation at 13,000 xg for 3 minutes.
3- The supernatant (extracted DNA) was transferred into another clean eppendorff.

3-DNA amplification (polymerase chain reaction):
DNA samples were tested (in 25ml. reaction volume in a 0.2 PCR tube. The reaction mixture consisted of 12.5 ml. master mix (Thermo
Scientific), 3 ml. Bacterial DNA, 0.25 ml. of each primer (Table 2) (conc. 25 pmol) and nuclease free water up to 25 ml., then thermal cycling in a programmable heating block (Coyvorporation, Grasslake, Michan, USA) was done.

4-Molecular identification of Salmonella spp. gene:

a) PCR protocol of invA gene:
- Initial denaturation at 94° C/ 5 min.
- Denaturation at 94° C / 0.5 min.
- Annealing at 64° C / 0.5 min.
- Extension at 72° C / 45 sec.
- Cycles repeated for 35 times with final extension at 72°C / 7 min.

b) PCR protocol of tet A and tet B genes:
- Initial denaturation at 94° C / 5 min.
- Denaturation 94°C / 30 sec.
- Annealing 55° C / 30 esc.
- Extention 72° C / 45 sec.
- Cycles repeated for 35 times with final extention at 72 °C / 7 min.

c) PCR protocol of sul1 and sul2 genes:
- Initial denaturation at 94°C/ 3 min.
- Denaturation 94°C/1 min.
- Annealing for sul1 gene 51°C/ 1 min., and for sul2 gene 57°C/1 min.
- Extention at 72° C / 1 min.
- Cycles repeated for 35 times with final extention at 72°C / 10 min.

5-Identification of the PCR products:
Following amplification, 10 of each reaction products taken for electrophoresis on 1.5% (W/ V) agarose gel containing 1 x TAE buffer ( 0.01 m Tris acetate 0.002 M EDTA) and ethidium bromide (0.5 mg/ml) The electrophoresis at 100 volts for 35 minutes in an electrophoresis unit. The presence of specific amplified DNA bands was detected by visualization with UV light at wave length 421 run and compared with molecular size marker (Ladder) with MW 100 bp and measure MW100-1000 bp.
Table (1): Antimicrobial susceptibility testing by disc diffusion method:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Code</th>
<th>Conc.</th>
<th>Standard zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>sensitive</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AMP</td>
<td>10μg</td>
<td>≥ 17</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>AML</td>
<td>10μg</td>
<td>≥ 17</td>
</tr>
<tr>
<td>Amoxicillin / clavulanic acid</td>
<td>AMC</td>
<td>30μg</td>
<td>≥ 18</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>5μg</td>
<td>≥ 21</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GN</td>
<td>10μg</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Neomycin</td>
<td>N</td>
<td>30μg</td>
<td>≥ 17</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
<td>10μg</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE</td>
<td>30μg</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>DO</td>
<td>30μg</td>
<td>≥ 14</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>SP</td>
<td>100μg</td>
<td>≥ 24</td>
</tr>
<tr>
<td>Sulfa./trimethoprim</td>
<td>SXT</td>
<td>25μg</td>
<td>≥ 16</td>
</tr>
</tbody>
</table>

Molecular detection of Salmonella isolates:

Table (2): Oligonucleotide primers encoding for inv A gene and antibiotic resistant genes: tetracycline and Sulfonamides:

<table>
<thead>
<tr>
<th>Primer</th>
<th>DNA sequences (5’ to 3’)</th>
<th>Target gene</th>
<th>Amplicon size</th>
<th>Annealing Temp.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inv A gene F</td>
<td>5’ GTGAAATTATCGCCAGTTCGGC-3’</td>
<td>Inv A</td>
<td>284 pb.</td>
<td>64 °C</td>
<td>Rahn et al. (1992)</td>
</tr>
<tr>
<td>Inv A gene R</td>
<td>5’-TCATCGCAGGTAAGGACC-3’</td>
<td>inv A</td>
<td>284 pb.</td>
<td>64 °C</td>
<td>Rahn et al. (1992)</td>
</tr>
<tr>
<td>tet (A) F tet (A) R</td>
<td>GTGACGCAGTGGTACACGCTACGTGCTG</td>
<td>tet (A)</td>
<td>741 bp.</td>
<td>55 °C</td>
<td>Ma et al. (2007)</td>
</tr>
<tr>
<td>tet (B) F tet (B) R</td>
<td>CAATTGATGATGATGTGTAACGATG</td>
<td>tet (B)</td>
<td>571 bp.</td>
<td>55 °C</td>
<td>Ma et al. (2007)</td>
</tr>
<tr>
<td>Sul1 F Sul1 R</td>
<td>TTCCGCATTGATGATGATGTAACGATG</td>
<td>Sul1</td>
<td>547 bp.</td>
<td>51 °C</td>
<td>Harel et al. (1991)</td>
</tr>
<tr>
<td>Sul2 F Sul2 R</td>
<td>CGGATCATTGATGATGATGTAACGATG</td>
<td>Sul2</td>
<td>543 bp.</td>
<td>57 °C</td>
<td>Ma et al. (2007)</td>
</tr>
</tbody>
</table>

Result and Discussion

Psittacine birds are frequently commercialized in illegal wildlife trade and when apprehended by the responsible public departments are often found in poor sanitary conditions. In these cases, these birds become susceptible to several pathogens, such as enterobacteria and can cause intestinal and extraintestinal opportunistic infections. (Lopes et al., 2015).

Source of positive Salmonella psittacines in the present study was illegal trafficking during period of import ban which demonstrate, that psittacines from illegal trafficking can be an infected with Salmonella and this result agreed with...
Dalia Elsayad et al. (2010); Hidasi, et al. (2013) and Matias et al. (2016). These birds were under stress condition that, may be enhance the presence of Salmonella that is an opportunistic microorganism, this agreed with Dorrestein et al. (1997) who stated that, Salmonella are ubiquitous microorganisms and, under suitable conditions can survive and multiply in environment for a long time. Many animals can be subclinical carriers and birds may become infected by ingestion of contaminated food and / or drinking water or contact with carriers (e.g., rodents, wild birds, or domesticated species).

In addition the presence of enterobacteria not considered normal, but actually categorized as a contamination. (Marietto et al., 2010).

There have been many reports on isolation of Salmonella from caged birds such as psittacines birds (Sawa et al. 1981). Asymptomatic Salmonella carriage in wild birds is thought to be high, as many species acquire the organisms and become intestinal carriers without showing any visible signs and can be considered apparently healthy birds (Gamal-Eldein et al., 2008).

The present study was conducted upon captive bred, species of order psittaciformes from different sources, private wildlife farms, pet shops and households .This study has been carried out on a total number of 300 psittacine birds belonging to 15 different species (257 apparently healthy, 34 diseased and 9 died during period of sampling).

At necropsy of (9) dead psittacines birds, { (1) budgerigar, (2) lovebird} from farm (A), { (3) Amazon and (3) lovebird} from farm (C), (Table 3), that died during period of sampling, no focal necrosis were seen in the post mortum samples, However no Salmonella was isolated in these birds, this result disagreed with each of Oros, et al., (1998) who isolated Salmonella Airozonea from the liver with multifocal necrotic hepatitis in Sulpher crested cockatoo (Cacatus galerita) with no previous clinical signs, Vigo, et al. (2009) who isolated Salmonella Typhimurium from liver, spleen, heart, lung, kidney and intestine of Blue and gold macaw (Ara ararauna) chicks died of fatal Salmonella and Piccirillo et al. (2010) who isolated Salmonella Typhimurium from 2 Molucan cockatoos (Cacatus moluccensis) and at post mortum reported necrotic foci surrounded by a hyperemic halo were observed in lungs, heart, liver, spleen, kidneys and intestine. In addition contrast to each of Cardona et al. (2016) who reported a case of unusual salmonellosis in female African grey parrot and at necropsy, revealed sever fibrinous pericarditis, moderate hydrocoelom, diffusely reddened lungs, and a green discoloration of the liver and
Siqueira et al. (2017) who isolated *Salmonella* Typhimurium from dead pet pittacine birds with multifocal necrotic hepatitis. *Salmonella* spp. are not regular members of the intestinal microbiota of psittacine and, therefore isolating these bacteria from asymptomatic or immunosuppressed individuals indicates a possibility of disease (Goncalves et al., 2010).

As shown in Table (3), the prevalence of *Salmonella* was in apparently healthy psittacine birds in farm (A), (10/80), 12.5% and 10/257, (3.89%) to the total investigated apparently healthy birds. This result go in hand with Lopes et al., (2015) and Suphoronski et al. (2015), they declared that, gram negative bacteria may be isolated from healthy psittacines. Also, Evance (2011) stated that, *Salmonella* spp. were isolated from several captive psittacines birds, whether they are asymptomatic carriers or clinically diseased. Previous studies proved that the normal microbiotas of these birds are Gram positive bacteria and yeast and this was disagreeing with our result. In addition Lopes et al. (2014) stated that, in most birds presented negative in the study of *Salmonella* spp. in captive psittacines may not imply the absence of this pathogen in these birds, since the intermittent excretion is a well-known characteristic of this microorganism.

Meanwhile, in this study as shown in Table (3) there was no isolation of *Salmonella* from pet shops nor households and this result disagree with Seeperadsingh and Adesiyn (2003) who reported 6 spp. of *Salmonella* isolated from pet birds were obtained from pet shops and households which may pose a health risk to their owners and contacts.

As shown in Table (4) the bacteriological examination of 300 samples revealed that, 10 *Salmonella* isolates were positive from apparently healthy birds demonstrated a (3.33%) *Salmonella* prevalence rate in the total investigated psittacine birds and (10.33 %) among the examined birds in a private wildlife farm group, farm A, 92 psittacines while no *Salmonella* was isolated from 34 diseased psittacines nor 9 psittacine birds died during period of study.

This low prevalence Salmonellosis in the present study, (3.33%) almost agreed with the study of Matias et al. (2016) and Siqueira et al. (2017) who isolated a single *Salmonella* Typhimurium (1/75), 1.33% prevalence rate, while disagreed with (Akhter, et al. 2010) who isolates 21 *Salmonella* species. out of 45 samples with a prevalence rate (46.6%).

Among the investigated (Table 5), 15 Psittaciformes spp. *Salmonella* was isolated from 3 spp., Lovebirds
Green rosella {1/18, 5.55% of total rosella} and budgerigar {4/85, 4.7% of total budgerigar} and this result similar to Oros et al. (1998) who isolated Salmonella Arizonae from captive sulphur crested cockatoo (Cactus g.  

Tizard, 2004) stated that, the wild and exotic birds, such as budgerigars, may harbor Salmonella spp. in their intestine. Moreover, Abd-El-Latif and El-Said, (2003) isolated Salmonella spp. from 50 psittacines, Seepersadsingh and Adesiyun, (2003) isolated Salmonella from pet birds. Also, Allgayer et al. (2008) investigated 13 captive psittacines birds for Salmonella and the most commonly infected were orange-winged parrot (Amazona amazonica), (28%) and red-spectacled parrot (Amazona pretrei), (20%) and Enas (2008) isolated Salmonella from budgerigar. Also, Vigo et al. (2009) isolated Salmonella strains from 2 blue and gold macaw (Ara ararauna), Akhter et al. (2010) isolated 21 Salmonella spp. from a total of 45 samples were collected from 5 types of caged parrots (Gray cockatiels, Rose ringed parakeet, Alexandrine parakeet, Red breast parakeet and Blossom head parakeet) of Dhaka Zoo and Evance (2011) isolated Salmonella spp. from caged parrots. Cardona et al. (2016) isolated Salmonella spp. from an adult female African grey parrot and Siqueira et al. (2017) isolated one single Salmonella in Amazon parrot (Amazona aestiva). Ten positive Salmonella were isolated from cloacal swabs,(10/34), 29.4% to the total no. of cloacal swabs in farm (A) and 13.88% (10/72) to total no. of cloacal samples, (Table 6), this similar to Akhter et al. (2010), who reported that, irrespective to the types of parrots, the higher percentage of different bacteria was isolated from cloacal swabs and also similar with Bezerra et al. (2013), Lopes et al. (2014) and Lopes et al. (2015).

While contrast to Sareyyü póglu et al. (2008) reported that, 5 (2.7%) fecal samples were found to harbor Salmonella spp. out of 108 fecal samples collected from pet birds in Ankara and Hidasi et al. (2013) isolated one Salmonella spp. from fecal samples. Salmonella Typhimurium was detected by serological identification in all 10 Salmonella isolates. This go in hand with Piccirillo et al. (2010) who said that, Salmonella spp. serotype most frequently isolated from psittacines is S. Typhimurium. Meanwhile, in previous studies are agreed to our findings that isolated Salmonella Typhimurium from psittacines as Ward et al. (2003) isolated 4/45, 8.88% Salmonella Typhimurium in a population of 45 lorikeets and lories, Piccirillo et al. (2010) reported 2 fatal cases of
Salmonella Typhimurium in Moluccan cockatoos and Krawiec et al. (2015).

However, disagreed with others who isolated other serotypes, Oros et al. (1998) isolated Salmonella Arizonae from captive sulphur crested cockatoo (Cacutusgalerita), Seepersadsingh and Adesiyun (2003) isolated 6 isolates of Salmonella species with 2 isolates of serotype Aberdeen and one isolate each of Thompson, Rubislaw, Panama and Newport, Allgayer et al. (2008) isolated Salmonella spp. from 13 different spp. psittacines birds but specific tests for Salmonella Typhimurium were negative, one Salmonella Lexinton from (31) Blue – fronted Amazon (Amazona aestiva), one Salmonella Saintpaul from (16) Red- and – green Macaw (Ara macao) and one Salmonella Newport from (06) Budgerigar and Akhter et al. (2010) isolated (5) Salmonella Pullorum from caged parakeets. In addition, Goncalaves et al. (2010) isolated Salmonella Enteridity in 3 captive specimens of Amazona aestiva out of 103 investigated birds, 2.9% and Enas (2015) isolated Salmonella Paratyphoid, S. Chester, S. Infantis, and untypable S. strains Moreover, Lopes et al. (2014) and Matias et al. (2016) isolated 2 Salmonella Panama strains from 2 chestnut capped black birds (Chrysomurufi capillus).

There is scarce information about antimicrobial resistance and diseases in pet birds, however there are reports involving free-living birds as potential disseminators of E. coli and Salmonella spp. resistant to cefalosporins, ampicillin, streptomycin, sulfoxazole and tetracycline isolated from passerines (Andres et al., 2013).

Antimicrobial resistance in non-typhoidal Salmonella is common, and in some places, it has been increasing in recent years (Centers for disease control and prevention, 2013).

While a growing body of research has found evidence of AMR in Salmonella spp. isolates derived from free-living wildlife, including birds. Wildlife species possess antimicrobial resistance determinants and the prevalence rate of AMR genes in these isolates could be as high as 100% (Botti et al., 2013).

In this study the most effective antibiotics (100%) sensitivity were amoxicillin /clavulanic acid, ciprofloxacin and gentamycin. Sixty%, 40%, 30%, 30%, 30% and 20% susceptible to neomycin, streptomycin, ampicillin, amoxicillin, spiramycin and tetracycline respectively while 100% of the isolates exhibited complete resistance to doxycycline and Sulph./ Trimethobrim, 80% to tetracycline, 50% to spiramycin, 30% ampicillin, 20% amoxicillin,
20% streptomycin and 10% neomycin, Table (7) and Fig. (1). These results agreed with Meakins et al. (2008) who mentioned that, despite wide use of fluoroquinolones such as ciprofloxacin, the levels of resistance to these antimicrobials remain low. Moreover, agree with Rahmani et al. (2011) and Abd-El-Latif and El Said, (2003) whom reported that, most of isolated Salmonella strains were resistant to amoxicillin, fluoquine, streptomycin and penicillin. El Sharkawy et al. (2017) reported tetracycline resistance in the Salmonella Typhimurium isolates 58 (86.6%) in a total 615 broiler flocks. The study observed 20% resistance rate to amoxicillin, also, disagree with Leonard et al. (2012) who recorded a sensitivity rate of (86.7%) against amoxicillin and disagree with Enas (2008) who reported that, amikacin, chloramphenicol and tetracycline were the most effective drugs against Salmonella and the isolated Salmonella was resistant to amoxclavulanic acid, erythromycin and penicillin. Moreover disagreed with Vigo et al. (2009) who reported that, all Salmonella strains isolated from 2 blue and gold macaw (Ara ararauna) was sensitive to trimethobrim-sulfamethoxazole while in our study resistance to sulph./ Trimethobrim was 100%. In addition, disagreed with Matias et al. (2016) who reported that resistance of one strain of Salmonella Typhimurium and 2 strains of Salmonella Panama (isolated from wild birds) to multiple antimicrobial drugs, like ampicilin, ceftriaxone, cefitifur, tetracycline, gentamycin, enrofloxacin and ciprofloxacin.

It is assumed that, the multidrug resistance in this result might be due to their frequent application of these antibiotics which suggest paying more attention when using these antibiotics. The excessive use of a specific antimicrobial agent may explain the difference between the sensitivity profiles observed among the surveys. Since it is known that, continuous exposure of the bacteria to an antimicrobial agent tends to select this microorganism to resistance (Arias and Carrilho, 2012).

The presence of multidrug resistant strains, if not controlled, can be considered a condition of sanitary risk to the birds, as well as to free-living animals that may be exposed to the introduced birds. Birds carrying resistant strains may spread these bacteria and, consequently, affect other wild animals through direct or indirect contact with contaminated feces (Hebla et al., 2011).

The antimicrobial susceptibility tests on psittacines from illegal trade revealed that, the enterobacteria found in the intestinal microbiota of the studied birds presented high multidrug resistance rates, which the most frequent resistance was to
azithromycin among the various isolated strains and this may be a consequence inadequate use of this antibiotic at some part of the life of these birds (Lopes et al., 2015).

One of the earliest steps in the pathogenic cycle of the facultative intracellular pathogen Salmonella species was the invasion of the intestinal epithelium, inv A was a member of this locus, and it was the first gene of an operon consisted of at least two additional invasion genes Galan et al. (1992) and Lamb et al. (2014) recommended the use of invA primer due accuracy, sensitivity and uniform distribution among Salmonella.

To assess potential virulence of Salmonella isolates by the presence or absence of genes, Polymerase chain reaction (PCR) was used to detect Salmonella virulence genes. All samples tested positive using PCR, amplifying the invasion gene inv A gene, at 284 bp. Fig. (2), these results were in agreement with Krawiec et al. (2015) while nearly similar with Hudson et al. (2000) who detected 15 positive inv A gene in a total of 22 Salmonella isolates. Detection of resistant genes was tested by PCR targeting tetA, tetB, sul 1 and sul 2. PCR detected tetA gene, (740 bp) with an incidence rate of 60%, Fig (3)., tet B gene, (571 bp) with an incidence rate 70%, Fig. (4) and sul1 gene, (574 bp) with an incidence rate 70%, Fig (7) while there was no detection at all of sul2, Fig. (6) Which refers that in this study the incidence rate of tet A gene is higher than that of tet B and so it is disagreed in percentage of detection with Eid and Shalaby (2013), who reported the incidence rates detected in their study for tet A and tet B genes by PCR was 90% and 40%, respectively and (Hamada et al. (2003), Asai et al. (2006) and Shahada et al., (2006) who stated that, the most common tetracycline resistance determinant in chickens belonged to tetA gene. Moreover, agreed with El Sharkawy et al. (2017) reported tetracycline resistance in the S. Typhimurium isolates 58 (86.6%) in a total 615 broiler flocks, correlated with the presence of tet C (96.6%), and tetA gene (84.5%), (Sul1 and Sul3). All tested strains were negative for tetB codon. tet A codon was also found in all of the non-typable Salmonella strains.
Table (3): Prevalence of Salmonella in different Sources of collection:

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Apparently healthy</th>
<th>Diseased</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Farm (A)</td>
<td>80</td>
<td>12.5%</td>
<td>9</td>
</tr>
<tr>
<td>Farm (B)</td>
<td>49</td>
<td>0%</td>
<td>49</td>
</tr>
<tr>
<td>Farm (C)</td>
<td>62</td>
<td>0%</td>
<td>25</td>
</tr>
<tr>
<td>Pet shops</td>
<td>41</td>
<td>0%</td>
<td>41</td>
</tr>
<tr>
<td>Household</td>
<td>25</td>
<td>0%</td>
<td>25</td>
</tr>
<tr>
<td>Total No.</td>
<td>257</td>
<td>3.89%</td>
<td>34</td>
</tr>
</tbody>
</table>

Table (4): Source of examined birds and positive samples.

<table>
<thead>
<tr>
<th>Source of examined psittaciformes</th>
<th>No. of examined birds</th>
<th>No. of positive samples</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm (A)</td>
<td>92</td>
<td>10/92</td>
<td>10.9%</td>
</tr>
<tr>
<td>Farm (B)</td>
<td>49</td>
<td>0/49</td>
<td>0%</td>
</tr>
<tr>
<td>Farm (C)</td>
<td>93</td>
<td>0/93</td>
<td>0%</td>
</tr>
<tr>
<td>Pet shops group</td>
<td>41</td>
<td>0/41</td>
<td>0%</td>
</tr>
<tr>
<td>Household group</td>
<td>25</td>
<td>0/25</td>
<td>0%</td>
</tr>
<tr>
<td>Total No.</td>
<td>300</td>
<td>10/300</td>
<td>3.33%</td>
</tr>
</tbody>
</table>
Prevalence of Salmonella infection in examined psittacines

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Examined (No.)</th>
<th>Positive salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ara macao</em></td>
<td>Macaw</td>
<td>2</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td><em>Platycercus caledonicus</em></td>
<td>Green rosella</td>
<td>18</td>
<td>1/18 (5.55%)</td>
</tr>
<tr>
<td><em>Psephotus varius</em></td>
<td>Mulga parrot</td>
<td>5</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td><em>Psephotus haematotonotus</em></td>
<td>Red – rumpedparakeet</td>
<td>25</td>
<td>0/25 (0%)</td>
</tr>
<tr>
<td><em>Neophema splendida</em></td>
<td>Splendid or Scarlet-chested parrots (Splendida)</td>
<td>1</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td><em>Neopsephotus bourkii</em></td>
<td>Bourke's parrot</td>
<td>4</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td><em>Neophema pulchella</em></td>
<td>Turquoiseine parrot</td>
<td>2</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td><em>Psittacus erithacus</em></td>
<td>African grey parrot</td>
<td>17</td>
<td>0/17 (0%)</td>
</tr>
<tr>
<td><em>Melopsittacus undulatus</em></td>
<td>Budgerigar</td>
<td>85</td>
<td>4/85 (4.7%)</td>
</tr>
<tr>
<td><em>Nymphicus hollandicus</em></td>
<td>Cockateil</td>
<td>16</td>
<td>0/16 (0%)</td>
</tr>
<tr>
<td><em>Polytelis alexandreae</em></td>
<td>Princess of Wales (paralceet)</td>
<td>3</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td><em>Psittaculakrameri</em></td>
<td>Indian ring head parakeet</td>
<td>9</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td><em>Amazona amazonica</em></td>
<td>Orange-winged amazon</td>
<td>28</td>
<td>0/28 (0%)</td>
</tr>
<tr>
<td><em>Agaponis roseicollis</em></td>
<td>Rosy-faced love bird</td>
<td>60</td>
<td>5/60 (8.33%)</td>
</tr>
<tr>
<td><em>Agaponis taranta</em></td>
<td>Black-winged lovebird</td>
<td>6</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td><em>Agaponis fischeri</em></td>
<td>Fischer's lovebird</td>
<td>6</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td><strong>Total lovebird</strong></td>
<td></td>
<td>72</td>
<td>5/72 (6.94%)</td>
</tr>
<tr>
<td><em>Pionus senilis</em></td>
<td>White-capped parrot</td>
<td>9</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td><em>Cacatus goffiana</em></td>
<td>Goffin's cockatoo</td>
<td>4</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td><strong>Total No.</strong></td>
<td></td>
<td><strong>300</strong></td>
<td><strong>10/300</strong> (3.33%)</td>
</tr>
</tbody>
</table>

Table (6) Prevalence of Salmonella in all types of samples:
<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Source of sample</th>
<th>No. of samples</th>
<th>positive Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Fecal samples</td>
<td>Farm (A)</td>
<td>55 / 92</td>
<td>0/55</td>
</tr>
<tr>
<td></td>
<td>Farm (B)</td>
<td>40 / 49</td>
<td>0/40</td>
</tr>
<tr>
<td></td>
<td>Farm (C)</td>
<td>58 / 93</td>
<td>0/58</td>
</tr>
<tr>
<td></td>
<td>Pet shops</td>
<td>41 / 41</td>
<td>0/41</td>
</tr>
<tr>
<td></td>
<td>House holds</td>
<td>25 / 25</td>
<td>0/25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>219</td>
<td>0</td>
</tr>
<tr>
<td>Cloacal swabs</td>
<td>Farm (A)</td>
<td>34/92</td>
<td>10/34</td>
</tr>
<tr>
<td></td>
<td>Farm (B)</td>
<td>9/49</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>Farm (C)</td>
<td>29/93</td>
<td>0/29</td>
</tr>
<tr>
<td></td>
<td>Pet shops</td>
<td>0/41</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>House holds</td>
<td>0/25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
<td>10</td>
</tr>
<tr>
<td>Necropcies</td>
<td>Farm (A)</td>
<td>3/92</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>Farm (B)</td>
<td>0/49</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Farm (C)</td>
<td>6/93</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>Pet shops</td>
<td>0/41</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>House holds</td>
<td>0/25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>300</td>
<td>10/300</td>
</tr>
</tbody>
</table>

Table (7): *Antibiogram of isolated Salmonella Typhimurium:*

<table>
<thead>
<tr>
<th>Antimicrobial group</th>
<th>R</th>
<th>I</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>(%)</td>
<td>No.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3</td>
<td>30%</td>
<td>4</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>2</td>
<td>20%</td>
<td>5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>8</td>
<td>80%</td>
<td>0</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>10</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>5</td>
<td>50%</td>
<td>2</td>
</tr>
<tr>
<td>Amoxicillin /clavulanetic acid</td>
<td>0</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Sulph./ Trimethobrim</td>
<td>10</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Neomycin</td>
<td>1</td>
<td>10%</td>
<td>3</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2</td>
<td>20%</td>
<td>4</td>
</tr>
</tbody>
</table>

R: Resistant  I: Intermediate  S: Sensitive
Figure (1): Antibiotic resistance pattern of different Salmonella Typhimurium isolated from investigated Psittacine birds

Figure (2): Electrophoretic pattern of inv A gene PCR assay: lane 2 (negative control): E. coli ATCC 25922, lanes 3-12: positive inv A gene (284 bp) Salmonella isolates and lane 1: DNA ladder from 100-1000 (Jena Bioscience).

Figure (3): Electrophoretic pattern of Salmonella Typhimurium tetracycline resistance gene, (A) tetA gene 740 bp PCR assay: DNA marker GeneRuler (Thermo); 1 negative control; 2 positive control; from 3-12 examined Salmonella isolates.
Figure (4): Electrophoretic pattern of *Salmonella Typhimurium* tetracyclin resistance gene, *tet B* gene 571 bp PCR assay; DNA marker JenaBioscience (Germany) (B) M; DNA marker GeneRuler (Thermo); 1 negative control; 2 positive control; from 3-12 examined *Salmonella* isolates.

Figure (5): Electrophoretic pattern of *Salmonella Typhimurium* Sulfonamide resistance gene 1, *Sul 1* gene 574 bp PCR assay; DNA marker Gene Ruler (Thermo); 1 negative control; 2 positive control; from 3-12 examined *Salmonella* isolates.

Figure (6): Electrophoretic pattern of *Salmonella Typhimurium* resistance Sulphonamid gene2 show negative *Salmonella Typhimurium* Sulphonamide resistant gene, *Sul 2* gene 543 bp PCR assay; DNA marker Gene Ruler (Thermo); 1 negative control; 2 positive control; from 3-12 examined *Salmonella* isolates.
References


Centers for Disease Control and Prevention (2013): Antibiotic resistance threats in the United States, Centers for Diseases Control and Prevention, Atlanta, GA.


NCCLS (National Committee for Clinical Laboratory Standards). (2007): Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals approved standards. 22 (1).


عزل سالمونيلا تيفيميوريوم من بعض أنواع الببغاوات ورصد الجينات المقاومة للمضادات الحيوية في مصر

داليا محمد الصياد، (1) عاطف محمد كمال، (2) محمد عبد الحليم جمال الدين، (3) عزة سعيد أحمد جودة

قسم الحياة البرية- حجربيطرى مطار القاهرة - قسم الحياة البرية وحدود الحيوان، (1) قسم البكتريا والمناعة والطفريات- جامعة قناة السويس- (2) مركز بحوث الصحراء- القاهرة

تم عمل هذه الدراسة لعزل وتصنيف السالمونيلا كواحدة من أهم الأمراض البكتيرية المشتركة على أنواع مختلفة من طيور الببغاء. تم فحص 300 طائر تنتمي ل(15) نوع من طيور الببغاء من مصادر مختلفة (محال طيور الزينة، مزارع خاصة للحياة البرية، طيور مرباة في المنازل). كما تم تجميع (219) عينة برازا، (72) مسحة من فتحة المجم، وعينات من الأعضاء الداخلية (الامعاء، الكبد، الرئة، الطحال والكلي) من 9 طيور نافقة وذلك لعزل ميكروب السالمونيلا بالطرق التقليدية وتأكيد العزل باجراء تفاعل البلمرة المتسلسل باستخدام جين (inv A) مع أجراء اختبارات حساسية. نجحت جميع التفاعلات السلبية بالإضافة إلى ذلك، حيث تمت تجربة واختبار المضادات الحيوية المستخدمة لعزلها. أظهرت النتائج أن معدل الأصابات بالسالمونيلا كان 3.33% لعدد (10) عينة من إجمالي الطيور محل الدراسة وذلك من طيور سليمة ظاهرا تم شراؤها بطرق غير مشروعة أثناء فترة حظر استيراد الطيور: (5) طيور الحب، (4) طيور الدار الاسترالي، و(1) طائر الروزليا. كما تم تصنيف العينات المعزولة إلى سالمونيلا تيفيميوريوم وتم تأكيد العزل باستخدام البلمرة المتسلسل باستخدام جين (inv A) المستخدمة لعزلها. وكانت نتيجة اختبارات حساسية المضادات الحيوية أن المضادات الأكثر تأثيرا (100%) هي أموكسيسيلان/ حمض الكلافولينك، سيروفلوكساسين، جنتاميسين وأظهرت مقاومة (100%) لكل من دوكسيسكلين سلفوناميد 1، أظهرت النتائج وجود (6) عينات إيجابية لجين المقاومة للتراسيكلين (أ)، (7) عينات إيجابية لجين المقاومة للتراسيكلين (ب)، سلفوناميد (1) بينما ظهرت النتيجة سلبية لجين المقاومة للتراسيكلين (2) في جميع العينات.