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Phenotypic and genotypic characterization of *salmonella* microorganism isolated from one-day old imported ducklings.

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Abstract

A total of 900 imported one-day ducklings samples were collected which submitted to reference lab for veterinary quality control on poultry production in Egypt and tested for the presence of *Salmonella* microorganism. The results revealed that the *Salmonella* microorganism was detected in 36% of examined samples by using standard Isolation method. Examination of internal organ from one-day old imported ducks revealed that *Salmonella spp*. were isolated from pooled liver with gallbladder, yolk sac and cecum samples in ratios 20%,10%,15% respectively ,while examination of 20 samples from paper lining duckling's boxes yield 15% positive . *Salmonella* isolates serotyped into the following *S.* Buck, *S.* Entertidis, *S.*Kentucky *S.* Hindmarsh, *S.* Newport , *S.* Mara, *S.* Entebbe , *S.* Brenderup, *S.* Tishiongwe, *S*. Fortune, *S.*Shubra

Key words: *Salmonella, Ducks , Incidence, PCR ,inv A*

Introduction

Ducks plays an important role in poultry industry and it's considered a great source for meat and eggs. it plays a role in transmission of zoonotic bacteria to human beings.

Heavy economic losses occur due to infection with salmonellosis and it leads to high morbidity, high mortality, reduced egg and meat production in ducks **(Kumar and Kaushi 1988)**.

Although ducks are very resistant to systemic infection caused by Salmonella, they are potential reservoirs of this organism and may shed it in the feces, contaminating the environment (**Barrow *et al*., 1999**). Outbreaks of human salmonellosis caused by contact with ducks have been reported in some countries, such as Australia, United States, United Kingdom and Denmark **(Dansk, 1998; CDC, 2000; Public Health Laboratory Service, 2000; Merritt & Herlihy, 2003**).

Salmonella is gram negative non spore – forming, usually motile, facultative anaerobic bacilli belonging to the family Enterobacteriaceae. Salmonella are differentiated into over 2200 serologically distinct types (serotype) based on differences in somatic and flagellar antigens. Infection with salmonella may or may not lead to a sometimes fetal salmonellosis, a disease that can remain localized in the gastrointestinal tract as gastro-enteritis, or become generalized septicemia and affect several organ systems. **(Ekperigin and Nagaraja 1998)**.

S. Typhimurium can survive in ducks droppings for about 190 days, while in the duck yard substrate even up to 240 days**. (Szeleszczuk, 1998)** .

Many different serotypes of salmonella have been isolated from ducks, most of these being of public health significance but some, including S. gallinarum, S. pullorum, S.typhimurium, S. enteritidis and S. anatum, can cause considerable losses in birds of less than a few weeks of age **(Barrow *et al*., 1999)**

Ducks, duck eggs and duck environmental samples in recent times have been reported as

very important sources of Salmonella **(Pan *et al.*, 2010; Adzitey *et al*., 2012a, b);** and contact with ducklings or the consumption of duck eggs, meats or products contaminated with Salmonella have been associated with salmonellosis, hospitalization or death of affected persons **(Merritt and Herlihy 2003; Noble *et al.*, 2012).**

Materials and Methods

Samples.

A total of 900 different samples (liver- gall bladder -yolk sac-cecum-paper lining duckling boxes) were aseptically collected from imported 1-day ducks for salmonella isolation, that were submitted to the reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Dokki. All samples were examined as soon as possible.

Table (1). Number, source and types of examined samples.

Number of codes	Sub samples	Source	Types of examined samples	Number of samples
20 codes of Ducks	15 ducks from each code	Imported from France	Liver, gallbladder, cecum, yolk sac, and paper lining boxes	300 internal organ and 20 paper lining boxes

bacteriological examination for *Salmonella*. (ISO -6579: 2002)

Indentification was done according to ISO-6579:2002 standard (**Feldsine *et al.*, 2003**).

Detection depending on PCR.

Extraction of *Salmonella* DNA (Sara *et al.*, 2006).

It was carried out as recommended by the manufacturer of the Genomic DNA purification kit.

PCR for amplification of *inv*A gene from the DNA of Salmonellae

Salmonella specific primers, S139 and S141 according to **Salehi *et al.*, 2005** have respectively the following nucleotide sequence based on the *invA* gene of Salmonella 5′ GTG AAA TT A TCG CCA CGT TCG GGC AA - 3′and 5′ TCA TCG CAC CGT CAA AGG AAC C -3′ were used .

Results

In present study, It was found that (108) ducklings out of (300) 1-day old imported ducks (36%) were positive for salmonella while (192) ducklings were found to be negative for salmonella (64%).Also it recorded that (3) of paper lining boxes were positive with percentage (15%) while (17) were negative for salmonella with percentage (85%). the isolated serotypes were *S.* Buck, *S.* Entertidis, *S.*Kentucky, *S.* Hindmarsh, *S.* Newport, *S.* Mara, *S.* Entebbe, *S.* Brenderup, *S.* Tishiongwe, *S.* Fortune and *S.*Shubra.

Table (2). Incidence of salmonella isolated from ducklings.

Types of samples	Number of examined ducklings	Results			
		+ve	%*	-ve	%*
20 codes of day old imported ducklings	300	108	36	192	64
Paper lining boxes	20	3	15	17	85

And it showed that high percentage of isolation from liver and gallbladder 20% then yolk sac with 15% followed by cecum 10% while *Salmonella* isolated from paper lining box is 15%.
In this study PCR amplification of gene *invA* was carried out with fragment size amplified of 284 bp located on *Salmonella* Pathogenicity Island which highly conserved in members of genus *Salmonella*, whereas no amplification could be observed with the extracted DNA of negative control .All 13 *Salmonella* serovars in this study showed positive ampilification of 248 bp fragment specific for *invA* gene.

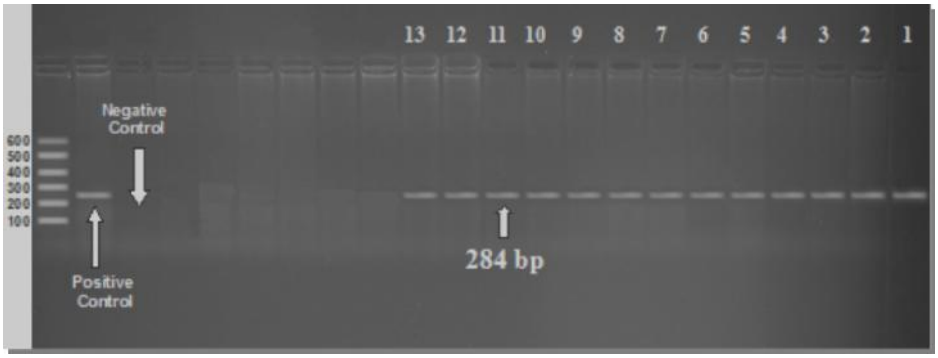

Figure (6). Agarose gel electrophoresis showing *Salmonella* specific PCR of *Salmonella* isolates using primer set for the *invA* (284 bp) gene.

L1. *S. Buck* L4. *S*. Newport L7. *S*. Brenderup L10. *S*. Fortune
L2. S. *Mara* L5. *S*. Newport L8. *S*. Tishiongwe L11. *S*. Entebbe
L3. *S. Entertidis* L6. *S*. Hindmarsh L9. *S. Shubra* L12. *S*.Kentucky
L13. *S*.Kentucky

Discussion

The high frequency of Salmonella recovery from imported day-old ducklings causes great concern because of the zoonotic potential of this pathogen and its economic importance to commercial poultry breeding. Ducks are probably asymptomatic reservoirs of Salmonella and, consequently, may act as infection sources to other species, including humans.
In this study 108 samples out of 300 one-day old ducklings were positive to salmonella (36%) and this result higher than **Shamoon *et al*., (1998)** who isolated Salmonella from digestive tract of ducks breed in open houses and it was 10 out of 60 birds (16.6%) ,on contrary –this results disagree with **Fallacara *et al.*, (2001**) found that the prevalence of Salmonella in water fowl was 0.2%.
The most Salmonellae isolated from present study were from the liver, gallbladder (20%) and yolk sac (15%) of 1-day-old ducklings were mostly evident from cecum(10%) followed by the paper-lined box samples (10%) and this in contrast with **Osman *et al*., (2010)** who isolated Salmonellae from the internal organs and paper-lined box of 1-day-old ducklings were mostly evident from the paper-lined box followed by caecal samples. But **Aho (1992) and Eriksson *et al., (*2001)** reported that drag swabs were more suitable than litter samples.
The results showed that *S.* Newport and *S.* Kentucky were the most prevalent serotyped with percentage (15.4%) for each one while the remained isolates were serotyped *S.* Enteritidis, *S.* Hindmarsh, *S*. Mara, *S*. Entebbe, *S*. Brenderup, *S*. Tishiongwe, *S*. Fortune, *S.* Shubra and *S*. Buck with percentage of 7.7% for each one of them **Asawy and El-Latif (2010)** examined 120 samples from freshly dead ducks and 40 faecal samples from clinically sick ducks and they isolated Salmonella at per-

centage of 3.3% and it's serotyping yielded three different serovars including *S*almonella Typhimurium, *S*almonella Derby and Salmonella Enteritidis while **Tsai and Hsiang (2005)** reported ten serotypes of *Salmonella enterica* were identified: *S.*Potsdam (31.9% of isolates), *S.* Dusseldorf (18.7%), *S.* Indiana (14.3%), *S.* Typhimurium (7.7%), *S.* Hadar (5.5%), *S.* Newport (4.4%), *S.* Derby (4.4%), *S.* Montevideo (2.2%), *S.* Schwarzengrund (2.2%), and *S.*Asinnine (1.1%). **Hoszowski and Wasyl (2005**) detected Salmonella in duck broilers with percentage of 14.3% and the most frequent serovars were *S.* Enteritidis, *S.* Infantis, *S.* Hadar and *S.*Typhimurium. Contrary to our results, **Simko (1988**) who isolated *S.*Typhimurium, *S.* Anatum, *S.* Meleagridis, *S.* Enteritidis, *S.* Infantis, *S.* Virehow (61%, 22%, 4%, 1%, 1%, 1%) respectively and *S.* Agaona, *S.*Bareilly, *S.* Gallinarum- Pullorum, *S.* Hadar, *S.* Heidelberg, *S.* London and *S.* Montevideo were found sporadically. *S. enterica* serovar Typhimurium has been frequently found in young chickens and ducklings in different countries **(Price *et al.,* 1962; Mario, 1990; Bisgaard, 1995; Henry, 2000; Duarte *et al.,* 2009)**. Although *S. enterica* serovar Typhimurium may not be a primary cause of disease, it could possibly cause septicemia in young ducklings with complications of other diseases (**Henry, 2000**). In normal birds, the highest prevalence of *Salmonella* was observed in 1-wk-old ducklings with a 37.5% (30/80) isolation rate (**Yu *et al.,* 2008**). In the USA, 491 *Salmonella* isolates from ducks were reported within 10 years period. The isolates were mainly *S.* Typhimurium(93%), *S.* enteriditis ,*S.* Saintpaul, and *S.* Newport (**Price *et al.,* 1962).** Paratyphoid infection in Egypt was recorded by **Sadek (1996)** with a total percentage of 17.8%. Serotyping revealed the isolates to be *S.* Typhimurium, *S.* Pullorum and Untyped groups. In a recent investigation **(Radwan, 2006)** in Egypt the percentage of *Salmonella* infection in local day old duckling was found to be 19.1% with new serovars (*S.* Shubra and *S.* Saintpaul). In Vietnam, the prevalence of Salmonella species in the ducks were reported to reach 8.7% with a predominance of S. Javiana and S. Weltevreden (**Tran *et al.,* 2004).**The distribution of serovars causing infections in Thailand differs markedly from those reported in other countries and seems to be related to the *Salmonella* serovars in the different food products and other reservoirs for infections. Of particular interest is the frequent occurrence of *S.* Weltevreden and recent increase in occurrence of *S.* Rissen, *S.* Stanley, and *S.* Schwarzengrund **(Kriengsag *et al.,* 1994; Bangtrakulnonth *et al.,* (2004).**

In this study, PCR assay was carried out for the detection of the *invA* gene in 13 *Salmonella* isolates has revealed that the gene was present in all of the isolates (100%) that were demonstrated by the presence of a 284 bp PCR amplified fragment which agrees with the previous studies **(Dias *et al.,* 2003; Mir *et al.,* 2010; Dione *et al.,* 2011)**. No amplified DNA fragments were obtained from non-*Salmonella* species. The *invA* gene encodes for a protein in the inner and outer membrane, which is essential for the invasion of epithelial cells **(Darwin and Miller, 1999)**. The *invA* gene is conserved among *Salmonella* serovars and is a useful marker for molecular detection of *Salmonella* by PCR (**Chiu and Ou 1996; Jenikova *et al.,* 2000; Salehi *et al.,* 2005).**

Conclusion

Duck is consider as reservoir of salmonella and attention should be taken in consideration to avoid human salmonellosis.

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