Cairo University
Faculty of Veterinary medicine
Department of Poultry diseases

Updating of Salmonellosis in chicken in some governorates in Egypt

A Thesis submitted
By

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(Poultry diseases)

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا

مَا عَلَّمْتَنَا

إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

(سورة البقرة آية: ٣٢)

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Abstract

In this study a total number of 655 samples were obtained from 117 different farms (breeder, layer, baby chicks and hatcheries) located in Giza, Qaliobya and Dakahlia Governorates from 2013 to 2015, the samples were collected under complete aseptic condition. The samples obtained from chickens suspected to be died due to avian salmonellosis. Incidence of *Salmonella* spp. was 2.13% (14/655). Serotyping of isolated salmonellae were *S.Typhimurium, S.Enteritidis, S.hindmarsh, S.amersfoort, S.cremieuz, S.newport, S.heistopdenberg,* and *S.Agona.* Ten of the isolated *salmonella* serovars were screened for different virulence genes (*invA, pefA, mgtC, avrA, spvC, sopB* and *stn*) by PCR. *invA* gene was detected in all isolates except one isolate in a percentage of 90%, *avrA, stn* genes were detected in eight isolates in a percentage of 80%, *mgtC, sopB* genes were detected in six isolates in a percentage of 60%, and *spvC, pefA* genes were not detected in any isolates in a percentage of 0%. There is variation in virulence gene profiles between strains belonging to an individual serovar. Ten of the isolated *salmonella* serovars were tested for antibiotic sensitivity, the tested strains were found to be 100% resistant to Tetracycline, Sulphamethoxazole and Ampicillin, also were 90%, 80%, 70% resistant to Doxycycline, Amoxicillin, and Ceftazidine respectively. Also 40%, 50% of isolated serovars were found to be sensitive to Chloramphenicole and Enrofloxacin respectively. There were relations between the virulence gene *invA, avrA, stn* and resistance pattern of isolated salmonellae to Tetracycline, Ampicillin, Sulphamethoxazole, Doxycycline and Ceftazidine. By using vero cells on tissue culture to test invasion ability of ten of the isolated *salmonella* serovars, Cytopathological changes on tissue culture were observed under the inverted microscope after 12 hour post infection and found cell vaculation, irregular in shape and 25% of monolayer sheet destroyed by *S.Amersfoort, S.Agona* and *S.Typhimurium,* also cell detachment and more than 50% of monolayer sheet were destroyed by *S.Newport, S.Hindmarsh, s.cremieuz* and *S.Enteritidis,* more than 70% of monolayer sheet were destroyed by *S.Heistopdenberg* and *S.Enteritidis* and 100% complete destruction of cell and monolayer sheet in *S.Newport*. The study aims to explore the current situation of Salmonellae in poultry farms in Egypt, so detection of *Salmonella* in chicken needs a greater concern for effective control programs in poultry farms and the epidemiological surveillance of this serovars would be necessary to evaluate its possible impact on human health, particularly in Egypt and other countries.

Key Words: Incidence – *Salmonella* - virulence genes - sensitivity test- Serotyping- Vero cell

Dedicated to ☺

My lovely princess who give me happiness

My mother who teach me how to love

My father who provide me with strength in this life

My sisters

(Noha, Nehal and Nada)

My lovely family

My lovely friends

And especially to My love who give me the happiness, love, support and life.

I thank all of you for your efforts with me and I hope to give all of you pleasure as you give me.

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Introduction

Poultry represents important source of cheap protein throughout the world. Avian salmonellosis is an important disease causing serious impediment to the development of poultry industry especially in developing countries of Asia and Africa (**Ramachandranpillai and Mangattumuruppel 2013**). It causes economic losses, particularly in those parts of the world where the poultry industries are continuing to intensify and where open sided housing is common through the death of birds and loss in production. On the other hand avian salmonellosis is an inclusive term designating a large group of acute and chronic diseases of poultry caused by any one or more member of genus *salmonella* under the family *Enterobacteriaceae*. *Salmonella* serovars may be encountered more frequently in one country than the other **(Capita *et al*., 2003 and Liljebjelke *et al*., 2005).** Infected poultry comprise one of the important reservoirs of *Salmonella* that can be transmitted to human. Isolation of *Salmonella* reported more often from poultry and poultry product than from any other species. This likely reflects not only the high prevalence of *Salmonella* infection in poultry, but also the very large number of commercially raised chickens and the application of active nationwide programs for identifying infected flocks **(Calnek *et al*., 1997**).

Poultry are usually exposed to these pathogens not only by contaminated food and water but also by secondary sources of exposure, such as ill contacts in a flock. *Salmonella gallinarum* and

Salmonella pullorum that cause fowl typhoid and pullorum disease respectively are non-motile. Also, motile salmonellae (paratyphoid group) infection cause salmonellosis in chickens and have zoonotic significance. **(Hofstad *et al.*, 1992)**. Over 2500 different *Salmonella* serotypes have been identified to date **(WHO, 2013).** Most serotypes aren't host specific, but a few species are restricted to one kind of animal such as *S.Pullorum* and *S.Gallinarum* in chicken **(Doyle *et al.*, 2009).** The pathogenicity of *Salmonella* depends on a series of factors associated with the bacterium, the bird itself and conditions in which the bird is raised. Association and penetration of the bacterium into digestive mucosa is a prerequisite for systemic infection **(Rychlik *et al.*, 1999).**

An infection with *Salmonella* usually starts by ingestion, followed by colonization in the intestine. After colonization, *Salmonella* is able to penetrate the mucosal epithelium which results in a systemic infection with colonization of the spleen and liver **(Henderson *et al.*, 1999).**The intestinal tract is the primary reservoir of *Salmonella* in poultry leading to contamination of chicken eggs in cloacal region through horizontal route. Transovarian transmission from infected chickens is another important route of contamination of chicken eggs leading to egg borne salmonellosis. Chicks can be infected with *Salmonella* by vertical transmission through infected parents or by horizontal transmission through hatcheries, sexing in contaminated hatcheries, cloacal infection, and transportation of equipment and feed **(Optiz *et al.*, 1993).** Once *Salmonella* has become established in a primary breeding flock, a cycle can be established by which the organism passes via the eggs to the progeny and even to chicks hatched from eggs laid subsequently by infected progeny **(Sharma, 2010)**.

Polymerase chain reaction (PCR) can reduce the time required to detect and identify the agent with high specificity and sensitivity **(Santos *et al.*, 2001).** For *Salmonella* to be virulent, the expression of numerous genes is necessary, which encode some factors with the ability to be located in transmissible genetic elements such as plasmids, bacteriophages and transposons and may be part of specific regions in the chromosome of the bacterium **(Hacker *et al.*, 1997).** Although some *Salmonella* serovar virulotyping studies **(Prager *et al.*, 2003; Herrero *et al.*, 2006; Soto *et al.*, 2006; Huehn *et al.*, 2010)** have been undertaken worldwide, little is known of the distribution of virulence determinants in strains in Egypt. Differences in virulence among *Salmonella* serovars and in the course of infections in various host species have been attributed to the variable acquisition and evolvement of virulence genes **(Falkow, 1996).**

The monitoring of drug resistance patterns among the *Salmonella* isolates not only gives vital clues to the clinician on the best therapeutic regime in each individual case, but is also an important tool in devising a comprehensive chemoprophylactic and chemotherapeutic drug schedule within a geographical area **(Murugkar *et al.*, 2005)**. Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals and selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance. Multi-drug resistance to critically important antimicrobials is compounding the problem (**WHO, 2005**). In recent years problems related to *Salmonella* have increased significally, both in terms of incidence and severity of cases of human and animal Salmonellosis, new concerns have been identified. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials have emerged and are threatening to become a serious public health problem .This resistance results from the use of antimicrobials both in human and animal husbandry **(WHO , 2006**).

Salmonellosis is one of the most common and widely distributed food borne diseases. It constitutes a major public health concern and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths **(WHO 2005)**. The occurrence of food borne diseases has been a matter of discussion over the last few years due to the worldwide concern on strategies for their control and for ensuring that safe food products reach the consumer. Since no "effective" immunoprophylactic measures are available for the disease till date, strict biosecurity is the only alternative to preclude the disease (**Rajagopal *et al*., 2013).**