Animal Health Research Institute,
Alex. Branch.

EPIDEMIOLOGICAL STUDIES ON SALMONELLOSIS IN BROILER CHICKEN FARMS IN ALEXANDRIA GOVERNORATE

(With 3 Tables)

By

F.A. EL-SHABOURY and OLA A.A. BASHA

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دراسة وبائية علي السالمونيلا فى مزارع دجاج التسمين فى محافظة الاسكندرية

فتح الله على الشابورى ، علا عبد العزيز باشا

تم عزل ميكروب السالمونيلا تيفيميوريم من دجاج التسمين فى محافظة الاسكندرية حيث تم فحص خمسين مزرعة من دجاج التسمين من أماكن مختلفة داخل المحافظة. كما أجريت دراسة بعض عوامل الضراوة للعترات المعزولة مثل خاصية الاتحاد بصبغة الكونغو الحمراء وافراز انزيم تحلل الدم. مع دراسة حساسية هذه المعزولات للمضادات الحيوية المختلفة وأفضل الطرق التشخيصية من العزل البكتريولوجى والفحوص البيوكيميائية والسيرولوجية لتعريف الميكروب المعزول.

SUMMARY

A survey on *Salmonella* infection was carried out on 5o broiler chicken farms located at Alexandria Governorate. A total of 5 *Salmonella* isolates were isolated and serotyped using different selective enriched media for isolation and a variety of biochemical and serological tests for identification. The *in-vetro* sensitivity test revealed that Neomycin and Ceftiofur gave the heighest activity against *Salmonella* isolates. On the other hand, *Salmonella typhimurium* isolates showed resistance against Erythromycin, Amoxycillin and Ampicillin. Moreover, *Salmonella* isolates were tested for their virulence using Congo red binding test and ability to produce hemolysin.

Key words: *Broilers, salmonellosis, S.typhimurium.*

INTRODUCTION

Salmonella is one of the most important microorganisms that cause diseases in man and animals and among common causes implicated in outbreaks of food borne infectious disease around the world, (Abou-Zeed *et al.*, 2000). Salmonellae have a wide host range, including human, animals and birds (Duce *et al.*, 1991). Poultry have been implicated as a major source of *Salmonella* contaminated food products as poultry eggs or meat that cause human salmonellosis (Tauxe, 1991 and Humaphrey, 1999). *Salmonella pullorum* has a long history of causing economically significant illness and mortality in young commercial chickens (Snoeyenbos, 1991). *Salmonella typhimuruim* found to be the main cause of *Salmonella* in human and animals (Groisman *et al.*, 1989). Much more, *Salmonella entritidis*, only arose to its predominant position in man and poultry in the mid- 1980s as a result of vertical and horizontal transmission within and between large poultry organization in many parts of the world (Hartung, 1992). Sensitivity of isolation of these organisms by culture depends on various factors including the method used for collecting the sample, the amount of sample analyzed and seasonal variation in shedding the organism (Cohen *et al.*, 1994). The growth inhibition by other bacteria can be a particular problem, especially with faecal samples which are often heavely contaminated with other microorganisms, moreover, in chronic *Salmonella* infection in fowl, *Salmonella* organisms are excreted only intermittently in faeces. Standerd procedures for isolation and identification of *Salmonella* using growth media are slow and often these tests fail to detect bacteria that may be present for that, numerous schemes for pre enrichment, direct enrichment and secondary enrichment have been examined for recovering and isolating *Salmonella* from meat, poultry and enviroment (Tate *et al.*, 1990). There is a need for *Salmonella* testing methods that provide more rapidly and sensitivity results. These rapid methods should be robust and reliable and have a specificity that minimize false positive results. (Blackburn, 1993).

Plasmid analysis has a great effect as an epidemiological tool in an outbreak of salmonellosis (Kapperud *et al.*, 1989). More, plasmid analysis is relatively simple to perform, does not require aspecialized reference laboratory and can be accomplished in a short period of time and not expensive (Mayer, 1988)

The purpose of this work was designed to study the incidence of *Salmonella* infections in broiler chicken farms by using different

methods of enrichment for isolation and applying biochemical and serological methods for identification, Moreover study some virulence factors for *Salmonella* microorganisms and finally isolated *Salmonella* were tested for antimicrobial drug susceptibility.

MATERIALS and METHODS

1 - Bacteriological isolation:

A total of 50 broiler chicken farms of different ages at different localities in Alexandria Governorate were subjected for bacteriological studies , from each flock 5-7 live and freshly dead chicken aged from 1-5 week were taken for isolation and identification of *Salmonella* using enriched media as "Selenite F broth and Tetrathionate brilliant green broth" incubated at 37 – 42°C for 24-48 h. (Oxoid 1987) and delayed secondary enrichment for 5 days (Waltman *et al.*, 1991), then cultured on MacConkey's agar, Salmonella-Shigella (SS) agar and Brillient green agar incubated at 37°C for 24 h. (Oxoid, 1987).

The suspected colonies were examined morphologically and biochemically.

2 - Morphological identification:

Purified suspected *Salmonella* isolates were examined morphologically as described by Cruickshank *et al.* (1975).

3 - Biochemical identification:

Suspected *Salmonella* isolates were identified biochemically using criteria of Quinn *et al.* (1994).

4 - Serological identification:

Cultures biochemically identified as *Salmonella* were typed serologically according to Kauffmann White Scheme (Kauffmann, 1972) by slide agglutination test using polyvalent "O" *Salmonella* antiserum. Colonies which gave positive results were similarly tested using monovalent "O" and "H" *Salmonella* antisera.

5 - In vitro antibiotic sensitivity test:

It was done according to Finegold and Martin (1982) using Muller Hinton agar (Oxoid) and commercial antibiotic discs (Oxoid and BBL) including Neomycin (30µg), Gentamycin (10 µg), Amoxycillin (25 µg ug), Norfloxacin (10 µg), Stryptomycin (10 µg), Ampicillin (40 µg), Oxytetracyclin (30ug µg), Chloramphenicol (30 µg), Ceftiofur (30 µg), Doxycycline (30 µg), and Erythromycin (15 µg).

6 - Detection of virulence factors:

1 – Congo red binding activity (Agenta *et al.,* 1977). *Salmonella* strains were cultured on Congo medium. Congo red positive salmonella isolates were identified by appearance of red colonies. The reaction was best seen after 24 hours incubation at 37°C, followed by an addition of dys at room temperature. Congo red negative *Salmonella* colonies did not bind the dye (white colonies).

2 – Detection of haemolysin (Tange *et al.,* 1993). *Salmonella* isolates were inoculated into blood agar plates contaning 5% sheep blood, after 24 hours of incubation at 37°C positive B- haemolysis production was indicated by clear zone of haemolysis.

RESULTS

Incidence of Salmonella among broiler chicken flocks:

Out of 50 examined broiler chicken flocks, a total of 5 suspected *Salmonella* isolates were recovered. These isolates were subjected to further examination by polyvalent and monovalent *Salmonella* antisera. Five *Salmonella* isolates were obtained and identified as *Salmonella typhimurum*. (Table 1), and the biochemical characteristics of *Salmonella* isolates are illustrated in Table (2). The results of drug sensitivity was studied and summarized in Table (3). which indicated that *Salmonella typhimurum* was found sensitive to Neomycin, Ceftiofur and Gentamycin, while it was resistant to Ampicillin, Erythromycin, Oxytetracyclin and Amoxycillin, and showed variable degrees of sensitivity against other antimicrobial agents. Regarding to virulence activity of *Salmonella* isolates it was found that the examined isolates were bounded with Congo red dye giving red colonies and produce B-haemolycin on blood agar.

Table 1: Incidence of *Salmonella* among broiler chicken flocks.

Age of broiler chickens	No. of examined flocks	Positive flocks	No. of isolates	Serotype of salmonella	Incidence (%)
1st week	10	1	1	*S.typhimurum*	10
2nd week	10	2	2	*S.typhimurum*	20
3rd week	10	1	1	*S.typhimurum*	10
4th week	10	1	1	*S.typhimurum*	10
5th week	10	-	-	*S.typhimurum*	-
Total	50	5	5		10

Table 2: Biochemical reactions of suspected *Salmonella* isolates

Test	Result
Catalase	+ ve
Oxidase	- ve
Indol	- ve
Methyl red	+ ve
Voges proskauer	- ve
Citrate	+ ve
Urease	- ve
TSI	
Slant	Red
Butt	Yellow
H_2S	+ ve
Sugar fermentation	
Dextrose	+
Lactose	-
Sucrose	-
Mannitol	+
Maltose	+
+ positive	- negative

Table 3: Antibiotic sensitivity of isolated *Salmonella*

Drugs	Reactions		
	Sensitive	*Moderate*	*Resistant*
Neomycin (N 30 µg)	++++		
Gentamycin (GM 10 µg)	+++		
Amoxycillin (AML 25 µg)			++++
Norfloxacin (NOR 10 µg)		+++	
Stryptomycin (S 10 µg)		++	
Ampicillin (AMB 40 µg)			++++
Oxytetracyclin (T 30 µg)			++++
Chloramphenicol (C 30 µg)		+++	
Ceftiofur (CEF 30 µg)	++++		
Doxycilline (DO 30 µg)		++	
Erythromycin (E 15 µg)			++++

DISCUSSION

The incidence of salmonellosis has been increased as a major public health hazard (Rose *et al.*, 1991). Contaminated poultry meat and eggs have been frequently implicated as sources for human *Salmonella* outbreaks in many countries (Humphrey 1999, Gast 1993, and Gast 2001). In the present work 5 -7 freshly dead or alive bird were collected from each one of 50 broiler chicken flocks to represent as a sample of

the flock and were examined bacteriologically and serologically for isolation and identification of *Salmonella*. Results were tabulated (Table 1, 2) indicating recognition of 5 *Salmonella* isolates and serotyped as *Salmonella typhimurum*. The relation between *Salmonella* infection and age of broiler flocks (50 flocks) was also investigated. The result revealed that 2 isolates of *Salmonella typhimurum* were recovered at the 2nd week of age at a percent of 20 %, while 1 isolate as a percent of 10 % was recovered at the 1st, 3rd, and 4th week of age. In Egypt, different *Salmonella* serotypes were isolated from broiler chicken as *Salmonella typhimurum* and *Salmonella entritidis* (Shahata, 1980 and Abd- Alla, 1991). *Salmonella typhimurum* was isolated by (Abd El-Hamid *et al.,* 1984) who examined 555 autopsied chicks and isolated *Salmonella typhimurum* in 26.1%, Ibrahim and Abd El-Lateif (1997) recovered 7 *Salmonella* isolates which were identified as *Salmonella typhimurum* from diseased and dead baby chicks (5.6%) while Hegazy (2002) examined 138 broiler flocks and found *Salmonella typhimurum* at a rate of 5.41%. At the age of 5 weeks in 50 broiler flocks all samples were negative and non of the salmonellae were isolated which could be attributed to either large doses of antibiotics which were used till the 5th week, or to the fact that *Salmonella* colonization of birds decreased with increasing broiler age. This idea was supported by Barnes *et al.,* (1980), Williams (1984) and Rose *et al.* (1999). Since, one of the main purpose of such investigation is to find out the most suitable method for control, the antibiogram was carried out against *Salmonella typhimurum* isolate. The results showed that *Salmonella* was sensitive to Neomycin, Cetiofur and Gentamycin. However, they were moderately sensitive to Norfloxacin, Chloramphenicol and Doxycylin. Meanwhile, it was resistant to Amoxycillin, Ampicillin, Oxytetracyclin and Erythromycin. The high incidence of resistance could be attributed to the routein usage of subtherapeutic doses of antibiotics as feed additives to raise the feeding efficiency and the rate of weight gain. These findings were supported by Tourfrade (1984) and Coghlan (1991). Regardless of the different sensitivity patterns of *Salmonella,* the antibiogram is still the valied method for the drug of choice to control *Salmonella* infection. Abd El-Hamid *et al.*, 1984, Heffernan, 1991 and Hegazy, 2002).

Identification of virulence of *Salmonella* isolates had led to better understanding of pathogenesis of diarrheal disease caused by them and providing a new dimension to their diagnosis. Our results showed that *Salmonella* isolate recovered from broiler chicken bind Congo red and pathogenic *Salmonella* have envolved some unique cellular products

associatd with virulence of organism. Also, the result indicated that *Salmonella* isolate was hemolysin producer and this could be used as a phenotypic maker or virulence factor.

These results were supported and in agreement with Gorman and Adly (2004) and Sahar and Amany (2007).

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