STUDIES ON SALMONELLAE IN CHICKENS

By

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ABSTRACT

Chickens are known to be very sensitive to Salmonella during the first weeks of life because of delayed development of their intestinal flora. Salmonellosis is well documented as a debilitating disease in young poultry. Paratyphoid salmonella infection continues to affect the poultry industry and contribute to human food born disease. 16 cases (8.0%) out of 200 bacteriologically examined dead chickens and 5 chickens (5.0%) out of 100 ailing slaughtered chickens were found to be bacteriologically positive for Salmonella. The isolation rate of Salmonella from egg and eggshell samples was 3.0% of the total number of examined eggs (100 eggs). Out of 200 examined blood samples, 9 (4.5%) showed positive agglutination reaction. Serological examination of the recovered isolates revealed the recognition of 10 serovars comprising the following species: *S. entertidis* (4 isolates) and *S. infantis* (4 isolates) with incidence 16.66% of the total number of the recovered isolates for each followed by *S. typhimurium* and *S. cerro* (3 isolates for each) with an incidence of (12.50%). Meanwhile, *S. gallinarum-pullorum*, *S.reading* and *S.montevideo* was (2 isolates) with an incidence of 8.33% for each and one isolate of each of *S. anatum*, *S. vircho* and *S.neolands* with an incidence of 4.17% for each. Finally, one serologically unidentified isolate with an incidence of 4.17%. Experimental trials in baby chicks revealed that infected chicks showed anorexia, dehyderation, enteritis and depression with mortality rate reached40.0% and 70.0% when injected s/c and i/p respectively with *S. infantis* while in case of *S. typhimurium* the mortality reached 30.0% (s/c) and 70.0%(i/p) with reisolation rate 100%. On the other hand, the mortality caused by *S. cerro* decreased to 30.0% (S/c) and 50.0% (i/p) with 100% reisolation rate.

INTRODUCTION

Infections with bacteria of the genus Salmonella are responsible for a variety of acute and chronic diseases in poultry.

Infected poultry, moreover, comprise one of the most important reservoirs of Salmonellae that can be transmitted through the food chain to humans. Isolation of Salmonella is reported more often from poultry and poultry products than from any other animal species. This likely reflects not only the high prevalence of Salmonella infections in poultry, but also the very large numbers of commercially chickens and the application of active nationwide programs for identifying infected flocks.

The dimensions of the poultry Salmonella problem have expanded considerably in the recent years. In the past, the primary motivation for controlling Salmonella infection in poultry was to reduce disease losses. Today public health concerns, political pressures and consumer demands has increasingly made prevention of food-borne transmission of disease to humans and urgent priority for poultry producers **(Pomeroy and Nagaraja, 1991).**

This work was planned to through the light on:

1- Prevalence of Salmonella microorganisms in chickens as well as table eggs.

2- Screening serological examinations using pullorum test.

3- Pathogenicity test with the most prevalent isolates.

MATERIAL AND METHODS

1- Sanmples:

Samples used in this work were obtained from 300 Balady chickens, of different ages (one-week to 30weeks of age). Out of these, 200 chickens were clinically diseased and freshly dead and 100 ailing chickens with clinical signs of disease.

On the other, 200 blood samples were collected randomly from two hundred chickens ranging in ages from 4-35 weeks.

As regarding to Balady table eggs, bacteriological swabs were taken from the inner wall of the egg shell as well as from the yolk of 100 eggs.

2- Isolation of Salmonella organisms:

All bacteriological samples were pre-enriched into non selective tryptone soy broth as well as into selective tetrathionate broth and incubated for 18 hours at 37°C and 42°C respectively **(Krieg and Holt, 1984).**

A loopful was taken from each broth and spread onto each of the following solid plating media, namely, MacConkey bile salt lactose agar,

Salmonella-Shigella agar and Hektone entric agar media and incubated at 37°C for up to 72 hours.

3. Identification of the isolates:

All suspected colonies were examined for Gram staining technique, oxidase test, reaction on triple sugar and lysine iron agar before being identified using the API 20 E system according to **Murray and Yolken (1995)**.

4. Pullorum test:

Polyvalent crystal violet stained capsular antigen of *Salmonella pullorum* (Vet. Serum and Vaccine Institute Abbassia, Egypt), was used for pullorum test according to **Collee *et al.*, (1996).**

5. Serotyping of Salmonella:

Cultures that yield reactions similar to those given by Salmonella on TSI, LI agar, and API 20 E strips were examined serologically using the following antisera:

- Polyvalent antisera (group I, II and III)
- Individual monovalent (O) typing sera (Anti-O from factor 2-25)
- Individual monovalent (H) typing sera:
  Anti-H (a-z_{29})
- Anti H (1-7)
- These antisera provided in a liquid form from Behring Werke AG, Marburg, Germany

6. Experimental infection in chicks:

Preparation of bacterial inoculum:

Twenty four hour pure culture of each of the predominant isolated serovars of Salmonella was suspended in sterile saline at a concentration of $3X10^8$ CFU/ml. **(Christensen *et al.*, 1992)**

Inoculation of prepared serovars to day-old chicks:

Three groups (I, II, III) each of 30 chicks were used. (One group for each strain, namely *S. infantis*, *S. cerro*, and, *S. typhimurium*; respectively).

Each group was divided into three subgroups:

1-subgroup A: 10 chicks; infected S/C with $3X10^8$ bacteial cells for two successive days 0.5 ml each day.

2- Subgroup B: 10 chicks; infected I/P with $3X10^8$ bacteial cells for two successive days 0.5 ml each day.

3- Subgroup C: 10 chicks; left as control.

Chicks were observed daily to record their general health condition and to notice

any clinical symptoms. PM examination was performed for chicks died post infection with trials to reisolate the inoculated serovar. 14-days post infection, all living chicks were sacrificed for post mortem and reisolation procedures.

RESULTS AND DISCUSSION

Results of salmonellae recovery from chicken samples:

The material for this work was entirely collected from the internal organs, intestinal and cloacal swabs to get very efforts to isolate Salmonellae thoroughly.

Out of the two hundred bacteriologically examined dead chickens which were suffered from clinical signs of disease, 16 cases (8.0%) were found to be bacteriologically positive for Salmonella and out of 100 ailing slaughtered chickens, 5 chickens (5.0%) were recorded as Salmonella positive chickens.

The results obtained by **Schroder (1971); Parisis *et al.*, (1980) and Lee *et al.*, (1984)** who found that the incidence of Salmonellosis among adult chickens reached 6.9%. Moreover, **Lu *et al.*, (1986)** observed Salmonella infection which developed in 4.4% of 25,000 chicks aged 1-3 weeks. On the other hand, **Ozdemir (1996)** isolated Salmonella from 29 outbreaks of salmonellosis from commercial layers, broilers, hatchery, breeder layers and a broiler abattoir, also, **Pederson *et al.*, (2002)** added that the prevalence of Salmonella varied from 7.1 – 25% and 24 different serovars were detected.

Results of salmonellae recovery from egg and egg shell samples:

Regarding to the examined 100 egg samples, three eggs (3.0%) were found to harbor Salmonella. This seems to agree nearly with the results obtained by **Siddique *et al.*, (1985)** who isolated 47 strains of Salmonella from 2360 samples obtained from eggs, feeds, litters and drinking water of poultry farms in Romania with an incidence of 2.1%.

Results of rapid whole blood agglutination test using pullorum antigen:

Out of 200 examined blood samples, 9 (4.5%) showed positive agglutination reaction. Progress towards eradication demonstrated in a number of overseas countries **(Chart *et al.*, 1990 and Gast and Beard, 1991)**

Hence, the control of pullorum typhoid infection depends upon the rapid whole blood test which serves as an important control and eradication programs can be gauged by comparing the percentage of reactors detected **(Mahato *et al.*, 1990)**.

Bouzoubaa *et al.*, (1992) found that the overall seropositivity for pullorum disease was 6.0%. Also, **Hoop and Pospischil (1994)** recorded that

the rapid slide agglutination test with *Salmonella pullorum* antigen was positive in 6.4% of the examined chicken blood.

Results of serological examination of the recovered salmonellae:

Serotyping of the twenty four isolates of Salmonellae recovered from all bacteriologically examined samples revealed the recognition of 10 serovars namely:

S. entertidis (4 isolates), *S. infantis* (4 isolates), *S. typhimurium* (3 isolates) *S. cerro* (3 isolates), *S. gallinarum-pullorum* (2 isolates) *S. reading* (2 isolates), *S. Montevideo* (2 isolates), and one isolate of each of *S. anatum, S. vircho,* and *S. neolands.* There was only one serologically unidentified isolate (Table 1). These results nearly agree with those obtained by **Barnhart *et al.*, (1992); NovaK and Polaharova, (1993); and Uyttendaele *et al.*, (1998).**

Results of experimental infection in baby chicks:

S. infantis inoculated s/c caused death of 4 out of 10 chicks within 4-7 days post-infection with a amortality rate of 40.0% increased to be 70% in chicks inoculated i/p within 3-5 days post-infection. Before death, the *S. infantis* infected chicks showed the following symptoms: anorexia, dehydration, enteritis and depression.

Post mortem examination revealed congestion of the internal organs. Spleen was dark red and the ureters were filled with caseated urates. Reisolation was recorded from the liver and heart blood of dead chicks. While *S. typhimurium* when inoculated s/c caused 30% mortality and in group inoculated i/p mortality reached 70%.

The clinical signs and post mortem findings were identical to those obtained by *S. infants*. Reisolation rates were 100% from all dead chicks.

The virulence and pathogenicity of *S. cerro* was lower if compared with the other two experimentally examined serovars.

Three chicks dead after s/c inoculation and 5 chicks dead after i/p inoculation. Clinical symptoms recorded in the form of dehydration and diarrhea. Post mortem lesions were in the form of congestion of the intestinal tract and enlarged liver.

Reisolation rate was recorded in two out of the three dead s/c inoculated group and in four out of the five dead i/p inoculated group.

Symptoms and post mortem lesions were nearly similar to those recorded by **Adams *et al.*, (1983); Leuchtenberger (1983); Lee (1987); Humphrey *et al.*, (1990) and Ansuini *et al.*, (1993).**

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Table (1): Serotyping of Salmonella isolates:

Serotype of Salmonella	No. of isolates	Incidence of each serotype
S. entertidis	4	16.66 %
S. infantis	4	16.66 %
S. typhimurium	3	12.50 %
S. cerro	3	12.50 %
S. gallinarum-pullorum	2	8.33 %
S. reading	2	8.33 %
S. montevideo	2	8.33 %
S. anatum	1	4.17 %
S. vircho	1	4.17 %
S. neolands	1	4.17 %
Serologically Unidentifiable strain	1	4.17 %

الملخص العربي

دراسات عن السالمونيلا في الدجاج

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تعتبر الدواجن حساسة للإصابة بميكروبات السالمونيلا فى الأسابيع الأولى من العمر نتيجة تأخر تكوين الفلورا المعوية، فمرض السالمونيلا يعتبر من الأمراض التى تسبب الهزال فى صغار الطيور الداجنة وعدوى الباراتيفود لا تزال تؤثر على صناعة الدواجن وتعتبر من الأمراض التى تنتقل عن طريق الطعام. وبالفحص البكتريولوجى لعدد 200 من الدجاج النافق حديثا و 100 من ألدجاج المريض المذبوح وجد أن 16 (8%) و 5 حالات (5%)، على التوالى، كانت إيجابية لعزل السالمونيلا. وبالنسبة لعينات البيض كانت نسبة العزل (3%) من عدد 100 بيضة.

وبأجراء اختبار البللورم (اختبار تلزن للدم الكامل) لعدد 200 من الدجاج، وجد أن 9 عينات (4.5 %) كانت ايجابية.

أظهرت نتائج التصنيف السيرولوجى لعترات السالمونيلا المعزولة وجود 10 عترات سيرولوجية تشمل سالمونيلا إنترتيديس (4 عترات) و سالمونيلا إنفانتيس (4 عترات) بنسبة عزل (16.66%) لكل على حده من إجمالى العترات المعزولة ويلى ذلك سالمونيلا تيفيميوريم (3 عترات) و سالمونيلا سيرو (3 عترات) بنسبة عزل (12.50%) لكل على حده بينما تم عزل سالمونيلا جالينيرم بلورم و سالمونيلا ريدنج و سالمونيلا مونتيفيديو (عترتين) لكل منهم بنسبة عزل (8.33%) كما تم عزل سالمونيلا أناتم و سالمونيلا فيرشو و سالمونيلا نيولاندس (عترة واحدة لكل منهم) بنسبة (4.17%) بالإضافة إلى عترة غير مصنفة سيرولوجيا (4.17%).

أدت العدوى التجريبية فى الصيصان إلى ظهور أعراض فقدان الشهية والجفاف والإلتهابات المعوية وخمول ونسبة نفوق تصل إلى 40% و 70% فى حالة الحقن بسالمونيلا إنفانتس تحت الجلد وداخل التجويف البريتونى على التوالى. أما فى حالة سالمونيلا تيفيميوريم وصلت نسبة النفوق إلى 30% و 70% فى حالة الحقن تحت الجلد وداخل التجويف البريتونى على التوالى ، كما تم إعادة عزل الميكروبات بنسبة 100%. وعلى الجانب الأخر انخفضت نسبة النفوق المسبب بالسالمونيلا سيرو الى 30% و50% فى حالة الحقن تحت الجلد وداخل التجويف البريتونى على التوالى مع إعادة عزل الميكروب بنسبة 100%.