Prevalence of Salmonellae from broiler chicken

Reem, M. Reda*, Ahmed, M. Ammar* and Soad, A. Nasef***
*Central laboratory for quality control on poultry production, Animal Health Research Institute, Doki, Giza
**Microbiology Dept., Fac. Vet. Med., El Zagzagig Univ., Egypt.

Abstract
Prevalence of *Salmonella* among broilers farms was investigated in this study which covers different provinces in Egypt through examination of 1800 various samples as paper lining box, internal organs of chicks. ISO 6579 was used for isolation and identification of *Salmonella* and Kauffmann-White schemer was used for *Salmonella* serotyping .The result showed that percent of salmonella infection among imported baby chickens was 12.5% and in local baby chicks was about 65%.From imported chicks: *Salmonella Enteriditis* with percentage of 37% and From local baby chicks: *Salmonella Typhimurium, S.Essen, S.Bradfored,* and *S. Logos* 25%, 28.1%, 6.2% and 3.1% respectively. An experiment was done using salmonella *Enteriditis* in infection and immunohistochemical reaction for detection of salmonella organisms in liver and ceca was used.

Key words: *Salmonella Enteriditis , imported chicks, Salmonella serotyping .*

Introduction

Bacteria of the *Salmonella* genus are members of the *Enterobacteriaceae* family. They are Gram-negative, facultative anaerobes and inhabit the intestinal tract of animals and may be thus recovered from a wide variety of hosts, specially poultry and swine, humans, foods and environment. Besides, these bacteria may be pathogenic to wild domestic animals and humans **(Holt *et al.*, 1994).**

Infections with bacteria of the Genus Salmonella are responsible for variety of acute diseases in poultry. Infected poultry moreover, comprise one of the important reservoirs of *Salmonella* that can be transmitted through the food chain to humans. 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*etal*., 1997).**

Conventional culture methods used for detection and isolation of *Salmonella* include, are nonselective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspect colonies are then confirmed biochemically and serologically **(Fakhr*et al.*, 2006).**The complete test is laborious and consuming, generally requiring 3-4 days to obtain a negative result and up to 7 days to confirm a positive result **(Anon,1993 and Andrews *et al*., 1995, 2001)**.As an alternative to culture methods, immunological methods in different formats have been introduced for the rapid detection of *Salmonella infection* **(Patel,2000 and Fukuda *et al*., 2005)**.Immunological methods depend on binding of specific antibody to an antigen on the bacterium. Several immunoassay systems are commercially available for detection of *Salmonella* in food products **(DePaula*et al.*, 2002)**. Some of this immunoassay, which also requires pre-enrichment of samples, has been validated as rapid detection methods **(Andrews *et al*., 2001).**

Infection with *Salmonella* is usually started by oral ingestion of the pathogen and is followed by bacterial colonization in the gut and invasion of internal tissue. Little is known about the details of bacterial colonization and invasion. Knowledge about the mechanisms leading to invasion infections may ultimately lead

to new insights in prevention and therapy **(Amin *et al.*, 1994).**

The aim of the work is record the incidence of Salmonella infection in broilers and trials to detected the infected Salmonella in internal organs of experimentally infected birds

Materials and Methods:

Samples

A total number of 1800 samples as shown in table (1) was submitted to the laboratory to detect *Salmonella* species from different sources (local and imported baby chicks.

Table (1). Numbers and types of samples collected from different sources

Type of sample	No. of examined samples
Imported bird	1200
Local baby chicks	600
Total	1800

Materials used for the experimental infection in chicken

Chicken

Thirty five one day old Ross broiler chicks were selected for experimental infection. Five birds were examined to be sure they are free of Salmonella.

Bacterial strain

Salmonella Enteritidis field isolate recovered from locally produced day old chicks was used for experimental infection.

Experimental design

A total of 30 Ross broilers baby chicks were used in this experiment. That was divided into 2 groups. Group (G1) 20 birds were inoculated orally directly into crop with 0.2 ml broth culture 1.8×10^8 CFU/ml. The other group (G2) consisted of 10 bird not inoculated. They observed twice daily with recording of clinical signs up to 4 weeks. The fecal samples were collected every week for bacterial examination. At the end of the experiment, all birds were slaughtered and collected Liver, spleen and cecum, was collected for trials of re-isolation of inoculated organism.Liver and cecum were kept in 10% formalin for immunoperoxidase test.

Isolation and identification:

All collected samples were examined for detection of Salmonella using **ISO 6579(2002)** as well as samples for experimentally infected birds.

Immunohistochemical examination for detection of *Salmonella Enteritidis* in collected organ of experimentally infected the chicks (Desmidt*et al.*, 1998)

The ceaca and liver were kept in formalin 10%. Tissue section were made at 8 μm and stained with an immunoperoxidase test

Result and Discussion:

Examination of apparently healthy baby chicks revealed that detection of Salmonella in 30% of examined samples with percentage 12.5% Imported and 65%in local baby chicks.

Table (2). Incidence of *Salmonella* among examined samples.

Samples	No. of samples	No. of positive	Percentage
Imported baby chicks	1200	150	12.5%
Local baby chicks	600	390	65%

The result of *Salmonella* isolation from the local baby chicks was higher than the result of isolation from the imported chicks. Also, **Mousa *et al.* (2010)** said that *Salmonella* was relived a high incidence among the examined samples he local chicks was about 7.89% and the imported chicks was about 4.83%

Regarding *Salmonella* serotype isolated from examined samples the incidence of SE is about 12.5% from the imported baby chicks on the another hand the incidence of ST is about 25% from the local baby chicks, *S. Essen* 28.1%, *S. Bradfored* 6.2%, *S. Logos* 3.1%. So, the major strains is SE

Result of the experimental infection :

Two chicks were died on day fourteen post inoculation with percentage 10%.In died chicks the postmortem lesion in salmonellosis consists, spleenomegaly, hepatomegaly, necrotic foci and Petechiae in the liver and spleen, enteritis characterized by watery intestinal content. Symptoms usually seen in young chicks, it appears weakness, drooping wings, ruffled feathers and huddling together near the heat sources.

Many chicks that survived for several days was become emaciated and the feather around the vent was wetted with fecal materials diarrhea is occur due to enteritis. Similar result were reported by **(Sakia*et al.*, 2006)** indicated that in all animals inoculated with *Salmonella enterica serovar Enteritidis*, the *Salmonella* was detected in the faeces at all time points analyzed by bacterial planting .In contrast *Salmonella enteric serovar Enteritidis* not detected in the control animals. Also, **Ruby *et al.*, (2003)** recorded *Salmonella enteric servoar Enteritidis* can be rapidly speared among chicken through shedding *Salmonella enteric serovar Enteritidis* contaminated faeces. Compared with the controlled chicks it appears healthy and no symptoms of enteritis.

The rate of *Salmonella* isolate was higher in the litter it was 100% but in the cecum is 70% and liver is 50% as shown in table (3).

Table (3). Prevalence of *Salmonella* from experimentally infected chicks.

Organ	No. of samples	No. of positive samples	Percentage
Cecum	20	14	70%
Liver	20	10	50%
Litter	15	15	100%

Result of immunoperoxidase test: (photo1)

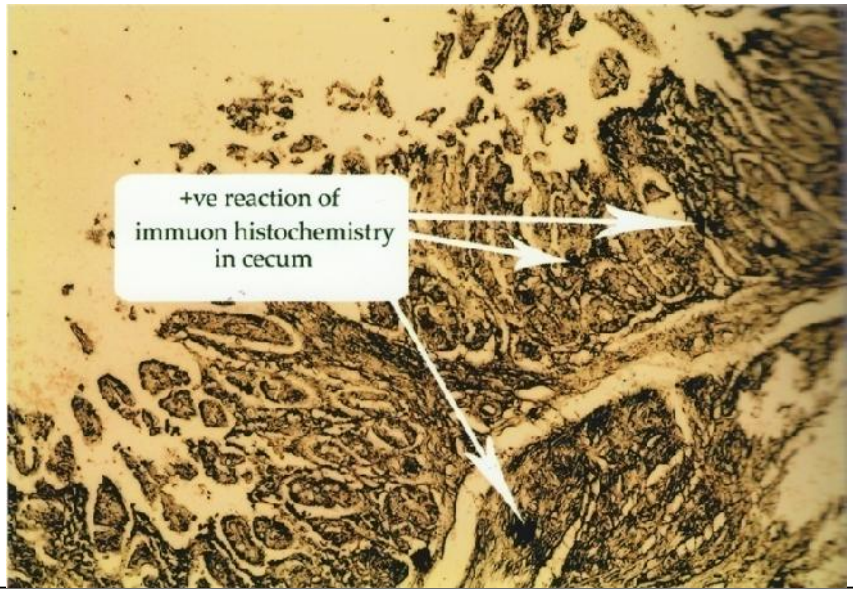


Photo (1) Showed labeled bacilli were associated with epithelial surface. Numerous *Salmonella* were adhering singly to the epithelium of the villi of the cecum. Found also immuonohistochemical observation in liver of chicken infected with *Salmonella Enteritidis.* The bacteria reach the intestinal tract can cross the intestinal epithelium after attachment to mucosa from there they can reach the lamina propria, where there replicated or penetrating to deeper tissue after reach to the blood stream, they infected the internal organ such as liver and spleen **(Sakia*et al.,* 2006)**.The attachment of microbial pathogens to the host cell surface is the essential step in pathogeneses **(Mizumoto*et al.,* 2005)**.

Oral investigation of *Salmonella* results in intestinal colonization especially in the ceca and shedding of pathogen in voided faeces. It is clear in table (3) that SE detected in all samples from infected group (G1) after 4 weeks the incidence of reisolation from ceca is about 70% while in liver is about 50%from liter is about 100%. On the other hand, SE could not be detect in voided faeces in non infected group (G2). This study deals with **Pieskus*et al.,* (2008)** who investigated a contamination of *Salmonella* in broilers about 470 samples, included faeces, and cecum content the result indicated that the most infected broilers samples were faeces and cecum (32.9 % and 23.1%), respectively.

In contrast **Vaugh*et al.,* (2008)** found the SE culture positive in cecum, liver and spleen were 100% at one week post inoculation and then the percentage of SE positives progressively decline overtime**.** Among the collected chicken samples analyses about 80 samples 20 (12.5%) contenting *Salmonella* on 10 case analyses that containing *Salmonella* 30% in the heart, 10% in the liver, 10% in the gizzard, 10% in the chest **(Kehi and Picoli 2009).** On contrast, **Sakia*et al.,* (2006)** indicated colonization in the liver started already 1day post inoculation with 40% of chicken is positive.

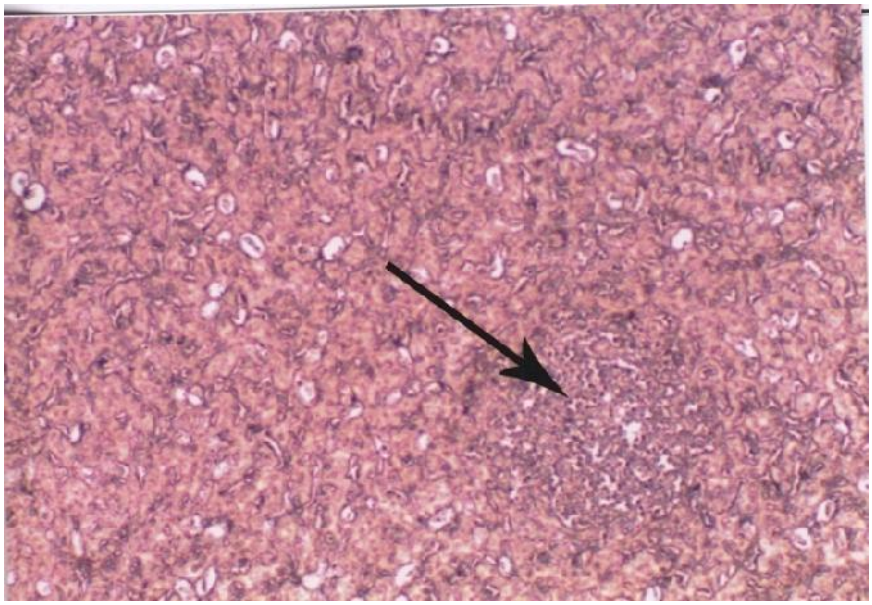
The frequency and duration of intestinal colonization in poultry flocks is influenced by the age, genetic line, immune status of the birds and by the strain and dose of *Salmonella* to which they are exposed. Infection of newly hatched chicks with SE by oral inoculation or horizontal contact exposure can lead to the establishment of intestinal colonization that persists into adulthood **(Gast and Holt, 1998)** when mature laying hens were infected with *Salmonella*, even at very large dose, the resulting frequency of intestinal colonization tends to decline much more steeply over time than in case for young chicks (**Gast*et al.,* 2004)**.Many serological tests can be used for detection of *Salmonella* infection. In this study we used immuonhistochemistry test for detection of *Salmonella* infection in the internal organs (liver and cecum).

After 4 week post inoculation the internal organs as (liver and ceca) were collected in formalin for immunohistochemistry test each organ in separated tube. In this study we found immuomohistochemically labeled bacilli were associated with epithelial surface. Numerous *Salmonella* were adhering singly to the epithelium of the villi as in photo (1) indicated the antigen-antibody reaction that appear in the tissue. Large macrophage like cell in the lamina propia contained numerous *Salmonella* organisms in the cytoplasm granulomatous nodules were present in the submucosa of cecum of bird at 14 day post inculcation. In constant, **(Pop *et al.,* 2009)** indicated a large necrotic area in the ceca and small intestine in newly hatching chicks after 7 days of infection of *Salmonella*.

After oral inoculation of SE in one-day old chicks, the organism speared rapidly from the ceca to the internal organ. The incidence of the invasion decreased rapidly with the age. Interluminal phagocytes of *Salmonella* organism were observed. A few organisms were seen in close associations with the epithelial brush border in the lower ilea lumina. The invaded organism were usually single and, on entering the tissues, were often surrounded by a halo phagocytosis, which wasn't observed in the

epithelium but seen in the lamina propria of the iliocaecal junction. Intratissue *Salmonella* detected in the ceca of 2-week old birds but was not detected in adult birds **(Turnbull and Snoeyenbos, 1973)**.

Photo (2)



Histopathological finding in chicks infected by SE were foci of necrosis and infiltration by heterophils and lymphocyte in the liver parenchyma **(Baskerville *et al.*, 1992)** as in photo (2) lymphocytes aggregation in hepatic cell containing positive staining bacilli

Photo (3)

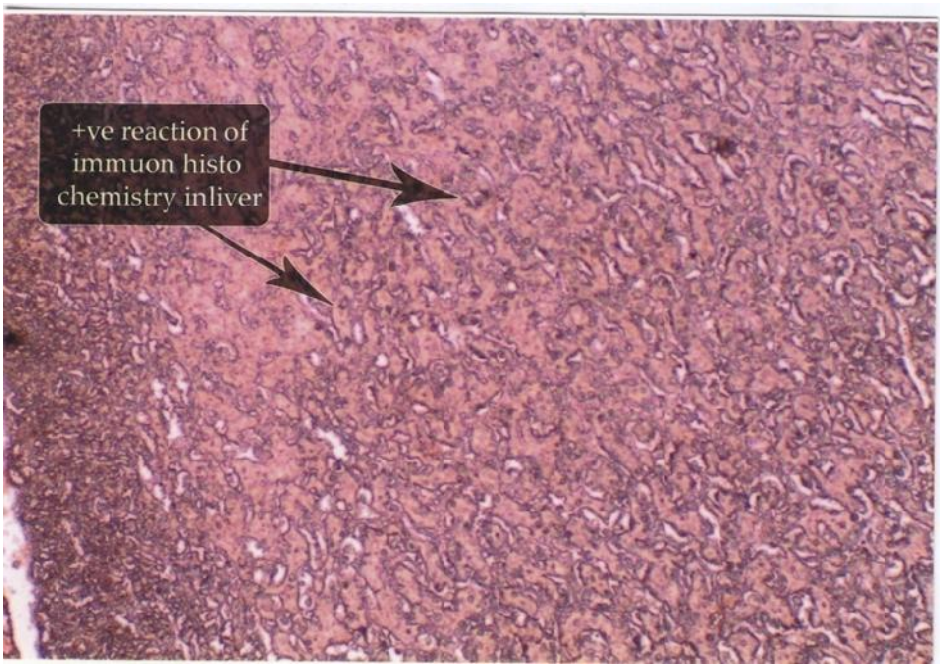


Photo (3). Necrotic area shown in hepatic cell with positive immunohistochemical reaction.

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رد الفعل السيرولوجى ضدالسالمونيلا الضارية فى الطيور المعدية اصطناعيا

أ.د .احمد محمد عمار* ، أ.د. سعاد عبد العزيز عبد الونيس، د. ريم محمد رضا***

* قسم الميكروبيولوجى كلية الطب البيطرى - جامعة القاهرة
** المعمل القومى للرقابة البيطرية على الانتاج الداجنى

الملخص العربى

ستظل الاصابة بميكروب السالمونيلا تمثل نسبة كبيرة فى الاصابة بامراض التسمم الغذائى وتعتبر الدواجن من اهم مصادر انتقال ميكروب السالمونيلا الى الانسان.

فى هذة الدراسة تم استبيان وبائية ميكروب السالمونيلا فى قطعان الدجاج التسمين فى مصر عن طريق اختبار ١٨٠٠ عينة تمثل الطيور المستوردة والطيور المحلية وتم ذللك باستخدام ISO ٦٥٧٩

وتشير النتائج الى تواجد عدوى السالمونيلا بنسبة ١٢.٥٪ فى الطيور المستوردة وكذللك بنسبة ٦٥٪ فى الطيور المحلية

وكانت التصنيف السيرولوجى للمعزولات كالاتى:

سالمونيلا انترتيديس بنسبة ٣٧٪ ، سالمونيلا تيفيميوريم ٢٥٪ ، سالمونيلا ايسن١.٢٨٪ ، سالمونيلا برادفورد ٦.٢٪ ، سالمونيلا لوجوس ٣.١٪. ولقد اجريت تجربة تهدف لتقييم تواجد عدوى السالمونيلا انترتيديس فى قطعان الدجاج التسمين وقدرتها على استعمار الامعاءفى هذة الدراسة تم استخدام ٣٠ كتكوت عمر واحد يوم وتم تقسيمهم الى مجموعتان كالاتى :

المجموعة الاولى تتكون من ٢٠كتكوت عمر واحد يوم تم اصابتها السالمونيلا انتريتديس عن طريق الفم بجرعة قدرها ٠.٢ مللى من ١.٨×١٠^{٧} . والمجموعة الاخرى تتكون من ١٠ كتاكيت متروكة من غير عدوي

ولقد وجد بعد الاسبوع الاول من الحقن علامات الضعف والاسهال المعوى على الكتاكيت المصابة بينما امكن الكشف عن سالمونيلا انترتيديس عند فحص الاعضاء الدخلية مثل الكبد بنسبة ٥٠٪ والامعاء بنسبة ٧٠٪ وكذلك وجد بنسبة ١٠٠٪فى للسبلة.

بعد الاسبوع الرابع من الحقن تم ذبح الكتاكيت المصابة وتم عزل الاعضاء الداخلية مثل الكبد والامعاء وكذللك تم تجميع السيرم لمعرفة الاجسام المناعية . تم قياس الجسام المناعية عن طريق اختبار الاليزا وتم استخدام اختبار Immuonhistochemisty لمعرفة اذا كان هناك اجسام مناعية الانسجة ووجدت بنسبة ٥٠٪ فى الكبد و بنسبة ٧٠٪فى الامعاء.