

STUDIES ON SALMONELLOSIS IN QUAILS

BY

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ABSTRACT

A total number of 225 samples were collected from priveta Quail Farm suffering from high morbidity and mortality rates with profuse and white diarrhoea with enlargement and congestion of all internal organs. Bacteriological examination revealed that isolation of 105 isolates of different Salmonella serotypes, among one day old, 30 days old, layers quails, eggs, ration and litters in an incidence of 50.0%, 50% 30.0%, 40.0%, 40.0% and 80.0% respectively. With an overall incidence of 46.6%. *S.pullorum* was the most predominant serovars isolated with an incidence 25.7%, followed by *S.typhimurium* where 25 strains were isolated with an incidence of 23.8%. On the other hand, *S.gallinarum* and *S.enteritidis* were the least serotypes isolated in an incidence of 16.2% and 9.52% respectively. In the meantime, 26 isolates were untypable serologically in an incidence 24.76%. The highest rate of isolation of salmonella species was from intestinal contents, caeca, liver and ovaries. Experimentally infected quails with both *S.pullorum* and *S.typhimurium* showed high mortality rate reached to 70% in comparing with single infection by either *S.pullorum* or *S.typhimurium* which induced lower mortality rates. The antibiotic sensitivity to the isolated Salmonellae, showed high sensitivity to chloromphenicol and enrofloxacin and highly resistant to erythromycin and so treatment of infected quails gave the best method for treatment of salmonellosis in quails.

INTRODUCTION

In the past, the tradition of quail production has been related to research work, nowadays, quails becomes widely distributed in Egypt as a source of meat and egg production, since their meat and eggs have become highly popular to the consumers, also occupied less space area per bird and consumed less feed with higher metabolic rate.

The main source of salmonella infection in poultry flocks are contaminated food, water, hatchary, and the environments (Smith, 1971).

Birds with high salmonella serovars may represent a secondary source of infection for humans (Sharp et al., 1983). Meanwhile, Murray (1991) investigated that birds acts as a source of salmonella carriage and spread the infection.

Like all creature quails may play a considerable role in the dissemination of many pathogens and their role for the transmission of mycoplasma organisms, *E.coli* and Salmonellosis were still a point of research (Hamouda, 1992a and Manal and Aisha, 1997).

Epidemiological analysis suggested that contaminated eggs or egg products are the major source of the infection (Hansenon et al 1992 and Hedberg et al 1993),

So this study was planned to investigate the role of quails as a reservoir for salmonellae and to throw more light on the different salmonellae serovars isolated, pathogenicity of isolated salmonellae and antibiotic biogram for the isolated serovars a trial for selected the most sensitive drug for treatment of experimentally infected quails

Moreover, experimental infection of quails by the most predominant salmonella isolates were done.

MATERIAL AND METHODS

1- Collection of samples:

A total number of 225 samples were collected from a Private Quail Farm in El-Kaloubia Governorate including 50 samples from quails aging one day old, 50 samples from quails 30 days old and 50 samples from layers. In addition to 25 samples from each of litters, feed stuffs and eggs during the period from October, 1999 up to February, 2000.

2- Bacteriological examination:

Various procedures used for Salmonella isolation were performed according to *Smyser and Snoeyenbos (1969)* and *Siebling et al (1975)*. Outer shell of each egg was disinfected by dipping it in 70% ethanol for 5 minutes and flaming it over a burner until dry. Each egg was then cracked aseptically, the shell and its contents were placed in separate sterile selenite F broth and incubated at 37(C for not more than 18 hrs. A loopful from inoculated incubated pre-enrichment fluid medium was seeded on to XLD and SS agar plates. The inoculated plates were incubated at 37(C for 24-48 hrs and examined for salmonella colonies. Litter and feed stuff samples were examined in the same manner.

Organs collected from diseased or freshly dead quails were dipped into sterile "F" broth directly and incubated for 18 hrs at 37(C. A loopfull was seeded onto the above mentioned media and incubated at 37(C for 24-48 hrs. Suspected colonies were picked up, purified and examined morphologically, culturally and biochemically according to *Cruickshank et al. (1975)* and *Miyamoto et al. (1997)*. Serological salmonella serotyping was done by using specific polyvalent and individual monovalent O and "H" antisera provided in a liquid form Behring Werke, AG, Marburg, Germany. Slide agglutination test was done according to *Kauffmann (1974)*.

Experimental infection: Fifty Quails 30 days old were used for experimental infection. The bird examined bacteriologically and serologically to be sure to be free from salmonellae. The quails were allotted into 5 groups, separately and infected experimentally as shown in table (1) and the infected birds were maintained in isolation to prevent cross-infection the quails were infected orally with the different Salmonella isolates at the dose 0.2 ml of 3×10^5 CFU/ml. according to *Buchholz and Fairbrother (1992)* All quails were kept under observation for 14 days. The survived and freshly dead quails were examined for reisolation of the organism .

Invitro sensitivity test by using disc diffusion for salmonellae were done using the following chemotherapeutic agents obtained from (Bio-Merieux and Oxoid),

namely, Nitrofurantoin (30 g), Gentamicin (10 g), Neomycin (30 g), Chloramphenicol (30 g), Amoxicillin (20g), Streptomycin (10g), Erythromycin (15g), Nalidixic acid (30g), Bacitracin (30g), Cephalothin (30g), Enrofloxacin (10g) and Norfloxacin (10g). the disc diffusion technique of sensitivity to different chemotherapeutic agents (Cruickshank *et al.*, 1975) was adapted.

Table (1): Experimental infection of 30 day old quails with the different Salmonella serotypes previously isolated from quails.

Group*	Dose	Type of inoculated Salmonellae
Group I	0.2 ml of 3×10^5 CFU/ml per os	<i>Salmonella typhimurium</i>
Group II	0.2 ml of 3×10^5 CFU/ml per os	<i>Salmonella pullorum</i>
Group III	0.2 ml of mixed culture 3×10^5 CFU/ml per os	<i>Salmonella typhimurium</i> and <i>Salmonella pullorum</i>
Group IV (infected and treated)	0.2 ml 3×10^5 CFU/ml per os of mixed culture	Infected with <i>S.typhimurium</i> and <i>S.pullorum</i> in a dose 0.2 ml 3×10^5 CFU/ml per os and treated with enrofloxacin 3 days after infection for 3 successive days
Group IV (control group)	Uninoculated and kept as a control	

*A total of 10 birds were used in each group.

RESULTS AND DISCUSSION

Quails played an important role in the dissemination of many microorganisms that have public health significance including salmonella microorganisms. As shown in table (2), one can conclude that the prevalence rates of salmonellosis among one day old, 30 days old, layers quails, eggs, feed stuffs and litters were 50.0%, 50% 30.0%, 40.0%, 40.0% and 80.0% respectively. With an overall incidence of 46.6%. *S.pullorum* was the most predominant serovars isolated with an incidence 25.7%, followed by *S.typhimurium* with an incidence of 23.8%. On the other hand, *S.gallinarum* and *S.enteritidis* were the least serotypes isolated in an incidence of 16.2% and 9.52% respectively. In the meantime, 26 strains were unable to be typed serologically in an incidence 24.76%.

S.typhimurium and *S.enteritidis* recovered in the present work are of public health hazard and can be transmitted to human beings through quails and its environments. Similar observations were recorded by *Socket and Roberts (1991)*. Moreover, *Sawsan (1996)* showed that quails acts as transmitting some zoonotic bacterial infection to man including *S.enteritidis*. Furthermore, *Buchholz and Fairbrother (1992)* and *Miyamoto et al. (1997 and 1998)* noticed that the cloaca and oviduct harboured *S.enteritidis*, in quails and the organism may be ascend through the oviduct to eggs to be contaminated. This might be due to the fact that salmonella infection was initially of endogenous sources or via yolks of quails.

As illustrated in table (3), the most predominant sites of salmonella infection in old quails were the intestinal contents, followed by liver specimens, then ovaries and lastly the caecal samples. On the other hand, among newly hatched one day old quails, the yolk sac was the organ mostly infected with salmonella serovars. These results coincide with that recorded by *Brown et al (1975)*. Also, the same results were obtained by *Hamouda (1992 b)* who isolated *S.pullorum* and *S.gallinarum* from the internal organs of 3 day old Quails. As shown in table (4), most of salmonella serovars isolated in the present work were highly sensitive to chloramphenicol, enrofloxacin, norfloxacin and neomycin. Meanwhile completely resistant to erythromycin. In contrast, moderate in its sensitivity to gentamicin, nitrofurantoin and cephalothin. These results are agree with those recorded by *Malya Mitra et al. (1997)*. There were slight variation in sensitivity of tested salmonella serovars to 12 different antibiotics and chemotherapeutic agents as shown in table (4).

Results of pathogenicity of salmonella serovars for Quails:-

The clinical signs observed among inoculated quails were: depression, dropping of the wings, dullness, sleepy appearance, closed eyes and severe diarrhea in most cases. Quails which died up to 48 hrs post infection showed no clinical symptoms but slight diarrhea may be observed (one Quails from each of group II and group III). Deaths begins to noticed from 72 hours post-infection. The post-mortem findings differed according to the duration of the disease. Quails which died after 72 hrs showed septicaemia, fibrinous pericarditis, perihepatitis, peritonitis, congestion of the liver and spleen, severe congestion and haemorrhages of the intestinal mucosa. Mortality rates reached 60.0%, 50.0% and 70.0% among group infected with *S.typhimurium*, *S.pullorum* and both *S.typhimurium* concurrently mixed with *S.pullorum* respectively. Similar findings were also observed by *Hamouda (1992b)* and *Manal and Aisha (1997)*.

Infected and treated group:

Ten, one month-old quails were inoculated with mixed culture of *S.typhimurium* and *S.pullorum* to concentration of 0.2 ml of 3×10^5 CFU/ml orally and treated with enrofloxacin 72 hrs post infection in the drinking water at a concentration of 100 mg/litre for 3 successive days and resulted in no clinical signs or mortalities among quails and all birds remains apparently healthy.

From the present study, it can be concluded that:-

- The most prevalent salmonellas among quails and their environments were *S.pullorum*, *S.typhimurium*, *S.gallinarum*, *S.enteritidis* beside others untypable salmonellas.
- All ages of quails were susceptible for salmonellas.
- The most predominant site of salmonella infection in quails were yolk sacs, intestinal contents, liver, and ovaries and can be transmitted through egg contents.
- Most local salmonellae serovars isolates proved to be highly sensitive to chloramphenicol, enrofloxacin, Norfloxacin, Neomycin and complete resistant to erythromycin.

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Table (2): Prevalence rate of salmonellae isolated from different examined samples.

Kind of samples	Total number of examined samples	*positive samples for salmonellae		**serotyping of salmonella isolates											
				<i>S.typhimurium</i>		<i>S.pullorum</i>		<i>S.gallinarum</i>		<i>S.enteritidis</i>		Untypable salmonellae			
				No.	%	No.	%	No.	%	No.	%	No.	%		
One day old quails	50	25	50.1	6	24	8	32	4	16	2	8	5	20		
One month old quails	50	25	50	4	16	7	28	5	20	2	8	7	25		
Layer quails	50	15	30	3	20	3	20	3	20	1	6.6	5	33.3		
Eggs	25	10	40	2	20	2	20	2	20	2	20	2	20		
Feed stuffs	25	10	40	4	40	2	20	1	10	1	10	2	20		
Litter	25	20	80	6	30	5	25	2	10	2	10	5	25		
Total	225	105	46.6	25	23.8	27	25.7	17	16.2	10	9.52	26	24.76		

* The percent was calculated according to the total number of samples.

** The percent was calculated according to the total number of positive samples.

Table (3): Sites and distribution of salmonellae recovered from various organs of diseased quails.

Type of Salmonella isolates	Eyes		Heart blood		Liver		Ovaries		Lungs		Intestine contents		Caeca		Yolk sacs		Total			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
One-day old quail																				
<i>S.typhimurium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	30	-	-	6	30
<i>S.pullorum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	50	-	-	8	50
<i>S.gallinarum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	20	-	-	4	20
<i>S.enteritidis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	10	-	-	2	10
One month old																				
<i>S.typhimurium</i>	1	5.6	-	-	1	5.6	-	-	-	-	1	5.6	1	5.6	-	-	-	-	4	22.2
<i>S.pullorum</i>	-	-	1	5.6	1	5.6	1	5.6	1	5.6	2	11.1	1	5.6	1	-	-	-	7	31
<i>S.gallinarum</i>	1	5.6	-	-	-	-	1	5.6	1	5.6	2	11.1	-	-	-	-	-	-	5	27
<i>S.enteritidis</i>	-	-	-	-	1	5.6	-	-	-	-	1	5.6	-	-	-	-	-	-	2	11
Layer-quails																				
<i>S.typhimurium</i>	-	-	-	-	1	10	-	-	-	-	1	10	1	10	-	-	-	-	3	30
<i>S.pullorum</i>	-	-	1	10	-	-	1	10	-	-	-1	-10	-	-	-	-	-	-	3	30
<i>S.gallinarum</i>	1	10	-	-	-	-	1	10	-	-	-	-	1	10	-	-	-	-	3	30
<i>S.enteritidis</i>	-	-	-	-	-	-	-	-	-	-	1	10	-	-	-	-	-	-	1	10
Total	3		2		4		4		2		9		4		20				48	

Table (4): Antibiogram of the isolated Salmonella serovars obtained from quails.

Chemotherapeutic	Concentration/disc**	S. Typhimurum (25)*		S. pullorum (27)*		S. gallinarum (17)*		S. enteritidis (10)*	
		No. sensitive	%	No. sensitive	%	No. sensitive	%	No. sensitive	%
Nitrofuran	30µg	14	56	15	55.6	10	58.8	5	50
Gentamycin	10µg	15	60	16	59.3	11	64.7	6	60
Neomycin	30µg	20	80	25	92.6	16	94.1	4	40
Chloramphenicol	30µg	24	93	25	92.6	16	94.1	8	80
Amoxicillin	20µg	6	24	6	22.2	7	41.2	6	60
Streptomycin	10µg	7	28	6	22.2	8	47.1	7	70
Erythromycin	15µg	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nalidixic acid	30µg	6	24	7	25.9	14	82.4	6	60
Bacitracin	30µg	6	24	6	22.2	8	47.1	5	50
Cephalothin	30µg	15	60	15	55.6	15	88.2	6	60
Enrofloxacin	10µg	23	92	24	88.9	15	88.2	9	90
Norfloxacin	10µg	23	92	24	88.9	15	88.2	9	90

*No between brackets indicates the total strains examined for its sensitivity.

**Concentrations are in micrograms.

Table (5): Results of experimental infection of one month old quails with tested salmonella serovars.

Groups	Total No. of inoculated quails	Time of death in hours or days post-infection							No. survived/total No.	No. dead/total No.	Mortality Rate	
		24 hrs	48 hrs	72 hrs	4	8	10	12				14
Group I (S.typhimurium)	10	-	-	2	1	-	1	1	1	4/10	6/10	60%
Group II (S.pullorum)	10	-	1	1	-	1	1	1	-	5/10	5/10	50%
Group III (both S.typhimurium and S.pullorum)	10	-	1	1	2	1	1	1	-	3/10	7/10	70%
Group IV (infected with S.typhimurium and S.pullorum) and treated	10	-	-	-	-	-	-	-	-	10/10	0/10	0.0%
Group V (control)	10	-	-	-	-	-	-	-	-	10/10	0/10	0.0%

الملخص العربي

دراسات على الإصابة بالسالمونيلا فى السمان

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*قسم البكتريا والفطريات والمناعة-كلية الطب البيطرى بالسادات-جامعة المنوفية

** قسم أمراض الدواجن-كلية الطب البيطرى بمشتهر-جامعة الزقازيق-فرع بنها

تم فحص عدد ٢٢٥ عينة من السمان من مزرعة خاصة بمحافظة القليوبية تعاني من ارتفاع فى معدلات الإصابة والنفوق مع إسهال أبيض وتضخم وأحتقان فى الأعضاء الداخلية. وبالفحص البكتريولوجى تم عزل عدد ١٠٥ عترة من ذرارى السالمونيلا المختلفة وكان معدل العزل بنسبة ٥٠% من كلا من السمان عمر ١ يوم وسن شهر ومن البياض بمعدل ٣٠% و ٤٠% من كل من العليقة والفرشة وبنسبة عامة تصل إلى ٤٦,٦% كما تبين من نتائج التصنيف السيروولوجى لعترات السالمونيلا المعزولة أن السالمونيلا باللورم هى أكثر العترات عزلا وكانت نسبتها ٢٥,٧% يليها السالمونيلا تيفي ميوريم وكان عددها ٢٥ عترة بنسبة ٢٣,٨% وعلى النقيض السالمونيلا جالينيرم والسالمونيلا أنتريتيدس كانت أقل المعزولات بنسبة ١٦,٢، ٩,٥٢% على التوالى، بالإضافة إلى عزل ٢٥ عترة من أصناف السالمونيلا التى لم تتم تصنيفها سيروولوجيا بنسبة ٢٤,٧٦%.

كما أشارت هذه الدراسة أهمية عزل ميكروبات السالمونيلا تيفي ميوريم و والسالمونيلا نتريتيدس لما لها من تأثير على صحة الانسان وذلك من خلال انتقالها عن طريق السمان أو البيئة المحيطة به وقد تبين أن أعلى معدل للعزل لهذه العترات كانت من مكونات الأمعاء والأعور والكبد والمبيض كما وجد أن أعلى معدل للعزل من السمان عمر واحد يوم كان من كيس الصفار وبإجراء العدوى الصناعية لميكروبات السالمونيلا باللورم والسالمونيلا تيفي ميوريم لوحظ أنها تسبب معدل على من النفوق تصل إلى ٧٠% وذلك بالمقارنة بالمجموعات التى تم حقنها بهذه الميكروبات كل على حدة، كما تم إجراء اختبارات الحساسية لعترات السالمونيلا المعزولة لمعظم المضادات الحيوية ومركبات السلفا فوجد أنها شديدة الحساسية لمركبات الكلورامفينيكول والنيومايسين والأنروفلوكساسين ومقاومة لمركب الأرترومايسين، ولذلك فإن العلاج بالأنروفلوكساسين للسمان المعدى بعترات السالمونيلا المعزولة سابقا أدى إلى عدم ظهور أعراض للإصابة أو نفوق خلال ١٤ يوم من العدوى، وكانت أقوى المضادات الحيوية تأثيراً على ميكروبات السالمونيلا فى السمان.