

Bacterial Causes Of Ducks Mortality In Sharika Province

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ABSTRACT

One hundred and thirty five bacterial isolates were recovered from 150 freshly dead and diseased ducks suffering from diarrhea and respiratory disorders (water fowl). *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella*, *Pasteurella multocida*, *Proteus vulgaris* and *Staphylococcus aureus* represented 26%,15.6%, 14%,12.6 %,11% and 10.4 % of the samples respectively . Bacterial isolates were identified morphologically, culturally, biochemically and serologically. *E.coli* strains were serologically identified to 4(O26),9(O78),6 (O86),5 (O125) ,6(O157) and 5 (untyped) , while the recovered *Salmonella* strains were identified to 8 (*S. typhimurium*),3 (*S. enteritidis*)and 6(untyped).

Experimental infection carried out with different bacterial isolates (*E.coli* – *Salmonella-Pseudomonas* and *Staph.*) in 3 – days old ducklings, revealed difference in mortality rate ranging from 60% for *Salmonella*, 50% for *E.coli* and *Pseudomonas* while *Staph* was 40%. The challenged ducks showed clinical picture and necropsy lesions similar to natural infection 24 hours post inoculation. *In vitro*- sensitivity pattern of isolated organisms proved that Ciprofloxacin, Enrofloxacin and Gentamycin were the most effective drugs.

INTRODUCTION

Ducks are hardly and relatively resistant to most diseases, but are susceptible to a number of infectious agents acquired from and common to other avian species. Some of these agents may pose a threat to public health significance as well as bacterial and viral agents (1). Many isolated microorganisms were associated with duck mortality as: *Salmonella*, *E.coli*, *Pseudomonas*, *Pasteurella*, *Proteus*, *Klebsiella* and *Staphylococci* (2)

The researches have focused on enumerating the intestinal load of pathogenic bacteria such as *Klebsiella spp.*, *Staphylococci spp.*, *Salmonellae*, *Escherichia coli* and *Arizonae spp.*, which are the preponderant bacilli in the intestine of ducks (3). Several microbial infections are responsible for the early mortality of duckling and losses of duck industry.

Colbacillosis is the commonest infectious disease of farmed poultry. It is most commonly seen following upper respiratory disease. It is caused by *Escherichia coli* and is seen worldwide in poultry. In ducks typically causes precarditis, prehepatitis and airsacculitis . Colbacillosis is characterized by moist, granular to curd like exudates on thoracic and abdominal viscera and surfaces of the air sacs, livers and

spleen of infected ducks are swollen and dark (4).

Salmonella infections in duck are probably the most important source of *Salmonella*-associated food poisoning in human. Recent information is available on the characteristics and pathogenesis of salmonella infection in commercial ducks (5). *S.gallinarum*, *S.pullorum*, *S.typhimurium*, *S. enteritidis* and *S. anatum* can cause considerable losses in birds of less than a few weeks of age (6).

This work deal with the investigation of bacterial agents causing death in ducks, and the pathogenicity of *E.coli*, *Salmonella*, *Pseudomonas* and *Staph* to experimentally – infected duckling. Moreover, the *in vitro* sensitivity of the isolated organisms to some chemotherapeutic agents was also investigated

MATERIAL AND METHODS

Sources of clinical cases

One hundred and fifty samples were obtained from apparently healthy and freshly dead ducks (1 week- 6 months). The samples were collected from different private farms and flocks of ducks in Sharkia Province. Clinical signs and gross pathological changes were recorded .

Collection of specimens

Samples from liver, lung, spleen, air sacs, kidneys, heart blood and intestine, were collected aseptically and were examined for bacterial isolation. A liver impression and blood smears were stained with Giemsa and Wright's stain for bipolar intercellular bacilli as a guide for the process of isolation, through microscopic examination.

Bacterial isolation

Samples were cultured on Nutrient broth, Rappaport broth, Brain heart infusion broth and Brilliant green bile broth and incubated at 25°C and 37°C for 24 hours. Subculturing was carried out onto nutrient agar, Eosin-methylene blue (EMB) agar, MacConkeys agar, Brilliant green bile agar, Xylose-Lysine-deoxycholate agar (XLD), Mannitol salt agar, and 10% sheep blood agar. The inoculated plates were incubated at 37°C for 24 hours. The growing colonies on various plates were examined morphologically, Gram staining and biochemical testes as described by (7).

Serological identification

A-Antisera of *E.coli* were used for serological identification of somatic antigen "O" using slide agglutination test (8).

B-Serological identification of the isolated strains of *Salmonella* was performed (8) using slide agglutination for identification of somatic antigen while flagellar antigen was identified by tube agglutination test.

Pathogenicity test in ducklings

fifty-five healthy duckling of one-day old white pekin duckling obtained from local private farm in Fakous-Sharkia governorate were used for testing the pathogenicity of recovered *E.coli*, *Salmonella*, *Pseudomonas* and *Staph* from naturally infected birds. A random sample of five ducklings were slaughtered and exposed to postmortem and bacteriological examination,

which revealed that they were in a healthy status and free from diseases. The ducklings were divided into 5 experimental groups each contained 10 ducklings. At age of 3- days, groups were inoculated subcutaneously with 0.2 ml suspension of 2×10^7 CFU/ml of salmonella, *E.coli*, *Pseudomonas* and *Staph* respectively. Ten ducklings were kept in parallel as uninfected controls. All inoculated duckling was kept under observation for 7 days. Dead and very sick ducklings were subjected for clinical examination, postmortem lesions and bacteriological re-isolation.

In vitro antibiotic sensitivity test

The disc diffusion technique was performed using Muller – Hinton medium (BioMerieux, france) on isolated bacteria (7). Available commercial antibiotic discs were obtained from Oxoid Lab. and Lincospectin was obtained from Pfizer Company.

RESULTS AND DISCUSSION

Clinical signs of examined ducks showed depression, off food, ruffled feathers, diarrhea, omphalitis and respiratory symptoms (coughing, sneezing and watery nasal discharge). The P.M examination revealed the presence of general congestion of internal organs (liver, spleen, intestine and enlarged gall bladder), focal necrosis of the liver, subcutaneous haemorrhages, enlargement of the spleen, caecal cores, congested lung with frothy exudates, catarrhal and necrotic enteritis, and swelling of the kidneys. Similar symptoms and P.M lesions were reported in several previous studies (9-11).

The results of bacteriological isolation (from 150 ducks) is shown in Table 1. The highest percentage of bacterial isolation recorded was *E. coli* (26%) followed by *Pseudomonas aeruginosa* (15.6%), *Klebsiella pneumonia* (14%), *Salmonella* spp. (12.6 %), *Pasteurella multocida* (11%), and 10.4% for both *Proteus vulgaris* and *Staphylococcus aureus*.

1. Incidence of different bacterial pathogens isolated from examined samples.

Bacterial isolates	Number of positive	Percentage %
<i>E.coli</i>	35	26
<i>Salmonella spp.</i>	17	12.6
<i>Proteus vulgaris</i>	14	10.4
<i>Pseudomonas aeruginosa</i>	21	15.6
<i>Pasterella mutocida</i>	15	11
<i>Klebsiella pneumonia</i>	19	14
<i>Staphylococcus aureus</i>	14	10.4
Total	135	100.0

High rate of isolates recovery was detected from heart blood followed by lungs, liver, intestine and kidney. Nearly similar results were recorded (10) where *Pseudomonas aernoginosa* (24.3%) *E. coli* (17.4%), *Salmonella spp.* (13.9%), *Staph. aureus* (13.9%), *Klebsiella spp* (11.1%) and *Proteus spp.* (9.02%), were isolated from ducklings at age of 1-21 days, in Dakahlia Governorate. Also, *E. coli* was isolated (16.2%) from duck suffering from diarrhoea (12). Meanwhile *E. coli* (42.5%), *Klebsiella pneumonia* (18.3%), *Pseudomonas aeruginosa* (15.8%), *Strep.feacalis* (9.2%), *Staphylococcus aureus* (6.7%) and *Salmonella* 3.3%, were isolated from 120 cases of duklings suffering from enteritis at the age of 1- 30 days (11). Also the highest rate of isolation was from liver,

intestine and then followed by faecal swabs. While *E. coli* (4%) was isolated with a low percentage from liver of dead duck (13). Moreover, *Pseudomonas aeruginosa* was isolated either alone or with *Staph. aureus* and *E. coli* from different ages of ducklings and chickens(14).

Serological identification of *E.coli* isolates

Thirty- five strains of *E.coli* belonged to five serogroups as 4 (11. 4%) O₂₆, 9 (26%) O₇₈, 6 (17%) O₈₆, 5 (14.3%) O₁₂₅, and 6 (17%) O₁₅₇ and 5 strains were untyped. A total of 17 isolates of *Salmonella* strains were serotyped as 8 (47%) *S. typhimurium*, 3 (17.7%) *S. entritidis* and 6 (35.3%) untyped as illustrated in Table 2.

Table 2. Serological identification of *E.coli* and *Salmonella* strains

<i>E.coli</i>			<i>Salmonella</i>		
Serogroup	No	%	Serogroup	No	%
O ₂₆	4	11.4	<i>S. typhimurium</i>	8	47
O ₇₈	9	26	<i>S. entritidis.</i>	3	17.7
O ₈₆	6	17	Untypable	6	35.3
O ₁₂₅	5	14.3			
O ₁₅₇	6	17			
Untypable	5	14.3			
Total	35	100		17	100

Similar results were previously obtained (10, 11) in stereotyping of *E.coli* isolates that belonged to serotypes O₂₆, O₇₈, O₈₆, O₁₂₅ and O₁₅₇. On the other hand the results obtained were different in *Salmonella* serotyping

percentage from our results (9-11, 15-17), this may be due to different in age of isolation and locality as illustrated in Table 3.

The extensive use of antibiotics as growth promoters and prophylactic agents for disease control in veterinary medicine has undoubtedly been responsible for large numbers of bacteria that have become resistant to different antibiotics.

Table 3. Results of salmonella stereotyping by some authors

Salmonella serotype	%	Reference	locality	age
<i>S.typhimurium</i>	41	<i>Bahgat et al(10)''</i>	Assiut	one month,
	61	<i>Sinko (16)</i>	Slovakia	-
	26	<i>Hegazy (17)</i>	Alexandria	-
	60	<i>Hatab et al (11)</i>	Dakahila	1-21 days
	50	<i>Asawy and Abd El- Latif (12)</i>	Dakahila	1-30 days
	29.8	<i>Ibrahim (18)</i>	Sharkia	1 w-2 mon
<i>S. enteritidis</i>	12.5	<i>Bahgat et al(10)</i>	Assiut	30 days
	15	<i>Sinko (16)</i>	Slovakia	-
	21	<i>Hegazy (17)</i>	Alexandria	-
	25	<i>Asawy and AbdEl-Latif (12),,</i>	Dakahila	1-30 days
	43.6	<i>Ibrahim(18)</i>	Sharkia	1 w-2 mon
<i>S. anatum</i>	25	<i>Bahgat et al(10),</i>	Assiut	30 days
	22	<i>Sinko (16)</i>	Slovakia	-
<i>S.gallinarum,</i>	8.3	<i>Bahgat et al(10),</i>	Assiut	30 days
	10	<i>Sinko (16)</i>	Slovakia	-
	26.6	<i>Ibrahim(18)</i>	Sharkia	1 w-2 mon

In-vitro testing of the proper antimicrobial agents against pathogenic microorganism is illustrated in Table 4. Most of these isolates were highly sensitive to Ciprofloxacin, Enrofloxacin, and Gentamycin. While variable results were recorded with the remaining antibiotics. The present results corresponded with the reported observation (5,10, 11). Contrary to these results, isolated strains of *Salmonella* were sensitive to tetracycline and trimethoprim (18).

Results of experimental infection of the susceptible ducks with different bacterial isolates via subcutaneous route to 3-day old ducklings, are illustrated in Table 5. The results revealed that 60% mortality in ducklings inoculated with *Salmonella* spp. followed by *E coli* and *Pseudomonas* with mortality rate

reached 50% then *Staphylococcus* where mortality rate reached 40% after 24hours of inoculation. Re-isolation trials from internal organs of dead and scarified birds were positive, while, no mortalities or re-isolation were recorded in control group.

The clinical observations of inoculated ducklings after 24h post inoculation were depression, ruffled feather, loss of appetite, profuse diarrhea, huddling together, increased thirst, sleepy appearance, gasping ,discharge from the eyes ,staggering gait and inability to stand . The gross lesions were severe congestion of the internal organs, enteritis, turbidity of air sacs ,distension of the gall bladder and nephritis. These results similar to several previous studies (5,16,17 ,19) .

Table 4. *In vitro* susceptibility of isolated bacterial recovered from examined samples

Antibiotic disc and their potency	<i>E.coli</i>		<i>Salmonella</i>		<i>Pseudomonas aeruginosa</i>		<i>Pasterella mutocida</i>		<i>Proteus vulgaris</i>		<i>Klebsiella pneumonia</i>		<i>Staph. aureus</i>	
	S*. isolate	%	S*. isolate	%	S*. isolate	%	S*. isolate	%	S*. isolate	%	S*. isolate	%	S*. isolate	%
Ciprofloxaci (10 µg)	28	80	17	100	18	85	15	100	12	92	14	75	14	100
Enrofloxacin (5µg)	35	100	17	100	19	90	15	100	12	92	17	87	14	100
Gentamycin (10 µg)	28	80	15	90	13	60	12	80	10	75	16	85	14	100
Amoxycillin (10 µg)	10	28	-	0.0	4	20	9	60	3	25	-	0.0	10	71
Pencillin (10 µg)	-	0.0	-	0.0	4	20	12	80	3	25	-	0.0	12	85
Trimethopri (25 µg)	28	80	-	0.0	-	0.0	10	70	-	0.0	-	0.0	-	0.0
Lincospectin	28	80	13	75	8	37	12	80	5	37	11	62	-	0.0
Neomycin (30 µg)	22	65	15	90	4	20	-	0.0	2	20	-	0.0	-	0.0
Oxytetracycl (30 µg)	10	30	4	25	-	0.0	12	80	3	25	14	75	7	50
Flumoquine (10 µg)	33	96	15	90	5	22	6	40	3	25	8	43	-	0.0
Chloromephi(30µg)	26	75	16	95	-	0.0	4	20	-	0.0	-	0.0	-	0.0
Erythromycci (15 µg)	-	0.0	-	0.0	9	45	10	70	6	45	9	50	7	50

* Sensitive

Table 5. Mortality associated with 0.2 ml 2×10^7 CFU/ml of different bacterial agents inoculated via s/c in 3-day old white pekin ducklings

Isolates	<i>E.coli</i>		<i>Salmonella</i>		<i>Pseudomonas</i>		<i>Staph.</i>		<i>Reisolation trials</i>	<i>Control</i>	
	Dead	%	Dead	%	Dead	%	Dead	%		Dead	%
1	2	20	3	0	2	20	1	10	+	0	0
2	2	20	2	0	2	20	0	0	+	0	0
3	1	10	0	0	1	0	2	20	+	0	0
4	0	0	1	0	0	0	0	0	+	0	0
5	0	0	0		0		1	10	+	0	0
6	0	0	0		0		0	0	-	0	0
7	0	0	0		0		0	0	-	0	0
Cumulative	5/10	50	6/10	60	5/10	50	4/10	40		0/10	0

Dead: Dead number.

PI: Post inoculation.

The results of mortality in case of *Salmonella spp* inoculations resembled to some extent with that previously observed (19) where death occurred within 24 h from the onset of symptoms, mortality rates were 65% for orally infected and 80% for s/c route 5×10^7 CFU to

one day old ducklings. On the other hand, one-day- old ducklings administered orally with 10^8 CFU, recorded mortality rate reached to 10% (16), these differences returned to difference in the route of inoculation. Meanwhile, it has been recorded that mortality reached 20% when one-day- old ducklings

were inoculated subcutaneously with 10^4 CFU of *Salmonella spp*, these differences is due to difference in the dose of inoculation (5).

Mortality reached to 50-60% from *E. coli* inoculation (16). On the other, hand the mortality recorded were 80% and 94% respectively ,when inoculated one - day old duckling with 10^9 CFU intera-tracheally (21, 22). This higher mortality could be attributed to the age of infection and difference in the dose and route of inoculation.

In the current study, the experimental infection with *Pseudomonas aeruginosa* showed 50% mortality. These results disagree with he work which recorded high mortality that reached 80% when inoculated one- day old ducklings intramuscularly (15). These differences could be due to the younger age of infection and difference in the route of inoculation.

In conclusion, the information given by the achieved results revealed that several microorganisms are incriminated in the problems of duck farms in Sharkia Governorate, their sensitivity for some antibiotics and pathogenicity of some of these isolates to 3-day old ducklings were studied.

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الملخص العربي

دراسة بكتريولوجية عن مسببات النفوق في البط

مها عوض الله السيد

المعمل التشخيصى لأمراض الطيور بمستشفى كلية الطب البيطرى التعليمى- جامعة الزقازيق.

اجريت هذه الدراسة لتبين مدى انتشار بعض الأمراض البكتيرية التى تصيب البط وقد تم جمع ١٥٠ عينة من البط الذى يعانى من أعراض تنفسية أو اسهالات أو حديث النفوق فى أعمار تتراوح ما بين أسبوع وستة أشهر من عدة مزارع خاصة فى محافظة الشرقية. تم فحص العينات أكلينيكية وتشريحيا وكذلك تم الفحص البكتريولوجى وذلك بزرعها على أوساط مختلفة للبكتريا وقد امكن عزل ١٣٥ عترة بكتيرية من ١٩٨ عضو داخلى. تم تصنيفها بالطرق المورفولوجية والبيوكيميائية الى ٣٥ (٢٦%) الاشيريشيا كولاى , ٢١ (١٧.٠٦%) سيدوموناس اريجينوزا , ١٩ (١٤%) كليبيلا , ١٧ (١٢.٠٦%) السالمونيلا , ١٥ (١١%) باستيريلا ملتوسيدا , ١٤ (١٠.٤%) لكل من البروتيس والميكروب السبى . تم تصنيف معزولات الاشيريشيا كولاى سيروولوجيا الى ٤ عترات (O26) و ٩ (O78) و ٦ (O86) و ٥ (O125) و ٦ (O157) و ٥ (untypable) . كذلك تم تصنيف عترات السالمونيلا سيروولوجيا الى ٨ عترات سالمونيلا تيغيميريوم ٣ عترات سالمونيلا انتيتريديس و ٦ عترات (untypable) .

تم إجراء العدوى التجريبية فى بط عمر ٣ أيام عن طريق الحقن تحت الجلد بميكروبات الاشيريشيا كولاى و السالمونيلا و سيدوموناس اريجينوزا والميكروب السبى وقد تم ملاحظة الأعراض الإكلينيكية والصفة التشريحية التى تشبه إلى حد كبير تلك التى لوحظت فى العدوى الطبيعية بعد ٢٤ ساعة من العدوى سجلت السالمونيلا اعلى نسبة نفوق حيث وصلت الى ٦٠% ويليها ٥٠% لكل من الاشيريشيا كولاى و سيدوموناس اريجينوزا و ٤٠% للميكروب السبى. وبإجراء اختبار الحساسية للمضادات الحيوية المختلفة اتضح أن الانروفلوكساسين والسيبروفلوكساسين والجنتاميسين كانت أكثر المضادات الحيوية تأثيرا على معظم المعزولات .