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BACTERIOLOGICAL STUDY ON SOME CAUSES OF EARLY MORTALITY OF DUCKLINGS IN DAKAHLIA GOVERNORATE

(With 3 Tables)

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دراسة بكتريولوجية عن بعض مسببات النفوق المبكر فى البط فى محافظة الدقهلية

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أجريت هذه الدراسة لمعرفة أسباب النفوق المبكر فى البط. تم جمع ١٠٠ عينة من البط عمر (١-٢١ يوم) وأنواع مختلفة من عدة مزارع خاصة والعينات التى تصل إلى المعمل الفرعى بالدقهلية وكانت كلها تعانى من النفوق المبكر. تم فحص هذه العينات بكتريولوجيا وذلك بزرعها على أوساط مختلفة للبكتريا وقد أمكن عزل ١٤٤ عترة بكتيرية تم تصنيفها بالطرق المورفولوجية والبيوكيميائية إلى ٣٥ (٢٤,٣٠%) سيدوموناس أريجينوزا ، ٢٥ (١٧,٣٧%) إيشيريشيا كولاى، ٢٠ (١٣,٨٩%) لكل من السالمونيلا والإستافيلوكوكس أوريس، ١٦ (١١,١١%) كليبسيلانيمونى ، ١٥(١٠,٤٢%) رايمريلاأناتيبتفر وبروتيس فالجاريس ١٣ (٩,٠٢%). تم تصنيف معزولات الإيشيريشيا كولاى سيريولوجيا إلى ٧ عترات ($O_{78}K_{80}$(B-)) و٦ ($O_{86}K_{61}$(B7)) و٥ ($O_{125}K_{70}$(B15)) و٧ (untypable). كذلك تم تصنيف عترات السالمونيلا سيريولوجيا إلى ١٢ عترة سالمونيلا تيفيميوريم و٨ عترات untypable. وقد أسفرت نتائج إختبارات حساسية المضادات الحيوية عن أن السيبروفلوكساسين والإنروفلوكساسين والجنتاميسين كانت أكثر المضادات الحيوية تأثيرا على معظم المعزولات.

SUMMARY

This study was conducted toward a problem of extend early mortality of ducklings. A total of 100 freshely dead and clinically sick ducklings of different ages (1-21 days) and types obtained from private farms at Dakahlia Governorate and also cases which arrive to the Mansoura lab.

The samples were dispatched to the laboratory to be examined bacteriologically for detection of the actual bacterial causes of early mortality problem in these farms. The obtained results pointed out that a total of 144 isolates were isolated. All the bacterial isolates were identified morphologically, culturally, biochemically and serologically for E. coli and Salmonella microorganisms. Pseudomonas aeruginosa was the most prevalent bacterial agent 35 (24.30%) followed by E. coli 25 (17.37) , Salmonella and Staphylococcus aureus 20 (13.89%) for each, Klebsiella pneumoniae 16 (11.11%), Riemerella anatipestifer 15 (10.42%) and Proteus vulgaris 13 (9.02%). The isolated E. coli were identified serologically into 7($O_{78}K_{80}$(B-), 6($O_{86}K_{61}$(B7), 5($O_{125}K_{70}$(B15) and 7 (untypable), while the recovered Salmonella strains were identified into 12 Salmonella typhimurium and 8 (untypable). In-vitro-sensitivity pattern of isolated strains proved that ciprofloxacin, enrofloxacin, gentamycin were the most effective drugs.

Key words: *Mortality, ducklings.*

INTRODUCTION

Nowadays, a great attention was payed toward ducks farming as a trial to fulfill excessive demand of the increased population from the animal protein. The ducks meat are considered to be of high protein content with high biological value.

Several microbial infections are responsible for the early mortality of ducklings and losses of duck industry. Many isolated microorganisms were associated with duck mortality as: Salmonella, E.coli, Pseudomonas, Pasteurella, Proteus, Klebsiella and Staphylococci (Bhowmik & Ray, 1987; EL-Gharib *et al.*, 1993 and Istania, 1993). Antibiotics are used therapeutically and for prophylaxis in intensive domestic birds farming. However of bacteria resistant to antibiotics emerge even under control use of antibiotics (Helm *et al.* 1999). This study was done to investigate the possible bacterial causes of the problem.

MATERIALS and METHODS

A- Samples

A total of 100 freshly dead and clinical sick ducklings of different ages (1-21 days) and breeds were obtained from private farms and also cases which arrive Mansoura Provincial Laboratory. The

samples were dispatched to the laboratory without delay to be examined bacteriologically for isolation and identification of microorganisms agents.

B- Clinical and PM examination:

Ailing ducklings were examined clinically, then sacrified and immersed in a disinfectant before being autopsied. Gross pathological changes were recorded, summarized and presented with results for both freshely dead & clinically sick ducklings.

C- Media:

- Nutrient agar media (Oxoid CM3).
- Blood agar media (Nutrient agar base Oxoide CM3 + 5-10% defibrinated sheep blood).
- Eosin methylene blue (Oxoid CM69).
- MacConkey agar (Oxoid CM7).
- Rappaport vassiliadis broth (Oxoid CM 669).
- Xylose lysine desoxycholate agar (Oxoid CM 469).

D- Serological identification of Salmonella and E. coli:

Samonella and E. coli isolates were identifed according to (Edward and E wing, 1972).

E- Bacteriological examination:

Bacteriological samples were collected aseptically from the deep tissue of liver, lung, bone marrow, heart blood and brain. Each specimen was divided into 3 portions under aseptic condition. The first part was streaked onto predried surface of Blood agar, Nutrient agar, MacConkey agar (Oxoid, CM7) and Eosin methylene blue (EMB) (Oxoid, CM69), incubated aerobically at 37°C for 24 hours. The second part was inoculated into Rappaport Vassiliadis broth (RV) (Oxoid, CM 669) incubated at 42°C, after 24 hours incubation, loopfulls from R.V. enrichement were streaked onto Xylose lysine desoxycholate agar plate (XLD) (Oxoid, CM 469) with incubation at 37°C for 24 hours. The third part was streaked over blood agar plate containing 0.05% yeast extract, 5% newborn calf serum and 5 mg/100ml gentamycine, incubated in a candle Jar at 37°C for 24-48 hours.

The growing colonies on various plates were examined morphologically and biochemically as described by Baily & Scott, (1974); Cruickshank *et al.* (1975) and Carter (1984).

The identified E. coli strains were tested for enterotoxin production through grown the E. coli isolate in trypticase soya broth at 37°C in stationary culture overnight. Culture was centrifuged at 4000 rev/min. for 20 minutes. The supernatant was tested using commercially

VET-RPLA kits (reversed passive latex agglutination) from Oxoid (TD 0920 A) following the manufacturer's direction.

The biochemically identified E. coli and Salmonella isolates were subjected for serological identification using avaliabe E. coli test agglutinating sera (BioMerieux, 1986) and diagnostic Salmonella agglutinating antisera (Denka Selken Co. LTD, Tokyo, Japan) according to manufacturer's instruction.

In vitro antibiotic sensitivity test:

The disc diffusion technique was performed using Muller-Hinton medium (BioMerieux, France) on isolated bacteria from examined samples according to National Committee for Clinical Laboratory Standards (1984) and Quinn *et al* (1994).

RESULTS

The clinical signs and PM lesions: The recorded clinical signs were diarrhea, ataxia, tremor of head and neck, coma, affected ducklings lie on their back, paddling their legs, septicemia. The P.M. examination of duckling revealed the presence of congestion in the internal organs (liver, spleen, intestine) and enlarged gall bladder.

Bacteriological examination:

The results of bacteriological examination were recorded in Tables 1, 2 and 3.

DISCUSSION

Various clinical signs were recorded, diarrhea, ataxia, tremor of head & neck, coma, affected ducklings lie on their backs, paddling their legs, septicemia. Bacterial infection of the examined ducklings is one of the main causes of early mortality and the present study deals with the pathogenic bacteria responsible for early mortality of ducklings. Pseudomonas aeruginosa, E.coli, salmonella, Riemerella anatipestifer, Kilebsiella pneumoniae, Proteus vulgaris and Staphylococcus aureus were isolated in percentage of 24.3, 17.37, 13.89, 10.42, 11.11, 9.02 and 13.89 respectively. Similar findings were investigated by (El-Gharib *et al*. 1993 and Istania, 1993).

Pseudomonas aeruginosa was the most prevalent bacterial agent 35 (24.3%). Pseudomonas infection of birds are of great important because epidemics may spread rapidly through flocks and is considered as one of the most dangerous disease, it causes morbidity and mortality

and the clinical signs including septicemia, diarrhea and respiratory signs (Soumaya, 1992 and Tanois & Samia, 1999).

Collibacillosis is a common systemic disease of world wide economic importance in birds, the major clinical signs of E.coli infection in young birds, acute septicemia that can cause sudden death (Leithnes and Heller, 1992).

In this study, all the isolated 25 (17.37%) E. coli strains from examined duckling samples were enterotoxigenic produce heat labile enterotoxin when tested by VET-RPLA kits, and serologically identified as 28% E. coli O_{78} K_{80} (B-), 24% E. coli O_{86} K_{61} (B_7), 20% E. coli O_{125} K_{70} (B_{15}) and 28% untypable strains (Table 2).

The results in Table (1) pointed out that Salmonella species were isolated from 20 (13.89%). This finding as nearly agree with the observations of El-Gharib *et al.* (1993). and Scott *et al.* (1984). The isolated Salmonella strains were serotyped as 12 (60%) Salmonella typhimurium and 8 (40%) untypable strains (Table2). Salmonella typhimurium had been reported as the most common species isolated from the examined ducks (50%) (Scott *et al.* 1984).

Generally, Riemerella anatipestifer is a Gram-negative, non-motile non sporforming rod, oxidase and catalase positive, not grow on MacConkey, negative indol and not hemolysis blood agar. R. anatipestifer is a contagious disease of domestic duck, it is known as duck septicemia, characterized by fibrinous pericarditis, perihepatitis, air saculitis, caseous salpingitis and meningitis (Sandhu, 1986). Results in Table (1) showed that Riemerella anatipestifer were isolated in 15 (10.42%), our results nearly similar with Ziedler *et al.* (1984), reported high mortality in the fattening flocks of ducks occurred due to Pasteurella anatipestifer outbreaks. Regarding the isolation of Klebsiella pneumoniae, Proteus vulgaris and Staphylococcus aureus,they were isolated from the examined duckling samples at the incidence rate 11.11, 9.02 and 13.89% respectively. This findings similar to El-Gharib *et al.* (1993) and Istania, (1993). The high mortality rate in duckling might be attributed to septicemic shock to the toxic effect associated with lipopolysaccharide fraction of the Proteus organisms (Wilison & Miles, 1975).

In vitro, the susceptibility distribution of each isolated pathogen to different antibiotics is presented in Table (3). The typical pattern of highly effective compounds were observed for zones of ciprofloxacin, enrofloxacin and gentamycin. These findings corresponded with those

reported by (Khodary & El-Sayed, 1997; Turbahn *et al.*, 1997 and Rahman *et al.*, 1999).

In conclusion, the information given by the achieved results revealed that several microorganisms were incriminated in the early mortality of the ducks and therefore strict hygienic measurements should be applied on the egg laying, hatcheries, and good management during the production.

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Table 1: The incidence of different bacterial isolates recovered from the examined samples.

Microorganisms	Age of ducklings / day						Total isolates	
	1-7		8-15		16-21		No.	%
	No.	%	No.	%	No.	%		
Pseudomonas aeruginosa	20	13.89	10	6.94	5	3.47	35	24.30
E. Coli	12	8.33	8	5.56	5	3.47	25	17.37
Salmonella spp.	10	6.94	8	5.56	2	1.39	20	13.89
Riemerella anatipestifer	3	2.08	4	2.78	8	5.56	15	10.42
Klebsiella Pneumoniae	6	4.16	6	4.16	4	2.78	16	11.11
Proteus vulgaris	7	4.86	3	2.08	3	2.08	13	9.02
Staphylococcus aureus	4	2.78	6	4.16	10	6.94	20	13.89
Total	62		45		37		144	100.00

* The percentage was calculated according to the total isolates (144)

Table 2: Serological identification of isolated E. coli and Salmonella strains .

E.coli (*25 strains)			Salmonella (20 strains)		
Serotype	No.	%	Serotype	No.	%
E.coli : O_{78} K_{80} (B-)	7	28	Salmonella typhimurium	12	60
E.coli : O_{86} K_{61} (B_7)	6	24	Untypable	8	40
E.coli: O_{125} K_{70} (B_{15})	5	20			
Untypable	7	28			

* All strains are LT toxin

Table 3: The in-Vitro susceptability of isolated bacteria recovered from examined samples

Antibiotic disc and their potency	E.coli (25)		Salmonella (20)		R.anatipestifer (15)		Proteus vulgaris (13)		K. pneumoniae (16)		Ps.aeruginosa (35)		Staph. aureus (20)	
	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent	Sensitive isolates	Activity percent
Ciprofloxacin 5 ug	22	80.00	20	100.00	15	100.00	12	92.3	12	75.00	30	85.71	20	100.00
Enrofloxacin 5 ug	25	100.00	20	100.00	15	100.00	12	92.3	14	87.5	32	91.43	20	100.00
Gentamycin 10 ug	22	80.00	18	90.00	12	80.00	10	76.92	14	87.5	20	57.14	20	100.00
Erythromycin 15 ug	-	0.0	-	0.0	12	80.00	-	0.0	8	50.00	16	45.71	10	50.00
Penicillin 10 ug	-	0.0	-	0.0	15	100.00	4	30.77	-	0.0	8	22.85	8	40.00
Ampicillin 10 ug	-	0.0	-	0.0	14	93.33	4	30.77	-	0.0	8	22.85	10	50.00
Flumquine 30 ug	24	96.00	18	90.00	12	80.00	2	15.38	7	43.75	8	22.85	-	0.0
Chloramephincol 30 ug	18	72.00	18	90.00	12	80.00	4	30.77	-	0.0	-	0.0	-	0.0
Oxyteteracyclin 30 ug	8	32.00	5	25.00	7	46.66	-	0.0	12	75.00	8	22.85	8	40.00
Lincospectin	20	80.00	15	75.00	12	80.00	4	30.77	10	62.5	15	37.14	-	0.0
Trimethoprim + sulphamethoxazol 1.25 ug + 23.75 ug	20	80.00	-	0.0	12	80.00	-	0.0	-	0.0	-	0.0	-	0.0