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SOME BACTERIOLOGICAL AND SEROLOGICAL STUDIES ON ENTERITIS IN DUCKS

(With 4 Tables)

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بعض الدراسات البكتريولوجية والسيرولوجية على النزلات المعوية في البط

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أجريت هذه الدراسة على عدد 120 عينه تم تجميعها من بط ميت حديثا (40 عينه من الكبد و40 عينه من الأمعاء) بالاضافه إلى 40 مسحه شرحية من البط المريض وكانت أعمار هم تتراوح من يوم إلى شهر وتم تجميع العينات من مزارع بط بمحافظة الدقهليه. بفحص العينات بكتيريولوجيا أمكن عزل 120 عتره بكتيريه تم تصنيفها بالطرق المورفولوجيه والبيوكيميائيه الى 51 (5و42%) ايشيريشياكولاى ، 22 (3و18%) كليبسيلا ، 19 (15,8%) سيدوموناس ايروجينوزا ، 11 (2و9%) الميكروب السبحى ، 8 (7و6%) الميكروب العنقودى الذهبى ، و3(2و4%) الميكروب السبحى الارو6%) سالمونيلا. وبإجراء الاختبارات السيرولوجية اتضح إنه يوجد 5 أنواع من الميكروب القولوني و21 نوع لم يتم التعرف عليها و3 أنواع من السالمونيلا (سالمونيلا تيفيميوريم ؛ سالمونيلا دربى ؛ وسالمونيلا انتيتريدس). وبإجراء اختبار الحساسية للمضادات الحيوية المختلفة اتضح أن الإنروفلوكساسين والجنتاميسين هما الأقوى حساسية مع جميع المعزولات. وجميع عترات الميكروب القولونى المعزولة من عينات البط تعطى سموم ممرضه.

SUMMARY

A total of 120 samples from freshly dead ducks and 40 feacal samples from clinically sick ducks of different ages (1-30days) obtained from a private farms at the Dakahlia Governorate. The samples were dispatched to the laboratory to be examined bacteriologically for detection of actual bacterial causes of enteritis in ducks. All bacterial isolates were identified morphologically, culturally, biochemically and serologically for *E.coli* and *Salmonella* microorganisms. *E.coli* was the most prevalent bacterial agent 51(42.5%) followed by *Klebsiella* spp. 22 (18.3%),

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Pseudomonas aeruginosa 19(15.8%), *Strept. faecalis* 11 (.9.2%), *Staph.aureus* 8(6.7%), *B-haemolytic streptococci* 5(4.2%) and *Salmonella spp.* 4(3.3%). Serological identification of the isolated strains of *E.coli* from diarrhoeic duck showed that they belonged to 5 different "O" serogroups which were 26, 78, 86, 125, 157 and 21 strains of *E.coli* were serological untyped. Meanwhile, serotyping of the isolated strains of *Salmonellae* yielded 3 different serovars including *S.typhimurium, S.derby* and *S.enteritidis*. The antibiogram study done on all the isolated pathogens revealed that all strains were sensitive to Enerofloxacin and Gentamycin. All the isolated *E.coli* strains from the examined samples were enterotoxigenic produce heat labile enterotoxin.

Key words: Ducks, enteritis, bacteriological examination, antibiogram.

INTRODUCTION

Duck are however 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 viral and bacterial agents (Huchzermeyer, 1997). The researches have focused on enumerating the intestinal load of pathogenic bacteria such as *Klebsiella spp., Staphylococci* spp., *Salmonellae, Escherichia coli* and *Arizonae spp. Salmonellae spp.* and *Escherichia coli* are preponderant bacilli in the intestine of ducks (Cao *et al.,* 2008). Infected ducks are listless, dehydrated, exhibit diarrhoea and show signs of incoordination and high mortality rate. The diseases condition are responsible for great economic losses in the poultry industry in many parts of the world (Brans and gross, 1997).

Like with, little information is available concerning the antibacterial resistance of common intestinal bacteria such as *E-coli* which isolated. Moreover, the manufacturing contaminated food, promotes recycle of *Salmonellae* into the meat production chain at farm level (Oosteram, 1991). Virulence factors associated with pathogenic *E.coli* of avain origin include the production of toxins (Dho-Moulin and Fairbrother, 1999).

The goal of this study was planned to identify and determine the incidence and types of aerobic, facultative anaerobic bacteria and serological identification of the isolated pathogens from digestive tract

of diarrhoeic duck and determined their spectrum of antimicrobial activity. In addition to detect the enterotoxigenic strains of *E.coli*.

MATERIALS and METHODS

Samples:

A total of 120 samples were collected from dead and clinically sick ducks of different age (1-30 days) from a private farms at the Dakahlia Governorate. 80 samples collected from liver and intestine of freshly dead ducks as well as 40 from feacals swabs from clinically sick ducks.

Bacteriological examination:

Bacteriological samples were collected aseptically and each sample was divided into two parts. The first part was streaked onto predried surface of Boold agar, Nutrient agar, MacConkey agar (Oxoid, CM7) and Eosin methylene blue EMB Oxoid, CM6, incubated aerobically at 37°C for 24hours. The second part was inoculated into Rappaport Vassiliadis broth RV (Oxoid CM 669) incubated at 42°C, after 24 hours incubation, loopfulls from RV.enrichement were streacked onto Xylose lysine desoxycholate agar plate XLD (Oxoid,CM 469) with incubation at 37°C for 24 hours. The growing colonies on various plates were examined morphologically and biochemically according to Ksteman et al. (1996). 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 0920A following the manufacturers direction.

Serological identification:

- a- Antisera of *E.coli* were used for serological identification of somatic antigen "O" using slide agglutination test according to Edward and Ewing (1972).
- b- Serological identification of the isolated strains of *Salmonellae* was performed according to Kauffman (1973) using slide agglutination for identification of somatic antigen while flagellar antigen was identified by tube agglutination test.

Antibiogram pattern:

Antibiogram was applied on the most of pathogenic isolated strains using in vitro disc diffusion technique according to Quinn *et al.* (1994) using Mueller Hinton agar plates. The results were interoperated according to Oxoid Manual company (Oxoid Manual, 2000).

RESULTS

Clinical signs and P.M. lesions:

The recorded clinical signs were diarrhoea, ataxia and off food. The p.M. examination revealed the presence of congestion in the internal organs (liver, spleen, intestine and enlarged gall bladder)

Bacteriological examination:

The results of bacteriological examination of 120 duck samples are given in Table (1). On the other hand, Table (2) shows the prevalence rate of various types of microorganisms isolated from samples and cloacal swabs of diseased duck. The most prevalent isolated bacteria were *E.coli* 42.5%, *Klebsiella* spp. 18.3%. *Pseudomonas aeruginosa* 15.8% followed by *Strept faecalis* 9.2% *Staph aureus* 6.7% *B-haemolytic streptococci* 4.2% and *Salmonella* spp 3.3%.

Serological identification:

Serogrouping of 51 strains of *E.coli* isolated from diarrhoeic duck belonged to 5 different serogroups and 21 strains were untypable as illustrated in Table (3). Moreover, serotyping of 3 *Salmonella* isolates showed that they belonged to 3 different serovars including *S.typhimurium, S.derby and S. enteritidis*.

Antibiogram pattern:

Antibiogram of the most pathogenic microorganism were recorded in Table (4) showing that all tested strains were sensitive to Enrofloxacin, also with Gentamycin.

Types of	No. of examined	Positive	samples		
Samples	samples	Single	Mixed	Total	%
Liver	40	12	26	38	95
Faecal swabs	40	22	12	34	85
Intestine	40	28	10	38	95

Table 1: Prevalence rate of bacterial pathogens isolated from diarrhoeic duck.

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Samples	Liver		Faecal		Intestine		Total	
Microorganisms	(38)*		Swabs(34)*		(48)*		(120)*	
	No	%	No	%	No	%	No	%
E-coli	13	34.2	16	47.1	22	45.8	51	42.5
Staphylococcus aureus	8	21.1	-	-	-	-	8	6.7
B- haemolytic streptococci	5	13.2	-	-	-	-	5	4.2
Strep.feacalis	-	-	4	11.8	7	14.6	11	9.2
Salmonella spp.	1	2.6	-	-	3	6.3	4	3.3
Klebsiella spp.	6	15.8	6	17.6	10	20.8	22	18.3
Pseudomonas aeruginosa	5	13.2	8	23.5	6	12.5	19	15.8

Table 2: Prevalence rate of different microorganisms isolated from samples of diarrhoeic ducks

*Percentage are calculated according to total number of isolates

Table 3: Serological identification of pathogenic strains isolated from diarrhoeic ducks.

E.col	Salmonellae				
Serogroup	No	%	Serovar	No	%
O ₂₆	4	7.8	S.typhimurium	2	50.0
O ₇₈	9	17.6	S.derby	1	25.0
O ₈₆ O ₁₂₅	5	9.8	S.enteritidis	1	25.0
O ₁₂₅	6	11.8			
O ₁₅₇	6	11.8			
Untyped strains	21	41.2			
Total	51	100		4	100

Table 4: Antibiogram pattern of the pathogenic microorganisms isolated from diarrhoeic ducks.

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Tested pathogens Therapeutic agents		S.aureus	B-haemolytic streptococci	St.faecalis	E.coli	Klebsiella spp.	Salmonella spp.	Pseudomonas aeruginosa
Enrofloxacin	"5 mg"	SS	SS	SS	SS	SS	SS	SS
Gentamicin	"10 mg""	SS	SS	S	S	S	S	S
Cefdroxil	"30 mg"	S	R	R	R	R	R	R
Pen & Strep	"200mg+250mg"	S	S	R	S	S	R	S
Ciprofloxacin	"5 mg"	SS	S	S	R	R	R	S
Colistin	"5 mg"	S	R	R	S	S	R	R
Doxycycline	"30mg"	SS	S	S	R	R	R	R
Erythromycin	"15mg"	R	S	S	R	R	R	R
Trimethoprim + sulphamethoxazole "1.25+23.75"		S	R	R	R	R	R	R
Florfenicol	"long"	S	S	R	SS	R	SS	S
SS = highly sensitive $S =$ se		ensitive	;	R =	resista	nt		

DISCUSSION

Nowadays, a great attention was payed toward duck farms as a trial to fulfill excessive demand of increased population from the animal protein. The ducks meat are considered to be of high protein content with high biological value. Several microbial infection are responsible for enteritis in ducks.

In the present study, bacteriological examination of samples from diarrhoeic ducks as shown in Table (1) revealed that 95% of liver, 95% intestine and 85% of faecal swabs were positive for one or more known, these results agree to certain extent to those reported by Welsh et al. (1997) who isolated various types of microorganisms from internal organs of ostriches, and recorded mortality of 40-100%. Various types of microorganisms have been isolated from examined samples as illustrated in Table (2). Those results are similar to those reported by Welsh et al. (1997) who isolated *Staphyloccous* spp. and *S.aureus* from ratite enteric samples. In the present study the most isolated organisms was E.coli 42.5%. Similar result was reported by Clark (1996) who reported that E.coli was the main cause of enteritis in poultry. While Terzich and Vanhooser (1993) isolated *E.coli* in low percent from liver of dead duck. Also Farooq et al. (2009) isolated enteropathogenic E.coli (4.0%) from duck. Moreover, Xingxiao et al. (2009) isolated E.coli (16.2%) from duck suffering diarrhoea.

The rate of *Salmonella* isolates isolates as shown in Table (2) was 3.3% this results nearly coincide with those reported by Tsai and Hsiang (2005) who isolated *Sal. spp.* (4.6%) from duck suffering diarrhoea. Meanwhile, Higgins *et al.* (1997) isolated one strain of *Salmonella* from the intestine of poultry, also Huchzermeyer (1997) isolated *Salmonella* from faecal swabs from ostrich. On the other hand, Gopo and Banda (1997) isolated *Salmonella* in higher percentage of 16.9 from the samples while 33.3% of the carcasses tested were positive of *Salmonella* they also mentioned that liver samples were negative for *Salmonella*.

In the present study the isolated *Pseudomonas aeruginosa* 15.8%. On other hand, Safwat *et al.* (1984) who isolated *Pseudomonas aeruginosa* (15%) from duckling suffering diarrhoea and high mortality. Moreover, Ibrahim *et al.* (2006) isolated *Pseudomonas aeruginosa* either a lone or with *Staphylococcus and E.coli* from different ages of chickens and ducklings. Jennes *et al.* (2000) who isolated *P. aeruginosa* from the intestinal tract of poultry.

Serological identification of the isolated stains of *E.coli* revealed 5 different "O" serogroups which were "26, 78, 86, 125, 157 and 21 strains were untyped as shown in Table (3). Moreover Knoble *et al.* (2001) who isolated eight isolates of *E.coli* from ostriches and poultry carcasses and were serogrouped as 2,7,8,9, and 21.

In the present study, serotyping of the isolated strains of "3" serovars Salmonella revealed that S.typhimurium(50.0%), S.derby(25.0%) and S.enteritidis (25.0%) were identified. On other hand Abd-EL Rahman and Mousa (2000) isolated S.typhimurum(12%), S.enteritidis(5.6%), and S.dublin(2.4%) from ducks suffering enteritis. Vanhooser and Welsh (1995) who mentioned that S.typhimurium was the most prevalent serovar isolated from ratites. Jennes et al. (2000) mentioned that S.typhimurium was common in Multi-species collection and causes mortality in chicken younger than three months. Moreover Cadman et al. (1994) who detected antibodies of S. enteritidis in poultry in Zimbabwe. Also Tsai and Hsiang (2005) isolated S. derby and S. montevideo from ducks.

Table (4) showed that antibiogram pattern of the isolated pathogens from diarrhoeic duck revealed variable results against different therapeutic agents which had been used. The tested pathogens were sensitive to Enrofloxacin and Gentamycin. While variable results were recorded with the remaining antibiotics. The present results coincide with observation reported by Higgins et al. (1997) who mentioned that in antimicrobiol susceptibility testing of Salmonella isolated strains were sensitive to Enrofloxacin, Gentamycin and Ampicillin, also Simpraga et al. (2005) recorded that S. typhimurium isolated from duck was sensitive to Enrofloxacin. In addition. Bastawrows (1997) found that P. aeruginosa isolated strains were sensitive to Gentamycin and Polymyxin B. Meanwhile, Summano et al. (1998) reported that Enrofloxacin was effective in treatment of natural infection of E.coli. Moreover, Galland et al. (2001) recorded that E-coli 0157:H7 was susceptible to Ciprofloxacin and Enrofloxacin.Futhermore Welsh et al. (1997) who reported that Enrofloxacin would be the drug of choice in treatment of Gram.negative bacterial infection in poultry while Amoxicillin would be a good choice for the most Gram-positive bacterial.

Contrary to these results, it was reported by Higgins *et al.* (1997) who mentioned that the isolated strains of *Salmonella* were sensitive to Tetracycline and Trimethoprim+ sulfamethoxazole.

In this study, all the isolated E.coli strains from examined samples were enterotoxigenic produce heat labile enterotoxin when tested by VET-RPLA kits, and serologically identified as 7.8% E.coli O₂₆, 17.6% E.coli O₇₈, 9.8% E.coli O₈₆, 11.8% E.coli O₁₂₅ 11.8% E.coli O₁₅₇ and 41.2% untypable strains (Table 3). Many investigators studied the toxigenicity of *E.coli* isolated from different species of diarrhoeic animal and poultry. The present study nearly coincide with observation of Joya et al. (1990) who isolated 42 strains of E-coli in diarrhoeic broiler chicks, they reported that 3 strains were enterotoxigenic by suckling mouse assay. Meanwhile, Wolk et al. (1992) found that the main enterotoxigenic strains belonged to serogroup; 10,9,8,90 and 56. Heekon (1999) in Korea reported that the most prevalent strains of E.coli were 20, also he mentioned that in Australia the most common "O" group currently encountered were 9,20 and 101, while 111,119 and 125 elaborated enterotoxin. Otherwise, our results were in contrast with these reported by Jorge et al. (1988) who isolated 73 E-coli strains from broiler and found that the isolated strains did not produce enterotoxin.

In conclusion, the information given by the achieved results revealed that several microorganisms were incriminated in the enteritis in ducks and E.coli is the most perevalent which treated by using Enrofloxacin beside strict hygienic measurements on the egg laying, hatcheries and good management during the production.

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