STUDIES ON PASTEURELLOSIS IN DUCKS AND TRIALS FOR TREATMENT

Maha E. Awad-Alla [1], and Fagr A, Mahmoud [2]

Dep.of Poultry and Rabbit Diseases [1]. Dep. of Pharmacology [2]. Central Lab .Vet. Hospital, Faculty of Vet. Med., Zagazig Univ.,

ABSTRACT

A total of 100 ducks having a history of respiratory disorders and mortality, in addition, 20 water and sediments samples each were examined in this study. Samples were collected from different private duck farms in a Sharkia Governorate for clinical, P.M and bacteriological examinations. Clinical signs of living ducks showed exhausted birds, loss of appetite, respiratory disorder (difficult breathing-coughing and watery nasal discharge), cyanosis of comb and wattles which were swollen and edematous, and watery green yellowish diarrhea. Postmortem examination revealed pneumonia, hemorrhages of various sizes in the heart, liver, and intestine. Also, congestion of the liver with necrotic foci. Bacteriological examination of these samples for the prevalence of Pasteurella spp. according to morphological characters and biochemical reactions , revealed isolation of 26 isolates of Pasteurella multocida { 21 isolates from ducks at a rate of 21%, 4 isolates from water samples at a rate of 20% and 2 isolates from sediments samples at a rate of 10% }. In addition , isolation of pasteurella haemolytica at a rate of 9 %. Sensitivity test revealed that pasteurella multocida were sensitive to Ceftifur, Enrofloxacin and followed by oxtetracycline, Erythromycin,

Doxycycline and Trimethoprim-sulphmethoxazole. Meanwhile, all isolates were resistant to Neomycin, Chloromephanicol, Flumequine and Streptomycin. Results of experimental infection with pasteurella multocida with trials for treatment showed complete disappearance of clinical signs in ducks when treated with Enrofloxacin and Ceftiofur, also, reduced mortality to 24% and 16% for Ceftiofur and Enrofloxacin respectively. In the same time, showed significant increase in body weight and weight gain when compared with infected non treated ducks.

INTRODUCTION

Avian cholera (Pasteurellosis) is a highly contagious disease caused by the bacterium *Pasteurella multocida* in a range of avian species including chickens, turkeys, and water fowl and is responsible for significant losses in poultry husbandry ***(Kardos and kiss 2005).*** *Pasteurella multocida* is a Grm-negative, rode-shaped bacterium, with a bipolar staining characteristic ***(Rimler and Glisson ,1997)***. Epizootics caused by *P. multocida* occur almost in waterfowl populations and causes annual mortality in various waterfowl areas. Sometimes, the disease occurs in the form of epornitics due to rapid spread and extraordinary virulence of the organism ***(Samuel et al., 2003)***. Pasteurellosis can ranged from acute septicemia to chronic and localized infections and associated with high morbidity and mortality of growing ducklings may be up to 100% **(*Rimler and Glisson 1997)***. The first outbreak of cholera occurred in a flock of Muscovey ducks in Okinawa Prefecture of Japan in November 1990 ***(Nakamine et al.,1992)***, and in Denmark in 2001 by ***Pedersen et al., (2003)***.

It was estimated that nearly 90% of all antibiotic agents were used in food animals, in a sub therapeutic concentrations as prophylactically or to promote growth,which lead to a cumulative resistance against antibiotics of many bacteria. Therefore, the development of new antimicrobial agents is of increasing interest ***(Weckesser et al., 2007).***

Enrofloxacin is one of *fluoroquinolones* antimicrobials used extensively in human and veterinary medicine. It has a broad- spectrum activity against both *Gram-negative* and *Gram-positive* bacteria. They initiate bactericidal activity primarily by inhibiting bacterial DNA gyrase. High potency, low incidence of resistance, high oral bioavailability, extensive tissue penetration and long elimination half-lives are consistent features of *fluoroquinolones* ***(Orcini and Perkons 1992).***

Cephalosporin antibiotics are one of the most newly developed antibiotics that seem promising in veterinary use. Ceftiofur is categorized as a third generation, broad- spectrum Cephalosporin. The antimicrobial effect of Cephalosporin is due to disrupt bacterial cell wall synthesis by inhibition of mucopeptide synthesis of growing bacteria, this result in defective and osmotically unstable cell wall. ***(Hornish and Susan 2002).***

The aim of this paper is isolation and identification of *Pasteurella spp*., detection of the antimicrobial susceptibility of *Pasteurella multocida* isolates. Also studying the pathogenicity of *P. multocida* by using experimental animals and birds. Evaluation the effect of Enrofloxacin and Cephalosporin in experimentally infected ducks with *P. multocida*.

MATERIAL AND METHODS

Samples:

A total of one hundred samples freshly dead and clinical sick ducks of different ages (4- 12 months),in addition , 20 water and sediment samples each were obtained from private farms and cases which arrived to Vet. Laboratory of Teaching Hospital,Zagazig Univ.The birds were subjected to clinical, postmortem and bacteriological examination for *P.multocida* investigation. Samples were collected from visceral organs such as lung, liver, spleen, air sacs, heart blood and intestine of birds. A liver impression smears and blood stained with Giemsa and Wright's stains for bipolar rods, through a microscopic examination.

Isolation and identification of *Pasteurella Spp.*:

Primary isolation was done by inoculated samples into dextrose starch agar and 10% sheep blood agar at37°C for 24-48 hours. The growing colonies were examined morphologically (hemolysis and dew drop like colonies). Suspected colonies were transferred to peptone water broth and incubated for 24 hours at37°C for biochemical identification. Carbohydrate fermentation, enzyme production and selected metabolite production were carried on the isolated strains according to (***Quinn et al., 2002***). Isolation of *Pasteurella spp.* from water and sediments samples by using the methods of ***(Samuel et al., 2003)***.

Pathogenicity test in mice:

Five Mice of 3-4 weeks old were obtained from Animal experimental unite, Education Vet.Hospital, Faculty of Vet.Med, Zagazig Univ. Animals were inoculated intraperitoneally with 0.2ml of $1x10^7$

C.F.U/ml of 18 hours broth culture of suspected *P. multocida* colonies. Inoculated mice were kept under observation, dead mice were recorded .Heart blood and liver smears from died mice were stained with Giemsa and trial for re-isolation of inoculated organisms was conducted. ***(Cruickshank et al., 1982)*.**

Antimicrobial sensitivity test:

The sensitivity of the isolated *Pasteurella multocida* to different antibacterial agents was done on dextrose starch agar by using available commercial antibiotic discs (Oxoid Lab.), The results were interpreted according to ***(Quinn et al.,2002).***

Antimicrobial agents used:

1- Enrofloxacin: (Baytryl) ®: It was produced by Bayer-Germany. Each ml contains 100 mg enrofloxacin .Its recommended dose in ducks is 10 mg/kg.B.wt. /day taken by I.M for 5 successive days. ***(Okerman et al.,1990)***.

2- Ceftioufur sodium(Excenel)®: It is manufactured by Upjohn , Pharmaceutical Industry -USA. Each ml of reconstituted solution contains 50 mg Ceftiofur sodium. Its recommended dose 2mg/kg body weight, taken I.M for 5 successive days ***(Abdel-Latif and Gamal El-Din, 1998)***.

Experimental infection:

One hundred and five white pekin ducks of 28-days- old were grouped into four groups (A, B, C, and D) 25 ducks each. Five ducks were slaughtered and exposed to postmortem and bacteriological examination, which proved their healthy status and free from diseases.

Group A left as control non infected non treated group, ducks of group B, C and D were inoculated at 30th day- old subcutaneously by 1ml (1x10^{8}cfu) CFU/ml of *P. multocida*. Group B infected- non treated, group C infected and treated with enrofloxacin in adose of 10 mg/kg body weight, intramusculary for 5 successive days**,** group D infected and treated with ceftifur in a dose of 2 mg/kg body weight, intramusculary for 5 successive days (Table 1)**.** All treatment started 48 h post infection. Five birds from each group were weighted at 30th, 38 th , 45nd and 52th day of age to study the effect of these treatment on body performance (weight, weight gain and gain percent) . Then sacrified for postmortem examination, and trials for bacterial reisolation .Efficacy of the drugs was evaluated by observation of the clinical symptoms, P.M lesions mortality rate, morbidity rate, and weight gain.

Table (1): Experimental design of one month old white pekin ducks subcutaneously infected with *P. multocida* by 1ml (1x10^{8}cfu).

No	Groups	No	Infected dose / duck	Treatment at 48 h post-infection			
				agent	Dose	Rout	Duration
A	Non infected non treated	25	-	-	-	-	-
B	Infected and non treated	25	1ml (1x10^{8}cfu). S/C	-	-	-	-
C	Infected and treated with Enrofloxacin	25	1ml (1x10^{8}cfu). S/C	Enrofloxacin	10 mg/ kg.B.Wt	I/M	5days
D	Infected and treated with ceftifur	25	1ml (1x10^{8}cfu). S/C	ceftifur	2 mg/kg B.wt	I/M	5days

Statistical analysis:

Analysis of data of different treatments using GLM (general linear model) of Statistical analysis system ***(SAS.1999).***

RESULTS AND DISCUSSION

Pasteurella multocida has been recognized as an important veterinary pathogens for over a century. The organism can occure as a commensal in the naso-pharyngeal region of apparently healthy birds and it can be either a primary or secondary pathogen in disease processes of a varity of domestic birds ***(Antony et al., 2007).*** As ,the world's poultry production continues to grow, so do a high light concerns about the control of pasteurellosis, which remains one of the most important diseases of ducks.

Clinical examination of diseased ducks of different ages showed depression, anorexia, ruffled feathers, cyanosis of comb and wattles and edematous, respiratory symptoms (coughing and watery nasal discharge), watery yellowish or greenish diarrhea, sinusitis and locomotory disturbances .Similar symptoms reported by ***Woo and Kim (2006)*** and ***Abdel-Rahman et al., (2009).***

Gross lesions of fowel cholera in ducks are not constant but vary in type and severity. The greatest variation is related to the course of the disease whether acute or chronic .The most prominent lesions were congestion of the carcases,ecchymosis petecial haemorrhages in heart,liver, and intestine. The liver were swollen, dark browen in color,with necrotic foci. Haemorrhages on the intestine particularly the duodenum and the lower part of intestine commonly contain thickened yellowish fluid. The cardiac airsac was filled with inflammatory material and congestion and edema of the lungs.These findings were similar to those described by ***Radad and Moustafa (2006),Woo and Kim (2006)*** and ***Abdel-Rahman et al., (2009).*** Microscopical examination of blood samples and tissue smears of organs revealed bipolar staining bacillus.

Table (2): Results of Biochemical test used for identification of *Pasteurella multocida*

Biochemical test	*P. multocida* (21) isolates	*P.haemolytica* (9) isolates
Haemolysis on blood agar	-	+
Indol production	+	-
Gelatin liquefaction	-	-
Hydrogen sulphide	-	-
Urease production	-	-
Citrate utilization	-	-
Methyl red	-	-
Voges-Proskauer	-	-
Motility	-	-
Lactose fermentation	-	+
Ornithine fermentation	+	-
sucrose fermentation	+	+
Catalase production	+	+
Oxidase production	+	+

Bacteriological examination revealed isolation of 30 isolates of Pasteurella spp., 9 isolates(30%) of them showed colonies which surrounded by single narrow zone of beta- hemolysis on sheep blood agar, (*P. Haemolytica* is the only pasturella which form soluble haemolysin). The other 21 isolates (70%) were pure colonies , showed transparent , glossy, and big colonies gave off a characteristic and sweet smell, colonies ranged from 1-3mm in diameter after 18-24 hours of incubation ,which is characteristic of *P. mutocida*.Results of biochemical

test of pure isolate illustrated on table(2). These results agree with those reported by ***Woo and Kim (2006)*** *and* ***Abdel-Rahman et al. (2009)***. Also we could isolated 4 isolates (20%) of *P. multocida* from 20 water samples and 2 isolates (10%) from sediment samples. These results are higher than that obtained by ***Samuel et al (2003)*** who isolate *P.multocida* from water and sediment in 7% and 4.5% respectively.

Results of the virulence of *P. multocida* in mice recorded 100% mortality within 24-48 hours post inoculation, while no death from control group. Direct smear from heart blood of dead mice were stained with Giemsa revealed the observation of bipolar staining bacillus in all specimens **.***P.multocida* could be reisolated from heart blood of freshly dead mice. These results agree with those previously reported by ***Ozben and Muz (2006).***

Result of the antimicrobial susceptibility pattern of *P. multocida* isolates is shown in Table 3. Our results indicated that a large proportion of the *P. multocida* isolates were highly sensitive to ceftifur (100%) and Enrofloxacin (96%) followed by oxtetracycline (80%), Erythromycin (80%), Doxycycline (70%) and trimethoprim-sulphmethoxazole (70%). Meanwhile all isolates were resistant to chlormephenicol, neomycin, flumequine and streptomycin with variable results ranged from (0%-20%**).**Similar results were obtained by ***Salmon and Watts (2000)*** and ***Olson et al., (2002)*****.** Meanwhile ***Shivachandra et al., (2004)*** indicated that the strains of *P. multocida* were most sensitive to Enrofloxacin (71.454%) followed by lincomycin (64.23%), norofloxacin (61.79%) and doxycycline (56.91%). On the other hand

Bhattacharya (2005) recorded that *P. multocida* were highly sensitive to Enrofloxacin, chlormephenicol and Gentamycin and resistant to trimethoprim, Ampicillin and cephalexin.

The results of experimental infection were summarized in table (4). Ducks of control group (A), non infected non treated, were healthy, and showing no clinical sings of illness or mortality during the experiment period. The recorded clinical signs in experimentally infected ducklings (groups B) with *P. multocida* appeared 36 hours post subcutaneous inoculation. They were in the form of ocular, nasal discharge, sneezing, mild coughing, general signs of an illness in the form of depression, anorexia, watery whitish diarrhea. Ducks in group (B) showed morbidity rate about 90%, and mortality rate reached to 80% , also showed inability to walk and lameness due to unilateral or bilateral arthritis of hock joints after 6 days post infection. These results were similar to that obtained by ***El-Banna 1998, Bhattacharya (2005), Ibrahim (2005), and Woo and Kim (2006).*** While in groups (C and D), which the treatment started post inoculation by 48 hours lead to reduction of morbidity to 30% and 25%for Enrofloxacin and Ceftifur respectively and reduction also in mortality rate to 24% and 16% for two drugs respectively. In the same time the symptoms were reduced by treatment with two tested antimicrobials agents. These results were in accordance with results obtained by ***Okerman et al., (1990),*** and ***Chao-Fu et al., (2003)*** who showed a significant improvement in rabbits and ducks experimentally infected with *P. multocida* and treated with Enrofloxacin and Ceftifur sodium.

Table (3): Sensitivity of *Pasteurella multocida* isolates (No. 30) to different antimicrobial agents.

Antibiotic Disccon	Standard sensitivity Zone (mm)	*P. multocida*					
		Sensitive		Intermediate		resistance	
		No	%	No	%	No	%
Ceftiofur 30 µg	16 or more	30	100	0	0.0	0	0.0
Enrofloxacin 5 µg	17 or more	29	96	1	4	0	0.0
Trimethoprim25 µg	19 or more	20	70	5	15	5	15
Penicillin10 µg	29 or more	15	50	6	20	9	30
oxtetracycline30 µg	20or more	24	80	6	20	0	0.0
Streptomycin 10 µg	17 or more	0	0.0	6	20	24	80
Doxycycline 30 µg	16 or more	21	70	3	10	6	20
Flumequine30 µg	18or more	5	17	0	0.0	25	83
Ampicillin10 µg	29 or more	18	60	3	10	9	30
Erythromycin15 µg	16 or more	24	80	3	10	3	10
Neomycin30 µg	15 or more	0	0.0	0	0.0	30	100
chlroamphenicol30 µg	21 or more	6	20	0	0.0	24	80

The gross lesions were in the form of hemorrhage in all parenchymatous organs, lung, intestine, coronary fat, and abdominal fat with pin point white necrotic foci on the liver and enlarged mottled spleen. These results were agree with ***El-Banna (1998) and Ibrahim (2005).*** The percentage of reisolation of *P.multocida* was higher from heart blood, liver and spleen of infected, non treated ducks more than infected -treated ducks , nearly similar results recorded by ***Chao-Fu et al., (2003)*** and ***Ibrahim (2005)***.

Table (4): Results of experimental infection of one month old white pekin ducks with *P. multocida*.

Groups	Dose and challenge route	IncubationePriod	Treatment for 5 days	Mortality	Morbidity	Re-isolation
				%	%	
A	-	-	-	0	-	-
B	1ml ($1x10^8$cfu). S/C		-	80	90	+
C	1ml ($1x10^8$cfu). S/C	36 hours	Enrofloxacin I/M	24	30	+
D	1ml ($1x10^8$cfu). S/C		Ceftifur I/M	16	25	+

Table (5): The effect of Enrofloxacin and Ceftifur sodium each alone on average body weight, weight gain and gain % on healthy and experimentally infected ducks with *P.multocida*.

Groups	Body weight (gm)									
	30^{th}Day	38^{th}			45^{th}			52^{nd}		
	gm	gm	Gain		gm	Gain		gm	Gain	
			g m	%		g m	%		gm	%
Non infected non Treated (A)	676.00 ±5.8	999.55 ±4.52^{a}	323.55	47.8	1450± 7.07^{ab}	450.45	45.06	1980.00 ±7.48^{a}	530	36.55
Infected and non treated (B)	676.00 ±5.8	850.05 ±5.78^{c}	174.05	25.75	1175.00 ±6.60^{c}	324.95	38.23	1554.25 ±7.48^{c}	379.25	32.28
Infected and treat With Enrofloxacin (C)	676.00 ±5.8	912.00 ±7.4^{a}	236.00	34.91	1312.00 ±5.78^{b}	400	43.86	1800.00 ±5.9^{b}	488	37.20
Infected and treat With Ceftifur sodium (D)	676.00 ±5.8	939.0± 4.52^{a}	263.00	38.91	1360.00 ±5.23^{b}	421	44.83	1900.00 ±4.08^{a}	540	39.71
significance	N.S	S			H.S			H.S		

Values have different litters (a, b, c) are significantly different from each other At $P \leq 0.05$ and vice versa.

Body weight, weight gain and gain percent of all infected and treated groups Pre and post experimental infection with *P.multocida* are presented in table (5).

Experimentally infected non treated group (B) showed a highly significant growth depression at the end of 45^{th} and 52^{n} days when compared with other groups (A, C and D). This might be due to negatively influence growth of *P.multocida*. Similar results were recorded by ***Ibrahim (2005) and Kamel (2009)***.

Enrofloxacin treated, infected ducks group (C) showed significant increase ($P<0.05$) in body weight when compared with infected, non treated group at the end of 45^{th} and 52^{nd} days. It attained (1312 gm and 1800 gm) versus (1175 gm and 1554 gm). These results were supported by ***Abd El-Galil and El-Naenaeey (1993)*** who indicated that Enrofloxacin was more effective than Gentamycin, in treatment of pasteurella multocida

Body weight of ducks that were infected and treated with Ceftifur sodium group (D) was statistically the highest among the experimentally infected and treated groups. Significant increase ($P<0.05$) in body weight of Ceftifur sodium treated group (1360gm and 1900gm) versus (1175 gm and 1554 gm) in infected non treated group respectively at the end of 45^{th} and 52th days. This may be due to a broad spectrum of antibacterial activity of Ceftifur sodium against *P.multocida* which reflected on healthy status of intestinal mucosa and reflected on body weight. Nearly similar results were recorded by ***Kamel (2009)***.

From the above mentioned results, it can be concluded that *Pasteurella multocida* and *Pasteurella haemolytica* causing a highly serious disease in ducks resulting in economic losses. These isolates in vitro sensitivity tests have shown that *Pasteurella multocida* was highly sensitive to Ceftifur,Enrofloxacin,oxtetracycline,Erythromycin,Doxycycline and Trimethoprim-sulphmethoxazole .From the challenge experiment , it is appear that Ceftifur sodium is more efficious than Enrofloxacin in treatment of experimental *P.multocida* infection in Pekin ducks.

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دراسات على الباستيريلا فى البط مع محاولات لعلاجها

مها عوض الله السيد* ***و فجر عبد الكريم محمود*****

أمراض الطيور والأرانب *, الفارماكولوجيا البيطرية ** المعمل المركزى- مستشفى كلية الطب البيطرى التعليمى- جامعة الزقازيق

تم اجراء هذه الدراسة على مائة من البط المريض والنافق حديثا (أعمار وأنواع مختلفة) من مزارع ومناطق مختلفة فى محافظة الشرقية حيث تم فحصها اكلينيكا وتشريحيا وكذلك تم اجراء الفحص البكتريولوجى وذلك بزرعها على أوساط مختلفة للبكتريا وقد امكن عزل ميكروب الباستيريلا (هيموليتكا وملتوسيدا) وكانت نسبة البط المصاب بالباستيريلا 30% من البط الذى تم فحصة (30 عينة ايجابية لميكروب الباستيريلا) وقد تم تصنيف هذه الميكروبات مورفولوجيا وبيوكميائيا وكانت أعلى نسبة عزل من ميكروب الباستيريلا ملتوسيدا حيث كانت نسبته 70% من نسبة المعزول (21 عينة ايجابية) بينما نسبة الباستيريلا هيموليتكا كانت 30% (9 عينة ايجابية).كما أظهرت نتائج اختبار الحساسية أن ميكروب الباستيريلا ملتوسيدا شديد الحساسية لكل من سيفتيفورصوديوم بنسبة 100%, إينروفلوكساسين 96%, اوكسىتتراسيكلين 80% والارثروميسين80% والدوكسسيكلين70% وأيضا الترامىثوبريم +سلفاميثوكسازول 70% .

تم دراسة تأثير الاصابة التجريبية بالباستيريلا ملتوسيدا على عدد 100 بط بكينى عمر شهر بعد تقسيمه إلى أربع مجاميع كل مجموعة 25 بطة (A,B,C ,D) حيث أن المجموعة A مجموعة ضابطة غير مصابة وغير معالجة و B مصابة وغير معالجة وC مصابة ومعالجة بالانروفلوكساسين و D مصابة ومعالجة بالسيفتيفيور صوديوم وتم وزن البط قبل العدوى وقد بدأت الأعراض فى الظهور بعد العدوى الاصطناعية ب36ساعة وبدأ العلاج بعد 48 ساعة من العدوى. تم أخذ عدد 5 بطة من كل مجموعة عند عمر 38 ,45 و52 يوم لوزنها وذبحها و تسجيل الأعراض الإكلينيكية والصفات التشريحية و إعادة عزل ميكروب الباستيريلا ملتوسيدا من البط النافق والمذبوح.

وأظهرت النتائج أن المجموعات المصابة تجريبيا بالباستيريلا ملتوسيدا والمعالجة بالانروفلوكساسين و سيفتيفيور صوديوم (C ,D) تم انخفاض نسبة الأعراض الإكلينيكية وانخفاض نسبة النفوق من 80% فى مجموعة B (المصابة والغير معالجة) الى 16% فى مجموعةC و24% فى مجموعة D أيضا تم تسجيل زيادة ملحوظة فى وزن البط المعالج بالعقاريين مع ملاحظة ان البط المعالج بالسيفتيفيور صوديوم أظهر تحسنا أكثر فى وزن البط المعالج ونسبة اقل فى النافق من البط المعالج بالانروفلوكساسين.