

Bacteriological and Biochemical Studies On Pekin Duckling Infected With *Pasteurella Multocida* with Trial For Treatment

Allam HH¹, Aly Salah ABS², Zaki R H³ and Nagwa A Bahar⁴
(Poultry¹ and Biochemistry^(2,3 and 4) Department)

Animal Health Research Institute (Zagazig^(1 and 3), Kafer El Sheik² and Ismailia⁴ Branches)

ABSTRACT

Samples from cloacal swabs, liver, heart, lungs, trachea, spleen and nasal exudate were collected from 150 pekin duckling ageing 1-30 days (75 apparently healthy 35 diseased and 40 freshly dead) for bacteriological examination. Out of 150 examined sample 43 (28.67 %) were positive for *Pasteurella Multocida* namely 5 from apparently healthy, 13 from diseased and 25 from dead ducklings. Antibiogram study of isolates revealed that florfenicol was the drug highly effective against *Pasteurella Multocida*

A total of 160, one day old pekin duckling (80 healthy & 80 experimentally infected with *Pasteurella Multocida*) were divided into 4 equal groups (40 birds each), the 1st group consisted of healthy ducklings (control), the 2nd group included healthy ducklings were treated with florfenicol (30mg/kg bwt.) in drinking water for 5 days, the 3rd group included infected non treated and the 4th group included infected and treated with florfenicol (same dose, period and route of administration). In the four groups the hemato-biochemical changes were studied.

The results revealed that healthy pekin ducklings treated with florfenicol displayed significant rise in body weight, leukocytic count, lymphocytosis, significant decrease in heterophils and insignificant increase in monocytes, eosinophils, basophils, total proteins, albumin, globulins, A/G ratio, AST, ALT, ALP, uric acid and creatinine.

Pasteurellosis in duckling induced anorexia depression, ruffled feathers, coughing, diarrhea, rise respiratory rate and 30% mortality rate. Monocytes, total, β , γ globulin, AST, ALP, uric acid, creatinine levels and significant decrease in weight gain, heterophils, albumin, α globulin, A/G ratio, insignificant decrease in leukocyte, lymphocyt, esinophils, total proteins and insignificant rise ALT

Florfenicol residues in examined liver and kidneys in both treated healthy and diseased ducklings were high at 1st day post treatment, very low at 6th day and completely negative from examined samples at 9th day post treatment. The highest levels of florfenicol residues were recorded in kidneys then liver.

Duckling suffering from pasteurellosis and treated with florfenicol showed no clinical signs, mortality rate 5%, reduced reisolation *Pasteurella Multocida* and Improved hemato-biochemical parameters.

It could be concluded that florfenicol is effective in treatment of *Pasteurella Multocida* in duckling

INTRODUCTION

Ducks are relatively resistant to some diseases (1). In duck farms in Egypt, poor management, inadequate diagnosis, control and

prevention of various infectious diseases play a vital role in high morbidity and mortality (2). Pasteurellosis is a contagious disease in ducks caused by *Pasteurella Multocida* (3) which is

associated with poor sanitation (4). Acute form occurs as septicemia of sudden onset with high mortality (5). While chronic form results in localization of infection in wattles, respiratory passages and joints (6). Symptoms of pasterulosis include anorexia, mucous discharge from mouth, diarrhea and laboured breathing (7).

Florfenicol is a novel broad spectrum bacteriostatic antibiotic belonging to family including also thiamphenicol and chloramphenicol (8). It has fluorine atom instead of hydroxyl group located at C-3 in the structure of chloramphenicol and thiamphenicol (9). Florfenicol inhibits protein synthesis (10), it has greater activity than chloramphenicol and thiamphenicol against *Pasteurella* (11).

The aim of this study was to evaluate effects of treatment in experimentally infected ducklings. Biochemical parameters.

MATERIAL AND METHODS

Isolation and identification

A total of 150 samples (35 diseased, 40 freshly dead, 75 healthy pekin duckling) 1-30 days old were obtained from different private farms at Sharkia Province. All samples were aseptically collected from cloacal swabs, liver, heart, lungs, trachea, spleen and nasal exudate and inoculated into nutrient broth aerobically at 37°C over night, subculturing on blood agar and MacConkey agar plates was performed for 24h at 37°C, suspected colonies were identified (12). Pathogenicity and virulence of isolated *P. milt* to mice were determined (13).

Antibiotic sensitivity test

Sensitivity of *Pasteurella Multocida* to different chemotherapeutic agents was tested by disc diffusion method (14).

Drugs

Florfenicol (Aviflor, 100 mg/ml) water soluble formulation for oral use was supplied by Avico (Jordan).

Experimental duckling

A total of 160, one day old pekin duckling were reared under hygienic conditions, Fed on balanced commercial starter ration free from antibacterial agent and water ad-libitum,

Pasteurella multocida infection

On day 30 of age 80 duckling were I/M inoculated with 0.2 ml/bird of 48 hr broth culture of *Pasteurella multocida*. containing (3x10⁸CFU) viable organism (15).

Experimental design

Duckling were divided into 4 equal groups (40 ducks each), 1st group included non-infected non-treated duckling (control group), 2nd group included non-infected duckling treated with florfenicol (30 mg/kg bwt) in drinking water for 5 successive day. The 3rd group included infected non treated duckling and the 4th group included infected duckling treated with florfenicol (same dose, period and route of administration). Treatment started at age of 32 day.

Body weight

From each group 5 ducklings were weighted individually at the start of the experiment and at the 1st day post treatment and consumed diets were recorded, calculation of weight gain and feed conversion rate.

Sampling

At 1st, 7th and 15th days post treatment two blood samples from all ducklings were taken, the 1st sample was taken in tube containing EDTA to estimate leukogram (16). The 2nd one was taken to obtain serum to estimate total protein (17). Protein fraction was performed using cellulose acetate electrophoresis (18). (AST-ALT) (19) ALP (20) uric acid (21) creatinine (22).

Re-isolation of *Pasteurella Multocida*

Samples were taken aseptically from cloacal, nasal exudates and internal organs from all groups post treatment then inoculated into nutrient broth at 37°C over night followed by subculturing on nutrient agar for 24h at 37°C, suspected colonies were identified (12).

Drug residues

Five ducklings from healthy treated and infected treated ducklings were slaughtered at 1st, 3rd, 6th & 9th days post treatment. Levels of florofenicol residues in liver and kidney were

determined by microbiological assay technique (23) using *Baceillus subtilis* as test organism

Statistical Analysis

Obtained data were statistically analyzed using T test (24).

RESULTS AND DISCUSSION

Table 1. Incidence of the isolated *Pasterulla multocida* from ducks

Source of sample	Total No. of sample	+ve samples		-ve samples	
		No.	%	No.	%
Apparently Healthy duckling	75	5	6.67	70	93.33
Diseased duckling	35	13	37.14	22	62.86
Freshly dead duckling	40	25	62.50	15	37.50
Total	150	43	28.67	107	71.33

Table 2. Incidence of the isolated *Pasterulla multocida* from internal organs in duckling

Source of sample	Healthy			Disased			dead		
	Total sample	+ve sample		Total sample	+ve sample		Total sample	+ve sample	
		No	%		No	%		No	%
Tracheal swab	35	3	8.57	5	2	40	8	4	50
Cloacal swabs	40	2	5	3	1	33.33	6	3	50
Liver	-	-	-	9	2	22.22	7	4	57.14
Heart	-	-	-	6	2	33.33	3	3	100
Lung	-	-	-	3	2	66.67	9	6	66.67
spleen	-	-	-	5	1	20	7	5	71.43
nasal exudates	-	-	-	4	1	25	-	-	-
Total	75	5	6.67	35	13	37.14	40	25	62.5

Table 3. Effect of *Pasterulla multocida* on mortality rate and reisolated *Pasterulla multocida* of duckling

Group	Parameter	Total No	Clinical Signs	Mortality rate		Reisolation	
				No	%	No	%
Healthy non treated (Control)		40	00	00	00	-	0%
Healthy Florofenicol Treated		40	00	00	00	-	0%
Diseased non treated		40	38	12	30	40/40	100%
Diseased treated		40	2	3	5	3/40	7.5%

Bacteriological examination revealed that 43 samples (28.67%) out of 150 collected samples to be positive for *Pasterulla multocida* (5 from healthy, 13 from diseased and 25 from freshly dead duckling). *Pasterulla multocida* was isolated from diseased duckling in a percentage of 25% (25). Experimental

pasteurellosis in duckling induce clinical signs mainly, depression, anorexia, ruffled feathers, increased respiratory rate, sneezing, coughing, diarrhea, mortality rate 30% and decrease body weight. Typical signs were previously recorded in duckling (26).

Table 4. In vitro susceptibility *Pasterulla multocida* to some commonly used antibiotics

Antimicrobial agent	Disk polancy	Inhibition zone diameters(mm)	Sensitive
Florphenicol	30ug	25	+++
Ceftiofur sodium	30ug	25	+++
Gentamycin	10ug	23	+++
Spectinomycin	10ug	22	++
Colistine	30ug	16	R

+++ = high sensitive ++ = sensitive R = Resistance

Antibiogram revealed that *Pasterulla multocida* was sensitive to florfenicol and ceftiofur sodium followed by gentamycin, spectinomycin and resistant to colistin. Same

reports showed that *Pasterulla multocida* were very sensitive to florfenicol (25). Other report that *Pasterulla multocida* were resistant to colistin (27).

Table 5. Effect of *Pasterulla multocida* and florfenicol on body weight gain (gm) and feed conversion rate in duckling (n=5)

Parameter	Control group	Healthy treated	Diseased Non treated	Diseased treated
Weight at 30 th day of age	410.32±1.49	418.58±1.37	405.08±1.40	415.41±1.86
Body weight at 1 st day PT	480.49±5.38	502.04±9.80*	464.22±3.50*	489.55±15.94
Weight gain	69.45± 1.98	83.46±3.97*	59.14± 2.13*	74.14± 1.85
Feed consumption	238.59	243.39	217.43	228.53
Feed consumption rate	3.45	2.92	3.68	3.08

*Significant at $P \leq 0.05$

PT= post treatment

The obtained results revealed that, florfenicol treatment in healthy ducklings displayed a significant increase in body weight gain in comparison to healthy duckling, meanwhile; pasteurellosis induced significant decrease in body weight gain and feed

conversion rate in comparison with healthy ducklings. The antimicrobials drugs induce rise in growth rate through inhibiting pathogenic organisms (28). Pasteurellosis in duckling induced significant decrease in body weight gain and increase in feed conversion rate (29).

Table 6. Effect of *Pasterulla multocida* and florfenicol on leukogram in duckling (n=5)

Parameter	Control group	Healthy florfenicol treated	Non Treated	Diseased			
				Day post treatment			
				1st	7th	15th	
TLC(X10 ³)	15.87± 0.50	17.31± 0.27*	14.90± 0.15*	15.06± 0.92	15.63± 0.99	15.75± 0.65	
Heterophils	4.12± 0.85	2.15± 0.19*	2.59± 0.37*	3.07± 0.40*	3.84± 0.63	4.08± 0.49	
Differential count(X10 ³)	Lymphocytes	8.09± 0.64	10.90± 0.98*	7.40± 0.89	7.51± 0.78	7.77± 0.56	8.02± 0.60
	Eosinophils	1.34± 0.35	1.57± 0.41	1.07± 0.55	1.10± 0.70	1.25± 0.69	1.30± 0.50
	Basophils	1.17± 0.22	1.33± 0.35	1.16± 0.32	1.18± 0.62	1.18± 0.71	1.15± 0.62
	Monocyte	1.15± 0.30	1.36± 0.34	2.68± 0.57*	2.20± 0.61*	1.59± 0.54	1.20± 0.93

*Significant at P ≤ 0.05

Florfenicol induced significant leukocytosis, lymphocytosis, significant decrease in heterophils, insignificant rise in monocytes, eosinophils and basophils. Pasteurellosis in ducklings induced insignificant decrease in leukocytic count, lymphocytosis, eosinophil, significant decrease in heterophils and monocytosis in duckling. Same

results were previously reported in healthy rats treated with florfenicol for 6 days (30). Our result agreed with those obtained in broiler chicken infected with *Pasterulla multocida* (31). Pasteurellosis in ducklings induces insignificant reduction in lymphocytosis beside significant decrease in heterophils and monocytosis (26).

Table 7. Effect of *Pasterulla multocida* and florfenicol on protein profile in duckling (n=5)

Parameter	Control group	Healthy florfenicol treated	Non Treated	Diseased			
				Day post treatment			
				1st	7th	15th	
T. protein (g/dl)	6.09± 0.10	6.87± 0.14	5.95± 0.18	6.03± 0.22	6.08± 0.15	6.13± 0.19	
Albumin (g/dl)	3.83± 0.42	4.35± 0.60	3.13± 0.28*	3.41± 0.16*	3.60± 0.13	3.79± 0.15	
Globulin (g/dl)	α	0.70± 0.19	0.77± 0.13	0.49± 0.10*	0.54± 0.08*	0.68± 0.17	0.73± 0.21
	β	0.83± 0.09	0.95± 0.16	1.25± 0.12*	1.20± 0.07*	1.00± 0.12	0.86± 0.17
	γ	0.73± 0.10	0.80± 0.18	1.08± 0.11*	0.88± 0.09*	0.80± 0.14	0.75± 0.15
Total	2.26± 0.37	2.52± 0.91	2.82± 0.14*	2.62± 0.19*	2.48± 0.17	2.34± 0.18	
A/G Ratio	1.69± 0.15	1.72± 0.42	1.12± 0.20*	1.30± 0.19*	1.45± 0.25	1.62± 0.33	

*Significant at P ≤ 0.05

In the current work, florfenicol displayed insignificant increase in total proteins, albumin globulins. Pasteurellosis in ducklings showed insignificant hypoproteinemia, significant rise in total, β , γ globulin, significant hypoalbuminemia, α globulin and A/G ratio. Similiar results were recorded in healthy broiler chicken treated with florfenicol (32-33). Changes in protein profile in ducklings suffering from pasteurellosis agreed *with* (34) in duckling and

(35) in chicken. Reduction in albumin in duckling infected with *Pasterulla multocida* may be due to effect of bacteria and its toxin in liver (sole of albumin synthesis) (36). Rise in β and γ globulin indicate activation of immune system due to infection (37). Reduction in globulin in infected duckling indicating immune defense mechanism against infection and enhanced immunoglobulin synthesis (38).

Table 8. Effect of *Pasterulla multocida* and florfenicol on liver enzymes and kidney function in duckling (n=5)

Parameter	Control group	Healthy florofenicol treated	Non Treated	Diseased		
				Day post treatment		
				1 st	7 th	15 th
AST ((μ /L)	30.28 \pm 1.50	32.04 \pm 1.78	36.04 \pm 1.41*	34.78 \pm 1.17*	33.20 \pm 1.44	31.08 \pm 1.48
ALT ((μ /L)	24.05 \pm 1.42	25.34 \pm 1.89	29.13 \pm 1.89	27.85 \pm 1.47	26.30 \pm 1.60	24.97 \pm 1.93
ALP (I.U/ml)	17.09 \pm 1.12	19.21 \pm 1.96	21.87 \pm 1.04*	21.03 \pm 1.10*	19.14 \pm 1.79	18.21 \pm 1.43
Urea (mg/dL)	4.92 \pm 1.22	5.58 \pm 1.60	7.88 \pm 1.10*	7.61 \pm 1.03*	6.05 \pm 1.02*	5.12 \pm 1.40
Creatinine (mg/dL)	1.14 \pm 0.11	1.25 \pm 0.21	1.69 \pm 0.17*	1.54 \pm 0.09*	1.34 \pm 0.13	1.20 \pm 0.15

*Significant at $P \leq 0.05$

Healthy duckling treated with florfenicol revealed insignificant increase in AST, ALT ALP, uric acid and creatinine. Pasteurellosis in duckling induce significant rise in AST, ALP, uric acid and creatinine levels beside insignificant rise ALT. Same results were obtained in healthy chickens treated with florfenicol (39). Florfenicol had no adverse

effects on liver and kidney functions (40). Same changes in uric acid and creatinine in duckling suffering from pasteurellosis were previously recorded (41). Rise in enzyme activities may be due to liver damage by infectious agent and its toxins (42). Elevation in uric acid and creatinine could be due to effect *Pasterulla multocida* or its toxin on kidney (43).

Table 9. Florfenicol residues (μ g/gm) in duckling liver and kidney

	Clearance period							
	Healthy duckling with florfenicol (days)				Diseased duckling with florfenicol (days)			
	1st	3rd	6th	9th	1st	3rd	6th	9th
Kidney	6.72 \pm 0.21	2.15 \pm 0.11	0.31 \pm 0.18	00	6.41 \pm 0.14	2.07 \pm 0.18	0.19 \pm 0.07	00
Liver	4.07 \pm 0.19	1.17 \pm 0.2	0.25 \pm 0.15	00	4.12 \pm 0.2	1.3 \pm 0.10	0.10 \pm 0.06	00

The obtained results revealed that florfenicol residues in liver and kidney in both healthy and diseased duckling treated with florfenicol were high at 1st day of clearance period and completely disappeared at 9th days of clearance period. High residue of florfenicol was found in kidney followed by liver. Florfenicol residues in liver and kidney disappeared at 8 day of clearance period (44, 45). Florfenicol residues in kidney more than in liver (46, 47).

Our findings revealed that duckling infected with *Pasterulla multocida* and treated with florfenicol showed disappearance of clinical signs, reduced mortality rate (5%), ameliorate the adverse effects and return leukogram and biochemical parameters to normal levels. This finding was previously recorded (26) which showed that duck pasteurellosis treated with florfenicol induce improvement of clinical signs and hematobiochemical parameters. This improvement may be due to antimicrobial effect of florfenicol (2).

From the obtained results in the current study, it could be concluded that florfenicol is effective against *Pasterulla multocida* in duckling

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الملخص العربي

دراسات بكتريولوجية بيوكيميائية على البط البيكيني المصاب معمليا بالباستريلا مع محاوله العلاج

د. حسام حسن علام^١ على صلاح بيومي^٢، رضا حسن نكي^٣، نجوى عباس بحر^٤
أقسام (الدواجن^١ الكيمياء^٢ و^٣ و^٤) معهد بحوث صحة الحيوان (فرع الزقازيق^١) كفر الشيخ^١ واسماعيليه^٤

تم تجميع عدد ١٥٠ عينة من الأعضاء الداخلية لبط بيكيني عمر ١ وحتى شهر (٧٥ بطه سليمة ظاهريا، ٣٥ بطه يظهر عليها أعراض متمثلة في الإسهال والكساح و ٤٠ بطه ناقه حديثا) للفحص البكتريولوجي. وبعد الفحص البكتريولوجي وجد ميكروب الباستريلا مالتوسيدا في عدد ٤٣ عينة (٢٨,٦٧%) من ١٥٠ عينة تم فحصها موزعه كالاتي (٥ بطه سليمة، ١٣ بطه مريضه و ٢٥ بطه ناقه). وبعمل اختبار الحساسية لتلك المعزولات وجد أن الفلوروفينيكول أكثر المضادات الحيوية المستخدمة تأثيرا على البستريلا مالتوسيدا المعزولة عن باقي المضادات الحيوية المستخدمة.

في هذه الدراسة تم استخدام عدد ١٦٠ من البط البيكيني عمر يوم واحد (٨٠ بطه سليمة و ٨٠ بطه مصابه اصابه اصطناعية بالباستريلا مالتوسيدا. قسم البط إلى أربع مجموعات متساوية. المجموعة الأولى بط سليم ظاهريا واكينيكا ولم يعالج (مجموعة ضابطة) والمجموعة الثانية بط سليم ظاهريا واكينيكا وتم اعطاؤه الفلوروفينيكول بجرعة ٣٠ مجم/كجم من وزن الجسم لمدة ٥ أيام متتالية. المجموعة الثالثة بط مصاب اصابه اصطناعية بالباستريلا مالتوسيدا ولم يتم علاجه إما المجموعة الرابعة بط مصاب اصابه اصطناعية بالباستريلا مالتوسيدا ويتم علاجه باستخدام الفلوروفينيكول بنفس الجرعة والمدته السابقه. يتم دراسة تأثير الباستريلا على نسبة الوفيات ووزن الجسم. يتم اخذ عينات دم من البط في كل المجموعات وذلك لقياس بعض الوظائف المناعية والبيوكيميائية. يتم ذبح عدد ٥ بطه عند فترات مختلفة إثناء وبعد نهاية العلاج ويتم اخذ عينات من الكبد والكلى لتعيين بقايا الفلوروفينيكول.

وأظهرت النتائج أن البط السليم والمعالج بالجرعة العلاجية من الفلوروفينيكول أدى إلى حدوث زيادة معنوية في وزن الجسم. العدد الكلي لكرات الدم البيضاء، الخلايا الليمفاوية ونقص معنوي في خلايا الهيتروفيل بجانب زياده غير

معنويه في معدل التحويل الغذائي الخلايا الملتهمه الكبيرة, الخلايا القاعدية, الخلايا الحامضيه, البروتين الكلى, الزلال, النسبه بين الزلال والجلوبيولين, حمض اليوريك والكرياتينين AST, ALT, ALP. حمض اليوريك والكرياتينين

اليط المصاب اصابه اصطناعية بالباستريلا مالتوسيدا ظهرت عليها أعراض مرضيه تتمثل في حدوث أصوات غير طبيعية والامتناع عن الأكل والإسهالات بجميع الألوان وارتفاع نسبة النافق ٣٠% ووجود زياده معنويه في عدد الخلايا الملتهمه الكبيرة الجلوبيولين الكلى والبيتا والجاما جلوبيولين, AST, ALP, حمض اليوريك والكرياتينين بجانب نقص معنوي وزن الجسم, خلايا الهيثيروفيل, الزلال, الفا جلوبيولين, النسبه بين الزلال والجلوبيولين ونقص غير معنوي في معدل التحويل الغذائي العدد الكلى لكرات الدم البيضاء, الخلايا الليمفاويه, الخلايا الحامضيه, البروتين الكلى وزيادة غير معنويه في ALT

وقد دلت نتائج الدراسة على أن الفلوروفينيكول له بقايا في الأنسجة أثناء وبعد العلاج وكان أعلى منسوب لبقايا الفلوروفينيكول في الكلى يليها الكبد, ولكن الفلوروفينيكول اختفي من الأنسجة بعد مرور ٩ يوم من الحقن

من كل ما سبق نلاحظ أن استخدام الفلوروفينيكول بالجرعة العلاجية له تأثير فعال في علاج الإصابة بالباستريلا وأدى إلى اختفاء الأعراض الظاهرية وأدى إلى عودة هذه الوظائف إلى المستوى الطبيعي.