

EVALUATION OF FOSBAC PLUS T (PHOSPHOMYCIN WITH TYLOSIN) FOR CONTROL OF PASTEURELLOSIS IN DUCKLING

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ABSTRACT

Efficacy of Fosbac Plus T for control of Pasteurella anallpestifer infection in Pekin duckling was evaluated by disc diffusion methods. Where P. anallpestifer strain were highly sensitive to this drug. In this study Fosbac plus T was given with drinking water at a dose of 160 mg/kg B. wt. for three successive days in naturally infected ducks with P. anallpestifer. The drug at this dose reduced mortality from 25% to 5% It improved the adverse effect of P. anallpestifer on haematological parameters as evidenced by improvement of hypochromic macrocytic anaemia present in infected birds. Also it improved the liver functions as evidenced by the decrease in serum levels of sAST, sALT and serum globulin meanwhile the albumin level was increased compared with the infected group.

There was improvement of the kidney functions of infected bird (7 days post treatment) evidenced by decreased levels of serum creatinine , uric acid and inorganic phosphorous with increased calcium levels compared with the infected group. The histopathological examination of the liver and kidneys confirmed these results.. It is concluded that Fosbac plus T is an effective and safe antibacterial drug for treatment of Pasteurellosis in pekin ducks. Moreover, it has no adverse effects when given to normal ducks.

INTRODUCTION

Avian Pasteurellosis or avian haemorrhagic septicaemia is a contagious disease affecting domesticated and wild birds. It usually appears as a septicemic disease associated with high morbidity and mortality, but chronic condition often occur (Calnek et al; 1991). Adverse environmental condition (that included sinusitis may be initiated by poor management, especially in brooding period of duck) often predispose the bird to out breaks of P. anallpestifer infection (Songserm et al; 2003). Morbidity rate is 5 - 100% and mortality may vary from 5% to

90% according to route of inoculation (Sokkar et al, 1986 and Sarver;et al; 2005).

Pasteurella (*Plemerella*) *anatipestifer* infection is a contagious disease of domestic ducks, turkeys and other birds. It is also known as a new duck disease, duck septicaemia, anatipestifer syndrome, anatipestifer septicaemia and infectious serositis. It occurs as an acute or chronic septicaemia characterized by fibrinous pericarditis, perihepatitis, air sacculitis, caseous salpingitis and meningitis (Slagh et al; 1982 and Cynthia 2005).

Many efforts are done for controlling the disease through medication and vaccination so continuous research for new drugs for controlling the disease is necessary. "Fosbac Plus T" is a broad spectrum antibiotic combination, that is used for the control and treatment of infectious bacterial disease of the digestive, respiratory and urinary system.

The aim of this work is to study the efficacy of Fosbac plus T in treatment of duck Pasteurellosis and to demonstrate its effect on some haematological and biochemical parameters. Also to study the histopathological changes in the liver and kidney of the sacrificed ducks.

MATERIALS & METHODS

The present study was carried out on a duck farm in Minet El - Kamil city, Sharkia Governorate. The farm consists of two compartments each containing one thousand white pekiny ducks. The ducks were maintained on commercial well balanced ration and vitamins were added to ration and water adlibitum.

During routine observation of the farm, 250 ducks suddenly died from one of them, post mortem examination revealed congestion in all parenchymatous organs and intestine, haemorrhage in the abdomen. Phi headed necrotic foci on the liver and spleen was observed.

Clinical observation of the rest of ducks in the same farm revealed general signs of illness, watery whitish diarrhoea, lameness, and increased respiratory rate. Ten affected ducks were sacrificed to examine lesions in the internal organ and blood films were prepared from each case and stained with Gram stain to confirm diagnosis. Microscopic examination revealed gram negative bipolar organisms. The organisms were isolated from heart blood, liver and spleen of freshly dead and diseased ducks on different media.

Media: Nutrient agar, MacConkey agar, blood agar, tryptose phosphate broth (oxoid), yeast, protease, cystine agar and peptone water. The different media were prepared according to the method of (Cruickshank et al; 1975).

Bacteriological examination was performed and identification of *P. anatipestifer* was done (Krieg and Holt; 1984).

Drug: Fosbac Plus T (which is a combination of Fosfomycin at 20% and Tylosin at 5%, fructose 1.6 Diphosphate 15%, magnesium sulphate 10%, sodium chloride 32%) supplied in bottle containing 100, 250, 500, 1000 gm in the form powder, produced by Bedson.. (Argentina), imported by PharmaChem International (Egypt).

In Vitro studies: The isolated *Pasteurella anatis* strain were tested for their sensitivity to Fosbac plus T with other antimicrobial agent by Disc diffusion method (**Cruickshank et al; 1975**).

Commercial sensitivity discs:

The discs were Enrofloxacin (10 ug) (Difco), Gentamycin (5 ug) (oxoid, Basing stoke, England), Cefuroxime Sodium (30 ug) (Upjohn), Spiramycin (120 ug) (Bio merieux france), Colistin, (30 ug) (oxoid) and Streptomycin (5 ug) oxoid.

Experimental Design: One hundred, 20 days old white pekkin ducks (80 were naturally infected and 20 were clinically healthy) were used in this study. The eighty infected ducks were obtained from the diseased compartment, and divided into two equal groups each of 40 ducks: Group 1 is infected none treated, group 2 is infected and treated with Fosbac plus T at daily dose 160 mg/kg B. wt for three consecutive days. Others twenty ducks from the second compartment apparently healthy and bacteriological free were divided into two equal groups (3 & 4). Group 3 was kept as control none infected none treated. Group 4 was given Fosbac plus T at the same mentioned dose and for the same duration. Ducks of all groups were observed daily for clinical signs and mortalities. Dead ducks were examined to observe post mortem lesions. Two blood samples were collected from ten birds in each group. The first sample was collected on sodium EDTA for haematological studies (**Jain, 1986**). The second blood sample was obtained and the serum was separated for determination of some biochemical parameters:- Aspartate aminotransferase (AST) and Alanine amino transferase (ALT) were determined according to **Reitman and Frankels, (1957)**, total proteins and albumin (**Doumas and Biggs (1972)**), serum creatinine (**Bartels, 1971**), serum uric acid (**Sanders and Pasman, 1980**), serum calcium (**Gindler and Klug 1972**) and inorganic phosphorous (**Golden berg 1966**) were estimated. Samples from liver and kidneys of dead or sacrificed ducks were fixed in 10% formaline solution & stained with H & E for histopathological examination (**Stevens, 1990**).

Reisolation of *Pasteurella anatis* from sacrificed ducks was done (**Krieg and Holt; 1984**).

The data obtained were statistically analyzed according to **Armitage and Berry (1990)**.

RESULTS

Bacteriological examination of the diseased ducks revealed the presence of *P. anatispestifer*.

The mean Zones of Inhibition for Fosbac plus T against *P. anatispestifer* are shown in table (1).

The observed clinical signs were disappeared relatively under the therapeutic effect of Fosbac plus T. Mortality rate in naturally infected ducks with *P. anatispestifer* was 25%. Fosbac plus T reduced mortality to 5% (Table 2).

The infected ducks with *P. anatispestifer* showed a significant decrease ($P \leq 0.001$) in RBCs count, Hb concentration and PCV, while MCV increased significantly ($P \leq 0.01$) compared with the control group (Table 3).

One and seven days post medication the infected ducks revealed a significant increase in RBCs count, Hb concentration and PCV ($P \leq 0.05$) whereas MCV was decreased significantly ($P \leq 0.05$) compared with the infected control (Table 3). At the same time medication of the normal ducks with Fosbac plus T, showed no significant difference in all examined haematological parameters compared to the control ones (table 3).

The infected ducks revealed a significant increase in SAST, SALT, creatinine, uric acid and serum globulin ($P \leq 0.001$). Inorganic phosphorous was increased significantly ($P \leq 0.05$). Calcium level and albumin were decreased significantly ($P \leq 0.01$) compared with the control group (Table 4). These results were confirmed by histopathological examination of affected ducks. The liver of affected ducks showed numerous inflammatory cells mainly lymphocytes and heterophils in the portal tracts with periportal hydropic degeneration and necrosis of some hepatic cells (Fig 1). The kidneys of affected ducks showed hyper-cellularity of the glomeruli with fibroblastic proliferation in the interstitial tissues (Fig 2). Also kidney of affected ducks showed focal area of fibroblast and few round cells replaced the degenerated and destructed renal tubules. (Fig 3). In addition to the kidneys showed cloudy swelling of some renal tubules and oedema of bowman's capsules with dilation of some renal tubules (Fig 4) other cases revealed coagulative necrosis and epithelial & hyaline casts could be seen inside lumen of some collecting tubules.

One and seven days post medication the infected ducks denoted a significant decrease in the levels of sAST, sALT, creatinine, uric acid, globulin and inorganic phosphorous ($P \leq 0.001$) but albumin and calcium levels were increased significantly especially at 7th days post medication, compared with the infected group (Table 4 & 5). Fosbac plus T improves liver and kidneys as seen histopathologically. The liver of treated ducks showed vacuolar degeneration of some hepatic cells (Fig 5). The kidney of treated duck showed vacuolar and hydropic degeneration of some renal tubules (Fig 6).

One and seven days post medication of normal ducks showed no significant difference of all examined biochemical parameters compared with the control group (Tab 4 & 5).

DISCUSSION

Fosbac plus T is a broad spectrum Bioenergizing antibiotic complex that is used for the control and treatment of bacterial disease of the digestive, respiratory and urinary systems in poultry, animals and fish. It is effective against Gram negative, Gram positive, aerobic or anaerobic organism and *Mycoplasma* spp. organisms.

In vitro susceptibility of isolated *P. anatipestifer* to Fosbac plus T and six others commonly used antimicrobials was determined. Our results indicated that, the isolated *P. anatipestifer* is highly sensitive to Fosbac plus T. These results were in agreement with **Lion et al. (1986)** who reported that Fosfomycin is active against *P. Multoskda Rachac & Vladik (1987)* and **Pathanasphon et al; (1994)** stated that all strains of *P. anatipestifer* were sensitive to tylosin.

In this study natural infection of ducks with *P. anatipestifer* led to death 25% of birds. Different mortality rates were recorded by **Sami et al, (1995)** **Hanan, (1998)** and **Sarver et al (2005)**. The variations of mortalities were related to route of infection, infective dose, predisposing factor and species. The use of Fosbac plus T in treatment of infected ducks reduced mortality rate to 5% this may be due to the drug contain fructose 1.6 diphosphate that assists in obtaining a quicker and more effective transport of the antibiotic molecule to the interior of the bacteria. The antibiotic gains access into the cell as soon as the bacteria feed on the fructose 1.6 diphosphate. Bacteria absorb very high levels of Fosfomycin through active transport and this causes the destruction of the bacterial cell wall. On the same ground similar improvement in general condition and mortality rate caused by *Pasteurella* spp. were recorded after treatment (**Aslan et al, 2002**).

Haematological examination of the infected ducks revealed hypochromic macrocytic anaemia, where Hb concentration, RBCs count and PCV decreased significantly ($P \leq 0.001$) whereas MCV increased significantly ($P \leq 0.01$). The obtained results may be due to hemorrhagic effect of the microorganism or its endotoxin as seen histopathologically in liver and kidney tissues and this in turn led to active erythropoiesis which led to reticulocytosis. These results agree with that reported by **Randa (1996)** in chickens, **Ahlan & Ibtisam (1998)** in chickens and **Rasha (2002)** in rabbits.

In this study treatment the infected ducks with Fosbac plus T improved the haematological picture of the bird, where RBCs counts, Hb concentration and PCV increased significantly

compared with the infected group. This may be due to the drug is small molecular size make it very powerful and quick in penetration to all tissues and ensures full contact with infecting microorganisms with rapid action against them.

Similar improvement were observed by use of Tihulcostin in treatment the infected ewes with *P. multocida* (Khodary and Abdalla, 2001).

At the same time there was no significant differences in all examined haematological parameters one and seven days post medication the normal ducks compared with the control group. Which is indicating no harmful effect of the drug on haematological parameters of the ducks.

Biochemical examination of serum of infected ducks in this study revealed a significant increase in sAST & sALT. This may be due to degenerative changes, necrosis and haemorrhage induced by the bacteria or its endotoxin with liberation of these enzymes from damaged liver cell into serum, as seen histopathologically. Similar results obtained by Amany (1997) in chicken and ducks, Amina et al (1997) in rabbits, khodary & Rizk (2000) in Calves and Rasha (2002) in rabbits.

The insignificant change in total proteins in infected ducks in this study in spite of hypoalbuminemia (which occurred due to liver and kidney damage) that can be explained by compensatory hyperglobulinemia, which could be associated with bacterial septicaemia.

The recorded hypoalbuminaemia in this study may be attributed to the destructive effect of bacteria and its toxins on the liver cells which is the main sources of albumin. Similar discussion was mentioned by Mepherston (1984). The hyperglobulinaemia in infected ducks indicated immune defense of birds in respiratory infection. These results agree with that obtained by Rhoads and Rimler (1991) in poultry, Nasser and El - Sayed (1997) in cattle and Rasha (2002).

Treatment the infected ducks with Fosbac plus T elicited improvement of liver function especially at 7th day post medication, where sAST & sALT and serum globulin were decreased significantly. Meanwhile the albumin level was increased significantly compared with the infected non treated group. This may be due to decrease liver cell damage, which was demonstrated histopathologically where it showed only slight vacuolar degeneration.

Similar results obtained by Kohan et al (1974) who found improvement in the condition of the liver after treatment of birds. Which may be attributed to the bactericidal effect of the drug which inhibit the formation of bacterial cell wall.

The affected ducks with *P. anatispestifer* showed a significant increase in serum level of

creatinine ($P \leq 0.001$), uric acid and inorganic phosphorous ($P \leq 0.05$) whereas serum calcium was decreased significantly ($P \leq 0.001$) compared with the control group. These changes may be attributed to the renal damage induced by microorganism or its endotoxin, as seen histopathologically where kidneys showed focal hemorrhage particularly in the renal cortex with focal coagulative necrosis. Our findings are in agreement with **Miller et al; (1991)** in sheep, **Amany, (1997)** in chickens ducks and **Rasha (2002)** in rabbits.

The hypocalcaemia in the infected ducks may be attributed to decreased calcium reabsorption from damaged renal tubules (**Coles, 1986**). On the same time the hyperphosphataemia in this investigation is always associated with hypocalcaemia and renal damage as the metabolism of calcium and phosphorous was closely related to each others (**Coles, 1986 & Harrison and Harrison 1986**).

Treatment the infected ducks with Fosbac plus T elicited an improvement in the kidney functions especially at 7th day post medication where serum creatinine, uric acid and inorganic phosphorous were decreased significantly but calcium level was increased significantly compared to the infected group. These results were confirmed histopathologically where the kidney showed mild cloudy swelling of some renal tubules attributed to the decreased damage in renal tissues (Fig 5).

There was no significant difference in all examined biochemical parameters one and seven days post medication the normal ducks compared with the control group.

It could be concluded that Fosbac plus T is safe and effective drug for treatment of duck Pasteurellosis and it has no adverse effect on normal pckin duckling when given at a dose of 160 mg /kg B. wt.

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Table (1): In vitro susceptibility of *P. anatipestifer* strains to different antimicrobial agents.

Antimicrobial agents	Disc potency	Zone of inhibition
Fosbac	Fos 50	26 ± 3
Enrofloxacin	En 10	23 ± 2
Gentamycin	G 5	21 ± 2.5
Cefiofur sodium	Cef 30	21 ± 1.8
Spiramycin	Sp 120	18 ± 1.6
Colistin	Cl 30	15 ± 1.2
Streptomycin	S 5	17 ± 1.6

Table (2): Effect of Fosbac Plus T (160 mg/kg b. wt) after its oral administration in drinking water on mortality rates of naturally infected ducks with Pasteurellosis.

Groups	No. of bird used	No. bird dead	Mortality %
Infected none treated (G1)	40	10	25
Infected & treated (G2)	40	2	5
None infected none treated (G3)	10	-	-
Healthy & treated (G4)	10	-	-

Table (3): Effect of orally given Fosbac plus T(160mg/ kg B.wt) in drinking water (M±S.E.) in normal and naturally infected Ducks with *P.anatipestifer*.on some haematological parameters.

Parameters Group	One – day post medication				Seven days post medication			
	RBCS x 10 ⁶ /ul	Hb gm/dl	PCV %	MCV CU ^o	RBCS x 10 ⁶ /ul	Hb gm/dl	PCV %	MCV CU ^o
Infected none treated (G1)	2.28±0.15 ^{***}	7.86±0.33 ^{***}	26.0±1.1 ^{***}	114.64±2.44 ^{**}	2.62±0.13 ^{**}	8.5±0.35 ^{***}	28.6±1.3 ^{***}	109.29±1.18 ^{***}
Infected and treated (G2)	2.78±0.22	9.0±0.44	29.4±1.8	106.35±2.38 ^{**}	3.04±0.10 [*]	10.50±0.5 [*]	36.03±16.5 ^{**}	119.62±4.11 [*]
Control group none infected none treated(G3)	3.79±0.99	11.8±2.25	39.8±0.66	105.18±1.02	3.34±0.12	12.0±0.35	40.6±0.82	121.8±1.9
None infected and treated(G4)	3.48±0.09	10.9±0.33	34.8±0.09	110.35±2.99	3.06±0.19	11.56±0.5	39.53±1.6	129.83±2.3

Table (4): Effect of orally given Fosbac plus T (160mg/ kg B.wt) in drinking water on some biochemical parameters (M ± S.E) in normal and naturally infected Ducks with *P.anatipestifer*.

Parameters Group	One day post medication								
	AST u/ml	ALT u/ml	T. P gm/dl	Albumin gm/dl	Globulin gm/dl	Calcium mg/dl	Inorganic phosphorous mg/dl	Creatinine mg/dl	Uric acid mg/dl
Infected none treated(G1)	55.0±2.46 ^{***}	25.6±0.93 ^{***}	4.2±0.11	1.8±0.15 ^{**}	2.4±0.18 ^{**}	7.0±0.35 ^{***}	12.26±1.066 [*]	1.76±0.15 ^{***}	8.24±0.89
Infected treated (G2)	48.6±1.36	20.4±0.92 ^{**}	4.5±0.07	2.1±0.16	2.4±0.2	8.5±0.48 ^{**}	10.5±0.33	1.46±0.07	6.9±0.44
Control group none infected none treated(G3)	32.4±1.16	15.52±1.05	4.0±0.35	2.68±0.23	1.32±0.15	11.18±0.41	8.0±0.45	0.9±0.07	5.5±0.18
None infected and treated (G4)	30.8±0.86	17.0±0.89	4.7±0.2	2.94±0.06	1.76±0.22	10.0±0.7	8.8±0.26	1.08±0.1	6.1±0.13

* Significantly at P ≤ 0.05 ** Significantly at P ≤ 0.001 *** Significantly at P ≤ 0.001

Table (5): Effect of orally given Fosbac plus T (160mg/ kg B.wt) in drinking water on some biochemical parameters (M ± S.E) in normal and naturally infected Ducks with *P.anatipestifer*.

Parameters Group	Seven days post medication								
	AST u/ml	ALT u/ml	T.P gm/dl	Albumin gm/dl	Globulin gm/dl	Calcium mg/dl	Inorganic phosphorous mg/dl	Creatinine mg/dl	Uric acid mg/dl
The infected none treated (G1)	*** 63.0±2.09	*** 27±1.14	4.4±0.2	*** 1.5±11	** 2.9±0.08	*** 6.18±0.39	*** 13.1±0.41	** 1.8±0.25	** 8.68±0.3
Infected treated (G2)	*** 41.0±1.8	*** 17±1.14	3.4±0.11	** 2.25±0.13	*** 2.05±0.1	*** 9.52±0.35	** 0.9±0.58	* 1.1±0.11	*** 6.04±0.16
Control group none infected none treated(G3)	35.6 ± 1.7	14.0 ± 0.7	4.0 ± 0.23	2.5 ± 0.07	1.58 ± .25	10.5 ± 0.5	8.8 ± 0.39	1.01±0.11	5.94 ± 0.27
None infected and treated (G4)	34.6±0.927	16±01.14	3.8±0.22	2.44±0.12	1.36±0.15	11.1±0.6	7.66±0.44	0.81±0.04	5.4±0.20

* Significantly at $P \leq 0.05$ ** Significantly at $P \leq 0.001$ *** Significantly at $P \leq 0.001$

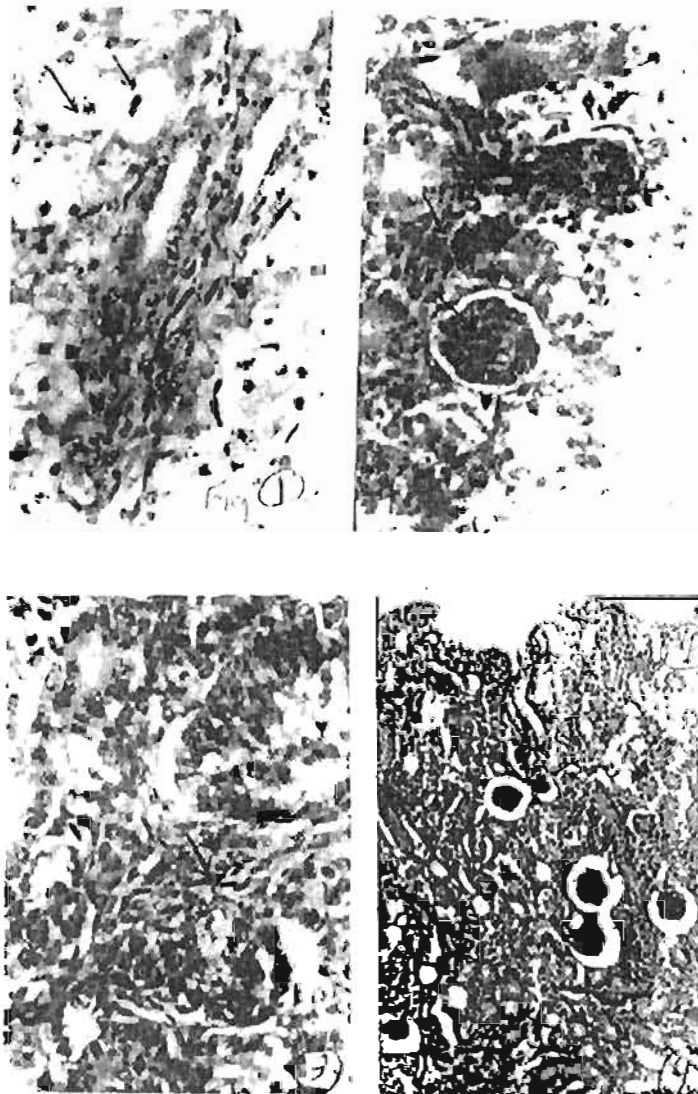


Fig (1) Liver of affected duck showing inflammatory cells mainly lymphocytes and heterophils with few fibroblast cells in the portal tract with periportal hydropic degeneration and necrosis of some hepatic cells (H.&E. X1200).

Fig (2) Kidney of affected duck showing hypercellularity of the glomeruli with mild fibroblastic proliferation in the interstitial tissues (H.&E X 1200).

Fig (3) Kidney of affected ducks showing focal area of fibroblast and few round cells replaced the degenerated and destructed renal tubules (H. & E. x 1200).

Fig (4) Kidney of affected ducks showing cloudy swelling of renal tubules and oedema of bowmans. capsules with dilation of some renal tubules (H. & E. x 120).



Fig (5) Liver of treated ducks showing vacuolar degeneration of hepatic cell (H.&E.x. 1200).

Fig (6) Kidney of treated ducks showing vacuolar and hydropic degeneration of some renal tubules (H. & E. x , 300).

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تقييم استخدام الفوسباك بلس تى (الفوسفوماسين مع الثايرلوزين) لعلاج الباستيريليا فى البط

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تم إجراء هذا البحث على مزرعة بط بكينى تتكون من عشرين أحدهما مصاب بالتسمم الدموى (البستيريللوزس) والآخر سليم لاتوجد به أعراض.

العنبر الأول : تم أخذ عينات من كبد وقلب البط النافق حديثاً وتم فحصه بالميكروسكوب بعد صبغة بصبغة جرام حيث تبين وجود ميكروب الباستيريليا فى هذه العينات وقد تم إجراء الاختبارات الخاصة بحساسية الميكروب وتم عزل ثمانون بطة وقسمت إلى جزأين :

الجزأ الأول : وشمل البط المصاب الذى لم يعالج وتم حساب نسبة الوفيات كذلك تم عد كرات الدم الحمراء وقياس نسبة والوفيات كذلك تم عد كرات الدم الحمراء وقياس نسبة الهيموجلوبين وحجم الخلايا المضفرطة حيث وجد بالبط المصاب أنيميا ذات الكرات الكبيرة كما وجد خلل فى وظائف الكبد والكلى حيث إقترن بارتفاع الترانسفيرز إنزيم والجلوبولين والفسفور الغير عضوى بينما وجد نقص معنوى فى الألبومين والكالسيوم كذلك ارتفع مستوى الكرياتينين وحامض البوليك ارتفاع معنوى فى السيرم.

الجزء الثانى : تم إعطاء الفوسباك بلس تى فى الماء بجرعة ١٦٠ مجم/كجم من وزن الدجاج لمدة ثلاث أيام متتالية. ثم أخذ العينات لأحص البكتريولوجى بعد إنتهاء العلاج كذلك تم حساب نسبة الوفيات وتم عمل صورة دم وقياس نفس الاختبارات السابقة الخاصة بوظائف الكبد والكلى ولوحظت النتيجة الآتية :-

- نقص فى معدل عزل الميكروب رقلت نسبة الوفيات من ٢٥٪ إلى ٥٪ وتحسنت الحالة الصحية للدجاج حيث زادت أعداد كرات الدم الحمراء وارتفعت نسبة الهيموجلوبين واختفت الأنيميا وتحسنت أيضاً كفاءة الكبد والكلى حيث انخفض مستوى الترانسفيرز والجلوبولين وارتفع مستوى الألبومين بينما ارتفع مستوى الكالسيوم وانخفض الفوسفور الغير عضوى كما تحسنت كفاءة الكلى وجد انخفاض معنوى فى مستوى الكرياتينين وحامض البوليك حيث إقترن التحسن الإكلينيكى بتحسناً باثولوجى فى صورة الكبد والكلى.

العنبر الثاني : تم فحص العينات بكتريولوجيا وتأكد خلوه من الميكروب وتم أخذ عشرون بطة وقسمت إلى قسمين :

الجزء الأول : كمجموعة ضابطة لم تأخذ أى دواء.

الجزء الثاني : تم إعطاء الفوسباك بلس تى بنفس الجرعة التى أعطيت للبط المصاب ولنفس المدة لمعرفة هل يوجد له

آثار جانبية أم لا ؟ فقد أظهرت النتائج عدم إختلاف معنوى فى صورة الدم ووظائف الكبد والكلى عند مقارنته

بالمجموعة الضابطة ومن هذا يتضح أن الفوسباك بلس تى آمن فى الاستخدام ويعالج بكفاءة البط المصاب بالباستيريلا.