Clinico-Pathological and Bacteriological Studies on Turkey Experimentally Infected With Pasteurella Multocida with A Trail For Treatment Using Spectinomycin

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Abstract

The present study aimed to investigate clinico-pathological changes on turkey poult infected with *Pasteurella Multocida* (*P. Multocida*), about 200 turkey poults, 15th day old, were subjected for bacteriological examination to obtain 80 healthy turkey poults free from any bacterial infection. Turkey poult were divided to 4 equal group, 1st Gp healthy poults non treated (control), 2nd Gp healthy poults injected with 20 mg/kg bwt. spectinomycin for 5 days, 3rd Gp infected non treated turkey poults and 4thGp infected poults treated with 20 mg/kg b. wt spectinomycin for 5 days. Clinical signs, mortality rate and post mortom examination were recorded. Effect of *P. Multocida* and spictinomycin on body weight was studied. At the end of treatment (1st day post treatment) 5 poults from each group were sacrificed, blood samples were taken for hemato-biochemical analysis. Samples from liver and kidney for detection of spectinomycin residue, beside samples from internal organs were taken for pathological study.

Turkey poults received spectinomycin showed significant increase in weight gain, improve feed conversion rate, significant decrease in RBCs, Hb, PCV% and insignificant effect in leukogram, total protein, albumin, globulin, AST , ALT, ALP, uric acid, creatinine, serum total lipid, cholesterol and triglyceride as compared with healthy non treated group

Pasteurellosis in turkey showed clinical signs of loss of appetite, depression, ruffled feathers, dropping of the wings, fever, diarrhea, poor growth, emaciation as well as cyanosis of comb and wattles and mortality rate was 25 %. Significant reduction in body weight, weight gain, RBCs, PCV%, Hb and significant elevation in feed conversion rate, WBCs, monocyte, globulin AST, ALT, ALP uric acid, creatinine and insignificant effect in heterophils, lymphocyts, Eosinophils, basophile, total lipid, cholesterol, triglyceride, total protein, albumin and A/G ratio .

Spectinomycin residues were detected in liver and kidney up to 9 days post treatment but not detected at 12 days post treatment and its residues in kidney higher than in liver.

The pathological examination of internal organs of different groups revealed, spectinomycin treated group (4) should mild pathological alteration in the internal organ, however *P. multocida* infection induced severe pathological lesions as hepatic

and lung tissue hemorrhage and congestion, spleen lymphocytic depletion, degenerative changes of renal tubules. cardiac muscle showed round cell infiltration, hemorrhage and degeneration of muscle fibers. The trachea showed mucosal desquamated epithelium and leukocytic infiltration but infected turkey poult treated with spectinomycin induced marked improvement in the pathological changes.

It could be concluded that pasterullosis in turkey poult induced many reversible adverse effect on body performance and hemato-biochemical parameters which is confirmed by pathological changes. Spectinomycin in therapeutic dose is considerable effective in treatment of pasteurollosis in turkey poult.

Introduction

Avian pasteurellosis is a contagious disease of domestic and wild birds caused by *P. multocida* (**Curtis and Ollerhead, 1990**). *Pasteurella multocida* (P. *multocida*) bacteria causes a variety of different disease syndromes in various animals (**Wilkie et al., 2012**). Pasteurellosis is a disease in a range of avian species including chickens, turkeys and ducks (**Shilpa, et al. 2015**). The disease occurs in two forms, acute septicemic form of sudden onset with high morbidity and mortality but chronic infection has been localised in joints and sinuses (**Rhoades and Rimler, 1991**). Turkeys are more susceptible to pasteurellosis than chickens and the susceptibility to this infection increases as the age advances (**Carpenter, et al. 1991**). Clinical sings of pasteurollosis include loss of appetite, mucous discharge from mouth, diarrhea, and labored breathing (**Carter and De Alwis, 1989**).

Aminoglycosides and aminocyclitols are antibiotics group that frequently used in Veterinary and human Medicine against gram negative and some gram positive bacteria. Spectinomycin is a one of the aminoglycoside aminocyclitol antibiotics, with broad spectrum antibacterial activity isolated from *Streptomyces spectalis* (Murray 1991). It is bactriostatic antibiotic which acts by inhibiting bacterial protein synthesis (Einstein et al 1994). It is active against G +ve and G-ve bacteria (Brander et al., 1982).

Present study was designed to clarify the effect of spectinomycin on body performance-, haemato-biochemical and pathological changes of *P. multocida* infected turkey poults.

Materials and Methods

Spectinomycin (**Spectam**)® **Spectam** is a liquid form of spectinomycin. It supplied by Amoun Pharmaceutical Comp., El-Obour City, Cairo. It available in bottles of 100ml its used in therapeutic dose of 20 mg/kg b. wt intramuscular injection for 5 successive days (**Einstein et al., 1994**).

Experimental turkeys Coloacal swabs were aseptically collected from 200, 15 day old, turkey poults, they were inoculated into nutrient broth at 37°C over night, sub-culturing on nutrient agar and MacConkey agar plates for 24h at 37°C and suspected colonies were identified (**Woldehiwet, et al. 1990**).this step was don to obtain 80 turkey poults free from any bacterial infection that using in this study.. Turkey poults were fed on

freshly prepared balanced ration and kept under hygienic conditions during experimental period.

Pasterulla multocida challenge

Pasteurella multocida strain that used in this study was obtained from bacteriological department Faculty Of Veterinary medicine Zagazig. University. Determination virulence of Pasterulla multocida was done by inculcation of mice (**Okerman**, et al 1979). On day 21 of age, 40 turkey poults were intranasal inoculated with 0.2 ml/ poult of 48h broth culture of P. multocida containing ($3x10^8$ CFU) viable organism (**Baron et al. 1992**)

Experimental design

About eighty turkey poults was divided into four equal groups (20 birds each). 1st Gp was served as control (non infected non treated), the 2nd Gp received spectinomycin (20 mg/kg bwt.) injected intramuscularly for 5 days, the 3rd Gp one was experimentally infected with *P. multocida* and 4th Gp were experimentally infected poults treated with injected intramuscular injection of spectinomycin (20 mg/kg bwt.) for 5 days. Treatment starts 24h. post infection. Clinical signs, mortality rate and postmortom lesions and body weight of all groups were recorded.

Re-isolation of P. multocida:-

cloacal swabs, tracheal swabs and nasal exudates swabs were collected aseptically from all turkey poults in the third and fourth groups, moreover beside post sacrifice of turkey poults at 1st day post treatment aseptically swabs from liver and heart blood were taken from Gp 3 and 4 for reisolation *P. multocida*. Samples were incubated on nutrient broth at 37°C for 24h, then subcultured on nutrient agar and MacConkey agar plates for 24h at 37°C, suspected colonies were identified (**Woldehiwet, et al. 1990**).

Blood samples

At the end of the treatment, 5 turkey poults in all groups were sacrificed, 2 blood samples were taken,1st one was taken on tube contain EDTA to estimate blood picture(Jain 2000)2nd one taken to obtain clear serum to estimate total protein (Doumas et al 1981). Albumin (Drupt 1974) and globulin were detected by substractin albumin from total protein, (AST-ALT) Reitman and Frankel (1957) ALP (John 982) Uric acid (Coalombe and Faurean 1963), creatinine (Husdan and Roporpot 1968).total lipid (Knight et al 1972) triglyceride (Royer, 1969) cholesterol (Richmond 1973) Catalas (CAT) (Singha 1972) superoxidase dismutase (SOD) determined spectrophotometerically (Paoletti and Mocali, 1990). Malonodialdehyde (MDA) Esterbauer, (1982)

Drug residue:

Three turkey poults from group 2 and group 4 were sacrificed at the 1st, 3rd, 6th, 9th and 12th day post treatment liver and kidneys were taken for spectinomycin residues by microbiological assay as Arret **et al.** (1971) using *Baceillus subtilis* as test organism.

Histopathological examination:

After the end of treatment 5 turkey poults were sacrificed from each group and carfull examination was done. Specimens were taken from liver, kidney, spleen, lung, trachea and heart then fixed in 10% neutral buffered formalin. Thin micron thick paraffin sections were prepared and stained with hematoxylin and eosin examined microscopically (**Bancroft et al., 1990**)

Statistical analysis Obtained data were analyzed Petrie and Watson (1999).

Results and Discussion

In the present study, the clinical signs that appeared on the challenged and non-treated turkey poults were loss of appetite, depression, ruffled feathers, dropping of the wings, diarrhea, mucous discharge, labored breathing and poor growth and emaciation beside cyanosis of comb and wattles and mortality rate was 25 %. Diseased turkey poults treated with spectinomycin showed disappearance of clinical signs and reduction in mortality rate up to 10% and the reisolation rate of *P. multocida* (table, 2). Same results were reported by **Sami and El-Oksha** (2006) in turkey poults infected with *P. multocida*. Similar results were reported by **Jain** (2000) in bird infected with *P. multocida*. Spectinomycin was very effective drug against pasteurollosis as it decreased mortality rate in cattle (**Schwarz et al. 2004**).

Healthy turkey received spectinomycin showed significant increase in body weight, weight gain and improve feed conversion rate. While *P multocida* infection induced significant decrease in body weight, weight gain and increased feed conversion rate as compared with control group (table, 3). Antimicrobials stimulate increase in weight gain due to suppression of bacteria (**Bryan et al 1998**). Spectinomycin induced increase in weight gain and improved feed conversion rate (**Bender et al. 1983**). Pasteurellosis induced decrease in weight gain and increase in feed conversion rate in duckling **El-Banna (1998)** in duckling pasteurollosis.

Our results revealed that, healthy turkey poult treated with spectinomycin showed significant decrease in RBCs Hb and PCV% beside insignificant effect in WBCs, heterophila, lymphocyts, monocyts ,eosinophils and basophile . Infected turkey poults showed significant reduction in RBCs, PCV% and Hb, significant increase in WBCs and monocyts associated with insignificant effect in heterophils, lymphocyts, eosinophils and basophile(table, 4). Similar changes in blood picture were observed by **Plumb** (1995) in birds received spectinomycin. Broiler chickens received spectinomycin showed significant decrease in RBCs Hb PCV% and insignificant effect in WBCs count (**Mohammed, Shorouk 2004**). Respiratory bacterial infection in ostriches resulted in significant decrease in RBCs, PCV and Hb besides increase in WBCs, heterophils and monocyts (**Ali and Youssef 2003**). Change in blood picture in diseased turkeys may be due to endotoxin produced by *P. multocida* (**Hassan, Randa 1996**). Elevated heterophilis and WBCs in diseased turkeys may be viewed as the

primary response to bacterial infection. (Ahamefule et al.2006).

In the present study, spectinomycin resulted insignificant effect in serum total protein, albumin, globulin and A/G ratio in healthy turkey but Pasteurella infection total significant increase in globulin (table, 5). Same changes in protein profile were reported in chickens received spectinomycin (**Abd El Kreem, Fagr 1988**). Our result is supported by **Bender et al. (1983)** as they stated that spectinomycin not affect protein picture. Same results were recorded by **Hassan, Randa (1996)** in chicken pasteurellosis. The increase in globulin in challenged turkey indicating the immune defense mechanism against infection and enhanced immunoglobulin synthesis (**Abd-Alla, Amany, 1997**).

Our results showed that, spectinomycin induced insignificant effect in AST, ALT, ALP, uric acid and creatinine, serum total lipid, cholesterol and triglyceride while challenged P. multocida revealed significant increase in AST, ALT, ALP, uric acid and creatinine as well as insignificant effect serum total lipid, cholesterol and triglyceride(table, 5 and 6). Spectinomycin induced insignificant effect in liver enzymes, uric acid and creatinine (Plumb, 1995). Also, spectinomycin induced similar changes in liver enzymes and lipid profile (Venning et al 1990). These changes may be due to spectinomycin does not have hepatotoxic or nephrotoxicity (Gary, et al.1988). same change in liver enzymes was recorded by Hassan, Randa (1996) in chicken pasteurellosis. Similar changes in lipid profile were reported by EL-Sayed et al. (2000) and Abd-Alla, et al. (2001) in duckling suffering from pasteurellosis. Elevated liver enzyme activities, uric acid and creatinine in diseased turkey poults may be due to pathological changes induced by P. multocida in liver and kidney (Kaneko, 1980). These results were confirmed by the histopathological changes as showed degenerative changes in liver and some kidneys tubules. Also, elevation liver enzyemes may be attributed to liver damage by infectious agent toxins (Halliwell, 1981).

Our study revealed that non challenged turkey poults received spectinomycin or suffering from pasteurollosis showed significant reduction in serum SOD and CAT beside significant elevation of serum MDA but poults suffering from pasteurollosis and treated with spectinomycin showed improvement in SOD, CAT and MDA(table, 6). Similar results were estimated by Yazar, et al. (2003) in rats received aminoglycoside. This change may be due to aminoglycoside antibiotics facilitate the generation of free radical leading to significant reduction in serum SOD, CAT and significant elevation of MDA (Enver, et al 2003). endotoxin produced by bacterial infection induced inflammation in different body organ causing imbalance between reactive oxygen species and antioxidant capacity leading to heightened free radical generation and impaired antioxidant system (SOD and CAT) (Vaziri 2008).

Obtained data in this study showed presence of spectinomycin residues in liver and kidney up to 9 days post treatment but not detected at 12 days post treatment and its residues in kidney were higher than in liver(table, 7). Spectinomycin residues in liver and

kidney were detected up to 7 day by many other authors (**Bergwerff et al. 1998** and **Hamamoto et al, 2003**). Also, **Mohammed (2015)** stated that kidney contain higher concentrations of spectinomycin than liver

Grossly, the infected turkey poults showed pinpoint necrotic foci on hepatic tissue, general congestion of the heart, trachea, lung, spleen and intestine. Microscopically, in case of healthy poults received spectinomycin, in the liver showing congested blood vessels and mild vacuolated hepatocytes (Fig. 1). Kidney of some cases undergoes focal lymphocytic aggregations and vacular degeneration lining tubular epithelium (Fig. 2). The turkey pasteurellosis, hepatic tissue undergoes multifocal areas of coagulative necrosis, hemorrhage and congestion (Fig. 3&4). The spleen with lymphocytic depletion (Fig. 5). The kidney undergoes nephritis, degenerative changes in the lining epithelium of renal tubules and inflammatory cell infiltration mainly neutrophils (Fig. 6&7). The cardiac tissue undergoes fibers degeneration, fibrinous pericarditis and severe congestion beside round cell infiltration and hemorrhage (Fig. 8&9). The trachea undergoes desquamated mucosa of epithelium and leukocytic infiltration beside severe congestion and hemorrhage (Fig. 10). The lung showing congestion, hemorrhages and interstinal pneumonia (Fig. 11&12). In pasteurellosis treated with spectinomycin, the small intestine undergoes hemorrhage, leukocytic infiltration and desquamated villi (Fig. 13). In pasteurellosis treated with spectinomycin, cardiac tissue showing extravasated erythrocytes among the cardiac fibers (Fig. 14). The hepatic tissue showed vacular and hydropic degeneration of hepatic cells (Fig.15). The lung shows mild bronchitis and hyperplasia of some lymphoid follicles (Fig.16). The kidney showed regenerative attempts of some renal tubules that represented by vesicular nuclei and basophilic cytoplasm (Fig.17). Similar lesions were observed in birds received spectinomycin (Cynthia, 2005). Also, Eleiwa, Naglaa (2000) reported that chickens received spectinomycin showed normal liver size with mild congestion and hyperplasia of bile duct epitheliurn and mild congestion and haemorrhagic spots on kidney surface. Similar pathological lesion was reported by Sami and El-Oksha (2006) in turkey poults suffering from pasturelosis and Abd El Rhman, Mona (2008) in poultry infected with P. Multocida. These observed lesions may be due to the effect of endotoxins excreated by P. multocida (Christensen andBisgaard, 2000). our observed lesion are in line with the finding of **Anitha and Mammen** (2013) who stated that P. multocida induced liver coagulative necrosis and haemorrhages. Degeneration of mild fibers with extensive intramuscular haemorrhages of heart with congested haemorrhagic lungs.

It could be concluded that pasterullosis induced many adverse effect on body performance and hematobiochemical parameters which was confirmed by pathological lesions. Spectinomycin is of considerable effective in treatment of pasteurollosis in turkey poults

Table 1: Incidence of the isolated bacteria from examined turkey poults (200) before experiment start

Total No of examined turkey poults	+ve s	amples	-ve samples		
Total No of examined turkey pourts	No.	%	No.	%	
200	110	55	90	45	

Table 2.Clinical signs, mortality rate. and re-isolation of p. multocida

Parameters	Total	Clinical		Clinical Mortality		Re-isolation of pasterulla multocida						
	Total No	sig	signs		rate		cload	cal swab	Trac	cheal swab	nasal	exudate
Groups	140	No	%	No	%	No	No	%	No	%	No	%
Gp 1	20	00	00	00	00	20	00	00	00	00	00	00
Gp 2	20	00	00	00	00	20	00	00	00	00	00	00
Gp 3	20	20	100	5	25	15	15	100	15	100	15	100
Gp 4	20	5	25	2	10	18	4	22.22	3	16.67	5	27.77

C = Control

H Sp T =Healthy spectinomycin treated turkey

INT =Infected non treated turkey

I Sp T = Infected spectinomycin treated turkey

Table 3. Effect of p multocida and spectinomycin on body performance of turkey(n=5)

Parameter Groups	Initial Body weight 21 th day of age)	Body at 1 st day post treatment (Gm)	Weight gain (Gm)	F.C. (Gm)	F.C.R.
Gp 1	595.14±4.61	787.04±5.76	191.90±4.31	315.62	1.64
Gp 2	602.25±3.43	808.37±5.69	206.12±6.21	307.37	1.49
Gp 3	596.83±4.82	729.93±6.57	133.10±8.73	294.03	2.21
Gp 4	599.49±6.55	776.73±6.94	177.24±6.28	297.24	1.68

F.C= feed consumption

F.C.R.= feed conversion rate

Table 4. Effect of p multocida and spectinomycin on Some hematological parameters of turkey (n=5)

Parameter		erythriogran	n	leukogram							
	RBCs	HB	PCV	WBCs (10 ³ / Diferenial count (103/ μl)							
Groups	$(10^6/\mu l)$	(Gm%)	(%)	μl)	Н	L	M	E	В		
Gp 1	5.09 ±	11.82 ±	33.37±	10,78 ±	3,52±	3,94±	1,12±	1,19±	1,01±		
Ор 1	0.22	0.38	0.79	0,48	0,22	0,58	0,15	0,30	0,12		
Gp 2	4.19 ±	9.94 ±	30,55±	11,27 ±	3,74±	4,02±	1,17±	1,26±	1,08±		
	0.31*	0.49*	0,68*	0,94	0,52	0,73	0,19	0,42	0,19		
Gp 3	4.02 ±	9.74 ±	30,04±	12,43 ±	4,44±	3,99±	1,71±	1,22±	1,07±		
	0.27*	0.65*	0,69*	0,40*	0,27	0,21	0,18*	0,16	0,09		
Gp 4	4.73 ±	10.88 ±	31,34 ±	11,66± 0,16	4,10±	3,95±	1,38±	1,20±	1,03±		
G p 4	0.42	0.89	0,83		0,19	0,34	0,22	0,19	0,12		

H=Heterophile B=Basophils L= Lymphocyte

M= Monocyte

E=Eosinophile

Table 5. Effect of p. multocida and spectinomycin on protein and lipid picture of turkey (n=5).

Parameter		Protein pro	ofile (g/dl)		Lipid profile (mg/dl)			
Groups	T. protein	Albumen	globulin	A/G ratio	T lipids	Chlosterol	Triglyceride	
Cn 1	4.94 ±	2.64±	2.30±	1.15±	163.12±	112.83±	81.04±	
Gp 1	0.27	0.17	0.05	0.23	2.87	1.98	1.45	
Gp 2	4.98 ±	2.66±	2.32±	1.16±	160.78±	109.13±	80.21±	
	0.19	0.18	0.16	0.12	2.93	2.08	1.83	
Gp 3	4.93 ±	2.33±	2.60±	0.90±	165.02±	114.41±	81.83±	
	0.19	0.21	0.11*	0.17	2.54	1.78	1.60	
Gp 4	4.96 ±	2.59±	2.37±	1.09±	164.12±	113.20±	81.04±	
Op 4	0.23	0.29	0.26	0.14	2.72	1.93	1.49	

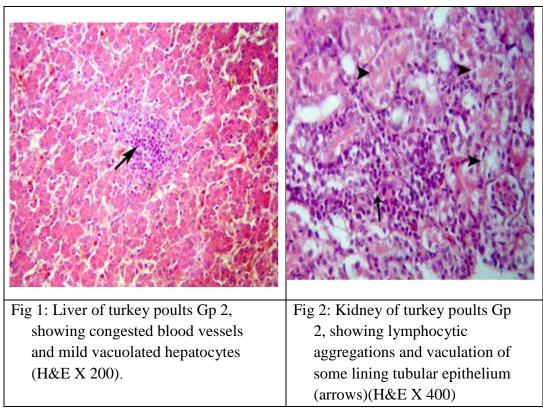
^{*} Significant at $P \le 0.05$

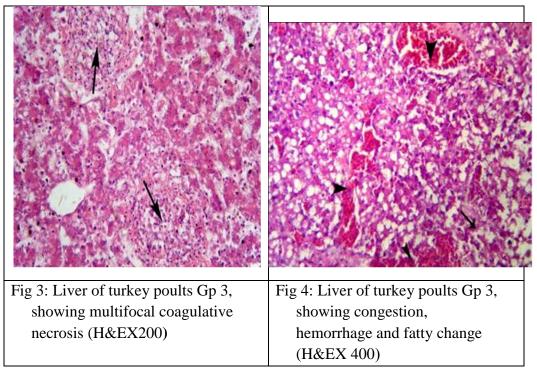
Table 6. Effect of p multocida and spectinomycin on liver enzymes and kidney function of turkey (n=5).

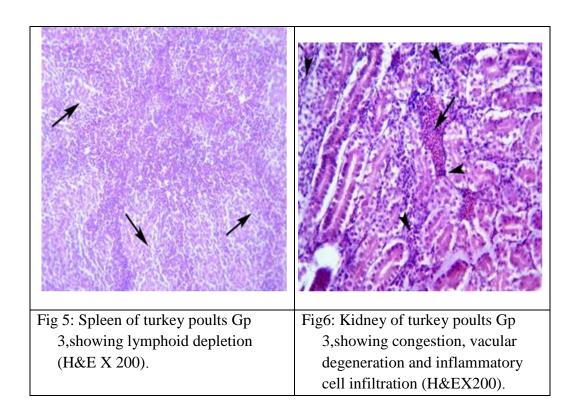
Parameter	Biochemical parameters								
	li	ver enzym	es	Kidne	y function	Antioxidant enzymes			
Groups	AST	ALT	ALP.	Urea	Creatinine	Catalas	SOD	MDA	
	U/L	U/L	I.U/ml	mg/dl	mg/dl	(U/mL)	(U/mL)	(ug/ml)	
Gn 1	76.51±	41.17±	89.16±	6.15±	1.07±	7.18±	30.93±	8.38±	
Gp 1	0.95	0.97	1.06	0.41	0.18	0.21	0.57	0.96	
Gp 2	78.06±	42.36±	90.42±	6.63±	1.15±	6.43±	29.17±	11.82±	
	1.35	1.54	1.23	0.83	0.25	0.16*	0.45*	0.87*	
Gp 3	81.21±	44.86±	93.41±	7.68±	1.66±	6.16±	29.04±	11.98±	
	1.06*	1.18*	1.12*	0.43*	0.14*	0.31*	0.42*	1.06*	
Gn 4	78.42±	42.51±	91.06±	6.48±	1.28±	6.73±	30.81±	10.61±	
Gp 4	1.27	1.25	1.43	0.30	0.18	0.52	0.68	0.97	

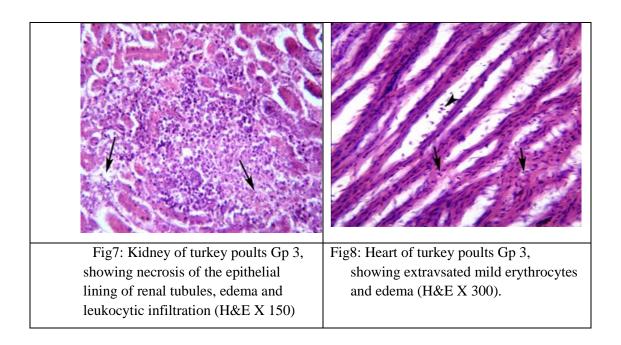
Table 7.Residues of spectinomycin (mg/kg) in poults

	day	Days post spectinomycin administration							
Tissues		1 st	3 rd	6 th	9 th	`12 th			
Gp 2	Liver	0.38±0.11	0.29±0.05	0.21±0.04	0.17±0.04	000			
	Kidney	0.61±0.33	0.43±0.21	0.23±0.12	0.10 ± 0.02	000			
Cm 1	Liver	0.42±0.08	0.36±0.09	0.24±0.07	0.16±0.06	000			
Gp 4	Kidney	0.73±0.28	0.48±0.10	0.27±0.10	0.11±0.04	000			

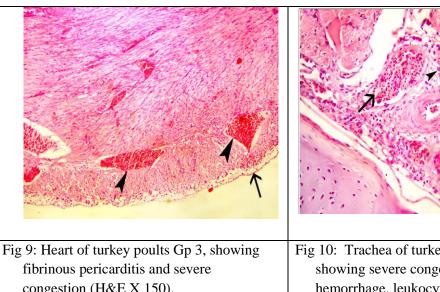








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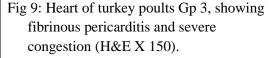


Fig 10: Trachea of turkey poults Gp 3, showing severe congestion, hemorrhage, leukocytic infiltration and desquamated mucosal epithelium (H&E X 300).

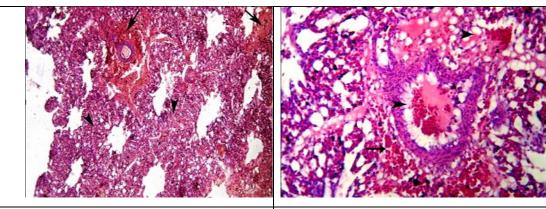
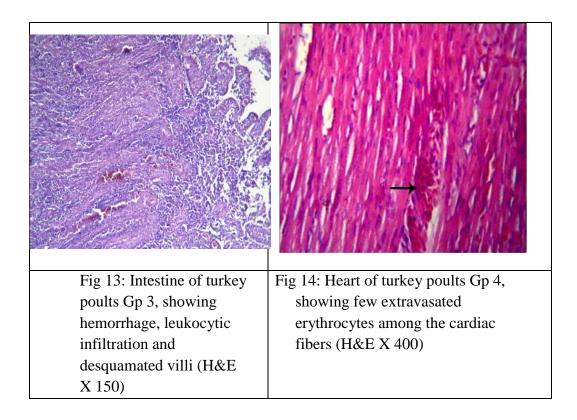


Fig 11: Lung of turkey poults Gp 3, showing congestion and thickening of interalveolar septa with leukocytic aggregations (H&E X 200).

Fig 12: Lung of turkey poults Gp 3, showing congestion, hemorrhage, perivascular edema and few leukocytic aggregations (H&E X 400).



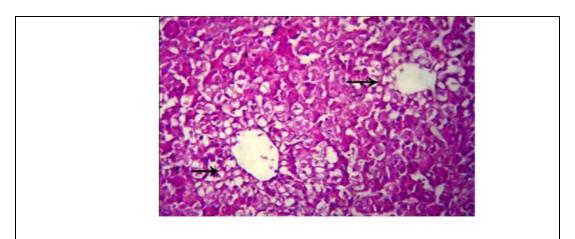


Fig 15: Liver of turkey poults Gp 4, showing vacuolar and hydropic degeneration (H&EX 400).

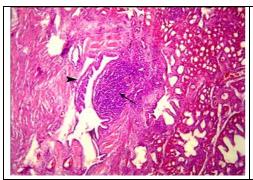


Fig 16: Lung of turkey poults Gp 4, showing hyperplasia of the parabronchial lymphoid follicles (H&E X 150).

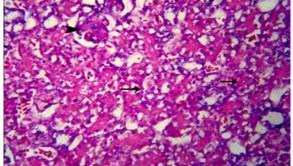


Fig 17: Kidney of turkey poults Gp 4, showing hydropic degeneration and regenerative attempts in the renal tubules (H&E X 400).

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