Effect Of Apramycin On Pathological, Hematological And Biochemical Changes In Turkey Infected With Coli-Bacillosis

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ABSTRACT

Eighty turkey poults 20 days old were equally divided into 4 equal groups to evaluate the efficacy of Apramycin against the colibacillosis; group1 was kept as control, group 2 received 25mg/kg.b.wt. of Apramycin in drinking water for 5 successive days, the group3was infected with 3×10° organisms of E.coli (O78)and group 4 was infected with E.coli and treated with Apramycin .Five poults from each group was sacrificed,1,15 and 20 day from the beginning of the experiment.

Two blood samples were taken and the serum was separated for hematological and biochemical studies. Tissue specimens were collected for drug- residues assessment and for pathological examination. The poults of group3 shows significant decrease in RBCs, Hb ,PCV%, total proteins ,albumin ,globulins with insignificant increase in A/G ratio. and significant elevation in WBCs ,AST ,ALT ,ALP, Uric acid and Creatinine These changes were lowered in group 4. The highest Apramycin residues were present in the kidneys ,liver, skin and thigh muscles ,respectively, then disappeared completely rom the examined organ samples after 20 days post treatment. The pathological examinations were coincided with the biochemical examination these changes included degenerative changes in hepatocytes, epithelial lining of renal tubules in addition to coagulative necrosis in both hepatic and renal parenchyma. Congestion and leukocytic infiltrations were noticed. Finally, it could be concluded that the Apramycin is effective against the Colibacillosis, in turkey poults.

INTRODUCTION

Escherichia coli, usually abbreviated to E.coli, is one of the main species of bacteria normally inhabitants of the lower intestines of warm-blooded animals (birds and mammals) (1). Colibacillosis affects poultry industry causing serious economic losses achieved by high mortality and loss of body weight (2). Colibacillosis was associated with various disease conditions (3). The acute form in poultry leads to septicemia and death (4).

Escherichia coli serotype O78 is highly pathogenic for chickens and can induce mortalities within short time (5).

Escherichia.coli infection in poultry is associated with pericarditis, perihepatitis, nephritis, air-sacculitis, peritonitis,

panophthalmitis and omphalitis. There were also degenerative changes in most organs of the affected chickens, represented by vacuolar and hydropic degenerations in addition to necrosis of hepatocytes and renal parenchyma. Blood vessels of most organs were congested, sometimes hyperplasia of their walls. Colibacillosis causes an elevation of AST, ALT and ALP, in addition to increase in Total proteins, Albumin, Globulins, Uric acid and Creatinine. (6).

Aminoglycoside antibiotics play an important role in treatment E.coli in poultry (7). Apramycin is a broad-spectrum aminoglycoside antibiotic produced by a strain *Streptomyces tenebrarius* strain (8). It is extracted from the fermentation medium as Apramycin sulphate. A microbiological assay is used to determine its

activity as equivalents of Apramycin base. Apramycin was used in the treatment of Colibacillosis in poultry. Apramycin leads to a decrease in RBCs count, Hb, PCV. An increase in WBCs count due to E.coli infection, decreased after Apramycin administration to infected chickens (9,10).

The present work was conducted to throw light on the efficacy of Apramycin in controlling E.coli infection in turkey poults and their effects on hemato-biochemical parameters and pathological changes in some internal organs associated with this infection and treatment, as well as detection of Apramycin residues in internal organs.

MATERIAL AND METHODS

Drugs

Apramycin: Apramycin sulfate soluble powder obtained from Unipharma Company for medical industry. Its traditional name is Apracin, each 150 gm of this powder contains 78 gm Apramycin sulphate. Apramycin is an aminoglycoside antibiotic produced by Streptomyces tenebrarius.

Experimental Turkey Poults

A total of 80 healthy one day old turkey poults were obtained from local commercial hatchery. Turkey poults were floor reared under hygienic condition, fed on balanced ration free from any medications and given water adlibitum. All turkey poults were subjected to bacteriological examination and proved to be free from infection before E.coli inoculation.

Microorganisms

E.coli strain (O.78) used in this study was isolated from a field case from broiler chickens farms infected with colisepticaemia in Sharkia Governorate. Identified and serotyped as O78 (11,12).

E. coli inoculum: Broth culture was standardized to give bacterial suspension containing 3X 10⁹ viable organism/ml of E.coli O78 using MacFerland tube. Each bird was given 0.3 ml via nasal route (13).

Experimental Design

At 20th day of age turkey poults, were divided into four equal groups (20 each). 1st group turkey poults were left non infected non treated (-ve control),2nd group healhy turkey poults treated with Apramycin (25 mg/kg B.wt in drinking water for 5 successive days), 3rd and 4th groups were expermintally infected with E.coli at the 20th day of age, 3rd group infected, non-treated turkey poults (+ve control),4th group infected turkey poults and treated with Apramycin at the same dose period. Treatment started 24 hrs post infection. All diseased turkey Poults were left under observations during the experimental period to record the clinical symptoms and mortality rate.

Sampling

Blood Samples

Five birds from each group were slaughtered at 1st,15th and 20th day post treatment for collection of 2 blood samples. The1st sample was taken in a tube containing EDTA and used for hematological studies, the 2nd sample was taken to obtain clear serum for estimation of AST, ALT and ALP, T. proteins, serum albumin, globulins mathematically, uric acid and creatinine according to (14).

Media Used: MacConkey's agar, nutrient agar, MacConkey's broth and Nutrient broth

Swabs for re-isolation of E.coli (O78)

Sterilized swabs from trachea, lung, heart, liver and air sacs were taken from sacrificed turkey poults for bacteriological examination for re-isolation of inoculated E.coli. Swabs were incubated in nutrient broth at 37°C for 24hrs, then subcultured into MacConkey's agar then incubated at 37°C for 24hrs (15). Re-isolated bacteria were identified biochemically and serology (16).

Tissue Samples for drug residues

Samples were taken from slaughtering turkeys post treatment from thigh muscles, liver, kidney and skin for determination of Apramycin residues (17).

Preparation of medium and test plates

Per 100 ml of Apramycin medium at 48°C, 1ml of micrococcus spore suspension (107 spore/ml) was added to obtain a density of 10⁴ spore /ml. The medium was shaked well and 13 ml³ of the prepared medium was poured into a number of petri dishes (1cm depth). The plates were left at room temperature tell complete solidification, then 6 pores were made on each plate using sterile borer with outside diameter 8 mm.

Procedure

Two plates spilled with different concentrations of Apramycin were used, and

then plates were incubated at 30°C for 24hrs. The width of inhibition zones were recorded then marking a curve between concentration of Apramycin and width of inhibition zone. The same procedure was done for turkey Poults tissues and the concentration of Apramycin was determined by comparing with those obtained by calibration curve.

Specimens for histopathological studies

Specimens from the liver and kidneys were taken from sacrificed birds then fixed in 10% neutral formalin solution and embedded in paraffin. Five microns thick sections were prepared and stained by H&E then examined microscopically (18).

5) Statistical analysis: The obtained data was analyzed (19).

RESULTS

The obtained results were tabulated in tables 1, 2, 3, 4 and 5 and Figures 1 to 6.

Table1. Effect of Apramycin on the mortality rate, pathological lesions and reisolation in healthy and E.coli infected turkey poults

Parameter	Total	Mor	tality			Lesion	scores	%	
Group	No	No	%		ecculitis		ırditis	Perihe	patitis
Non-inf., non treated	20	00	00	No	%	No	%	No	%
Non Inf. Treated	20	00	00	00	00	00	00	00	00
Inf. Non treated	20	5	25	00 15	00	00	00	00	00
Inf. Treated	20	1	5	4	75 20	17	85	13	65
Call a D				Т	20		10	3	15

Table 2. Re-isolation of E.coli from infected non treated and infected treated turkey poults

Parameters	No. of Examined	Re-isolation of E. coli for internal organs									
Groups	poults	Tracilea		lung		Heart		Liver		Air sacs	
Non-inf., non	pourts	No	%	No	%	No	%	No	%	No	%
treated Non	20	0	00	00	00	00	00	00	00	00	00
Inf.,Treated Inf., Non	20	0	00	00	00	00	00	00	00	00	00
treated Inf., Treated	20	20	100	16	80	12	60	09	45	15	75
mi., i reated	20	2	10	1	5	1	5	1	5	3	06

Table 3. Effect of Apramycin on Blood picture of healthy and infected turkey poults with E.coli

Group	Healthy	Healthy		Diseased tu	rkey (n=5)		
	turkey (n=5)	treated turkey	Non	Post treatment (days)			
Parameter	(11 0)	(n = 5)	treated	1	15	20	
RBCs (106/μl)	5.73±0.37	4.06±0.48*	3.82±0.32**	4.12±0.42*	4.32±0.29*	5.54±0.49	
Hb (gm/dl)	10.70±0.58	8.33±0.63*	7.94±0.77*	8.31±0.62*	8.93±0.50*	10.55±0.49	
PCV%	28.42±0.94	25.14±0.92*	24.55±0.99*	142002 144031 per 150 o			
WBCs(106/µl) *Significant at < 0.0	15.38±0.91		19.21±0.99*		-0.00=0.51		

Table 4. Effect of Apramycin on liver and kidney functions in healthy and infected turkey poults with E.coli (n = 5)

D		Livor	00000000]	Liver Fund				Kidney	Functions
Param	eters	ACT	enzymes		P	rotein pro	file (gm/dl)		Uric	Creatinine
		AST	ALT	ALP	Т.	Albumin	Globulin	A/G		(mg/dl)
	Non	77.10	00.0		proteins				(mg/dl)	(mg/di)
hy ts	reated	77.12	30.21	110.04	5.26	3.01	2.25	1.34	3.90	1.52
= =	reated	± 1.05	± 0.58	± 0.93	± 0.29	± 0.12	± 0.15	± 0.12	±0.22	±0.21
H G 1		00.00	33.04	115.13	3.84	2.41	1.43	1.69	4.87	2.42
	Non	± 0.90*	±0.72**	±1.64*	± 0.40*	± 0.19*		± 0.20	±0.16**	±0.30*
		81.15	33.11	115.06	4.16	2.30	1.86	1.24	4.97	2.59
pouts	1	± 1.13*	± 0.83*	±1.92*	± 0.28*	± 0.21*	all and a second	± 0.18	±0.19**	±0.36*
jd (SV	1	80.10 ± 0.70*	3253	113.41	4.58	2.68	1.90	1.41	4.73	2.15
Diseased p	15	79.04	± 0.52*	±1.30*	± 0.11*	$\pm 0.09*$	± 0.03*	± 0.15	±0.19*	±0.16*
sea: ed(13	± 0.45	32.10	111.39	4.79	2.81	1.98	1.42	4.32	1.97
Dis	20	78.88	± 0.39*	±1.55	± 0.17	± 0.10	± 0.11	±0.17	±0.16	±0.18
E	20	± 0.85	30.90	110.59	5.11	2.96	2.15	1.38	3.98	1.63
Tionic.		< 0.05	± 0.28	±1.28	± 0.21	± 0.14		±0.23	±0.10	±0.13

Table 5. Apramycin residues ($\mu g/g$) in fresh turkey poults tissues and organs

Organ	Шали	Healthy powler (days)									
- Sun		ny poults treated Apramycin	d with	Diseased Poults treated with							
	1	15	20	1	Apramycin 15	20					
Muscles	0.22±0.05	0.10±0.02	00	0.25±0.02		20					
Liver	1.07±0.11	0.10±0.03			0.12±0.03	00					
Zidna.		0.10±0.03	00	0.89 ± 0.10	0.06 ± 0.01	00					
Kidney	4.07±0.16	0.50±0.07	00	3.94±0.19	0.55±0.08	00					
Skin	0.31±0.05	0.10±0.02	00	0.27+0.07	_	00					
				0.37±0.07	0.08 ± 0.01	00					

^{* *}Significant at < 0.01

Pathological finding

Groups 1,2: Control and received Apramycin.

Neither Gross nor microscopical abnormalities were seen.

Group 3,4: Infected (non-treated) and infected (treated).

Macroscopically

The liver and kidneys of Group (3) showed severe congestion, firm and focal whitish necrotic foci, moreover, thickening of the hepatic and kidney capsules were noticed, in few cases, focal hemorrhagic spots were seen in the renal cortex. The liver and kidneys in most cases of group 4 were dark red to brownish in color and enlarged in size.

Microscopically

The liver of group 3: showed congestion of the hepatic blood vessels, the liver revealed thrombosis and thickening of the wall of the portal vein, besides leukocytic aggregations in hepatic parenchyma and hemorrhage. (Fig.1).Degenerative changes coagulative necrosis of hepatic parenchyma which represented by pyknosis karyorrhexis and karyolysis of the nuclei of hepatocytes. (Fig.2).

kidneys of group 3: showed hypercellularity of the glomeruli in addition to degenerative changes which represented by vacuolar and hydropic degenerations and perivascular edema (Fig.3). In some cases, focal coagulative necrosis of the epithelial renal tubules, hyperplasia thickening of the renal capsule was also observed (Fig.4). Congestion of the renal blood vessels and leukocytic aggregations in renal parenchyma was also observed. Most infected cases with E.coli, the lining renal tubules showed cloudy swelling and individual cell necrosis which represented by pyknosis and karyorrhexis of their nuclei (Fig.5).

The lesions of group4 were alleviated than those described in group3. Mild degenerative changes (vacuolar and hydropic types), slight congestion and few round cells infiltrations were visualized. (Fig. 6).

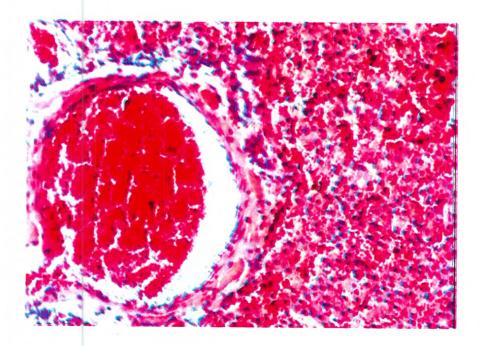


Fig.1. Electromicrograph of the liver of Group 3 showing congestion of the hepatic blood vessels and thrombosis and thickening of the wall of the portal vein in addition to leukocytic aggregations in hepatic parenchyma $H\&E \times 200$.

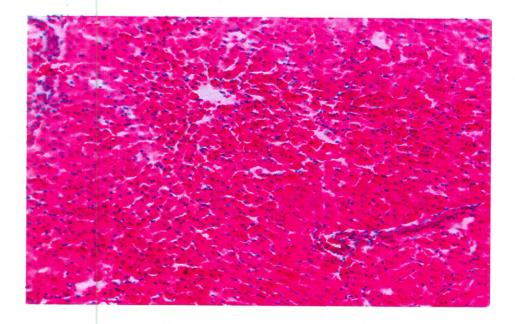


Fig.2. Electromicrograph of the liver of Group 3 showing focal coagulative necrosis of hepatic parenchyma $H\&E \times 100$.

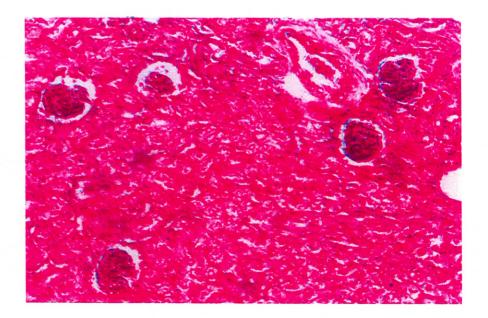


Fig. 3. Electromicrograph of the kidney of Group 3 showing vacuolar and hydropic degenerations of renal tubules in addition to hypercellularity of glomeruli and perivascular edema $H\&E \times 200$

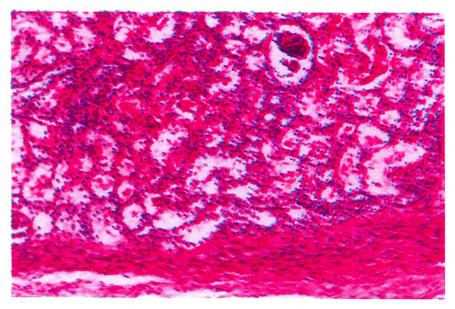


Fig. 4. Electromicrograph of the kidney from infected turkey poults treated with Apramycin showing Focal coagulative necrosis of the epithelial lining renal tubules with hyperplasia of the renal capsule $H\&E \times 200$

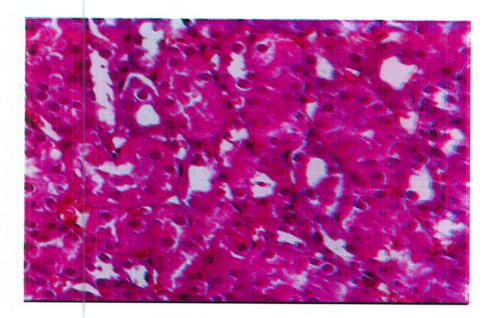


Fig. 5. Electromicrograph of the kidney: y of Group (3) showing cloudy swelling and individual cell necrosis of the epithelial lining of renal tubules which represented by karyorrehxis and karyolysis of their nuclei $H\&E \times 300$

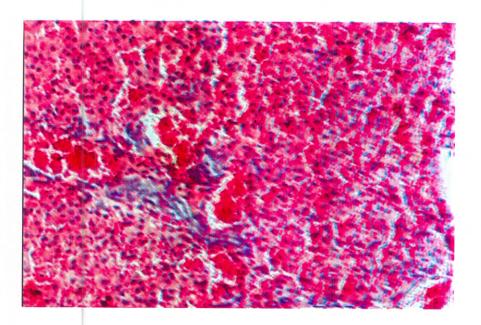


Fig. 6. Electromicrograph of the liver of Group 4 showing congestion of the hepatic blood vessels and sinusoids $H\&E \times 200$

DISCUSSION

Turkey poult experimentally infected with E coli displayed clinical signs as loss of appetite, weakness, depression, sneezing, cough, depression, watery diarrhea and 25% mortality The same signs were recorded in broiler chickens (12,20). Mortality rate in E.coli infected broiler ranged from 10-40% (21,22) who found that mortality rate in broilers infected with E coli was 40%.

Effects of Apramycin on blood picture in healthy and infected turkey poults with E.coli showed significant reduction in RBCs, Hb, PCV and leukocytosis. (Table,3). Same changes were recordedwhich showed that apramycin induced significant decrease in RBCs, Hb, PCV broilers. (25).Aminosidine another aminoglycoside was found to induce decrease in RBCs, Hb and PCV and leukocytosis (24). Our results proved previous observations which recorded that broilers infected with E.coli showed significant decrease in RBCs, Hb and PCV.(25). Rduction in erythrogram may be due to lipopolysaccharide toxin secrted by E.coli which inhibits bone marrow cells and nephrotoxicity decrease erythropoietin blood level which followed by decrease RBCs formation (26). Also, it has been shown that bacterial endotoxins cause intra-vascular destruction of erythrocytic cells leading to haemolysis with break down of Hb(27). Leukocytosis in E. coli infected birds may be to inflammatory response gastrointestinal tract due to bacterial infection (28).

Analysis of protein profile of the healthy turkey poults treated with Apramycin and those infected with E. coli showed significant decrease in total proteins, albumin and globulins (Table 4). Similar findings were previously cited by (29) which showed that apramycin evoked a significant decrease in proteins, albumin and globulins in rabbits. Decrease in serum protein picture in turkey poults treated with Apramycin might be due to damage in hepatic cells (30). Broiler chickens infected with E.coli showed significant decrease in serum total proteins, albumin and globulins (2,31, 32). Decrease in albumin in

birds infected with E. coli could be due to liver damage in which liver is the sole site of albumin synthesis (30).

In the present study ,healthy turkey poults treated with Apramycin and infected with E coli showed significant increase in serum AST, ALT and ALP, uric acid and creatinine (Table 4) .Our results were supported by previous study (23) which indicated that healthy rabbits treated with Apramycin showed significant increase in AST, ALT, ALP, uric acid and creatinine. Increase in uric acid and creatinine post administration of Apramycin may be due to accumulation and retention of Apramycin in proximal tubular cells inducing kidney damage (33). Our results are in accordance with the results obtained by previous authors (34,35), where they found that E.coli infection in broiler induce a significant increase in AST, ALT, uric acid and creatinine, in broilers infected with E.coli which may be due to liver damage induced by the organism and its toxins which lead to escape of these enzymes into serum (36).

Our findings revealed that, Apramycin residues in the examined samples of turkey poults liver, muscle, kidney and skin were very high at 1st day of clearance period, , very low at 15th days post treatment and completely disappeared from all examined organ at 20th day post treatment. The highest levels of Apramycin residues were recorded in the kidney followed by liver then skin and muscle(Table5).The obtained results nearly coincide with those previously reported (37) which showed that ,the average total residues of Apramycin at 1st day of withdrawal were 3.23, 0.42, 0.20 and 0.07 mg/ kgm in kidney, liver, skin and muscle, respectively. After 14 days, average of total residue had declined to 0.47, 0.08, 0.03 and 0.02 mg/kg in the same respective tissues. The highest Apramycin residue was present in the kidneys followed by liver (8).

Gross pathological lesions present in our study in group3 (infected group) were perihepatitis, pericarditis, air sacculitis misshaped and congestion these lesion scores percentages were decreased in group4 (infected

and treated). Poultry infected with E.coli. manifested the previously mentioned lesions Healthy turkey poults treated with (25).Apramycin showed pathological lesions as congestion of hepatic blood vessels with vacuolar degeneration of hepatocytes, kidney showed focal coagulative necrosis of renal tubular epithelium. The histopathological findings could be due to septicemic effect of E. coli upon the blood vessels, serous membranes and the parenchymatous organs (38). Same lesions were recorded previously (39) in Guinea pigs given Apramycin. Liver showed congestion of hepatic sinusoids, vacuolation in some hepatic lobules and hydropic degeneration, coagulative necrosis and lymphocytic infiltrations. Kidney showed cystic dilatation, hydropic degeneration of some renal tubules and desquamation of some epithelial lining of the renal tubules in turkey poults infected with E coli. Our results were nearly similar to that recorded by several authors (40 - 42).

CONCLUSION

From this study we concluded that, Apramycin is effective for treatment and improvement of the liver and kidney functions after infection by Escherichia coli (E.coli 078) in turkey poults. As E. coli infection has adverse effect on liver and kidney functions in addition to degenerative changes and necrosis of hepatocytes and epithelial lining renal tubules. Apramycin residues was detected in kidney, liver and skin respectively and removed completely from body organs after 20 days of Apramycin treatment. It is advised to use Apramycin for E.coli treatment of turkey poults.

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الملخص العربي

تاثير الأبراميسين على بعض التغيرات الباثولوجية و الهيماتولوجيه والبيوكيميانيه في الرومي المعدية بالميكروب القولوني العصوى

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استهدفت هذه الدراسة معرفة كفاءة الابراميسين في علاج االعدوى الاصطناعية بالميكروب العصوى ((708) في بدارى تسمين الرومي واستبيان بعض التغيرات في صورة الدم وبعض الوظائف البيوكيميائية اضافة التغيرات الباثولوجية . وقد استخدم في هذه الدراسه ٨٠ كتكوت رومي عمر يوم . ثم تقسيم كتاكيت الرومي الي ٤ مجموعات متساويه بالمجموعة الأولى كتاكيت رومي بصحة جيدة خالية من الميكروب القولوني العصوى (مجموعه ضابطه) والمجموعة الثانية كتاكيت رومي بصحة جيدة خالية من الميكروب القولوني العصوى وتم اعطائها الابراميسين بجرعه ٢٥ مجم/كجم من وزن الجسم في مياه الشرب لمده ٥ ايام, والمجموعة الثالثة كتاكيت رومي مصابة بالميكروب القولوني العصوى ولم يتم علاجها بينما المجموعة الرابعة كتاكيت مصابه بالميكروب القولوني العصوى بالميكروب القولوني العصوى ولم يتم علاجها بينما المجموعة الرابعة بحيث يبدأالعلاج بعد ٤ ٢ ساعه من العدوى وتم علاجها باستخدام الابراميسين بنفس الجرعه والمده السابقة بحيث يبدأالعلاج بعد ٤ ٢ ساعه من العدوى كتاكيت من كل مجموعه عند اليوم الاول الخامس عشر والعشرين بعد نهايه العلاج واثناء الذبح تم أخذ عينتين دم كتاكيت من كل مجموعه عند اليوم الاول الخامس عشر والعشرين بعد نهايه العلاج واثناء الذبح تم أخذ عينتين دم صورة الدم وبعض الوظائف البيوكيميائية وبعد الذبح يتم اخذ عينات من عضلات الفخذ الكبد بالكلي الجلد لتعيين بقايا الابراميسين في تلك الاعضاء ويتم اخذ عينات من الكبد والكلي, لدراسة تأثير الميكروب القولوني العصوي في كتاكيت الرومي الي والابراميسين على تلك الاعضاء ويتم اخذ عينات من الكبد والكلي, عدوث فوق بنسبة (٢٥٠%).كما ظهور أعراض تتمثل في فقدان الشهية ووجود نزلات معوية وهزال كمائدت إلى حدوث فوق بنسبة (٢٥٠%).كما

حدث نقص معنوى فى عدد كريات الدم الحمراء-وتركيز الهيموجلوبين- وحجم خلايا الدم المرصوصة-البروتين الكلى- الزلال-الجلوبيولين-مع حدوث زيادة معنوية فى عدد كريات الدم البيضاءاضافة لانزيم الاسبرتات امينوتر انسفيريز -الالانين امينوتر انسفيريز والفوسفاتيز القاعدى حمض البوليك والكرياتينين .

وقد دلت نتائج الدراسة على أن الابر اميسين له بقايا في انسجة عضلات الفخذ, الكبد,الكلى والجلد وكانت بنسب عالية بعد اليوم الأول وبنسبة منخفضة عند اليوم الخامس عشر بعد نهاية العلاج بينما اختفت بقايا الابر اميسين عند اليوم ٢٠ بعد نهاية العلاج. وكان اعلى مستوى للبقايا في الكلى يليه الكبد ثم الجلد واخيرا عضلات الفخذ. وبالفجص الظاهرى للكبد والكليه وجد تضخم واحتقان سواء في كتاكيت الرومي السليمه والمعالجة بالابر اميسين او المصابه بالميكروب القولوني العصوى. اما الفحص المجهري للاعضاء الداخلية لكتاكيت الرومي السليم والمعالج بالابر اميسين او المصابه بالميكروب القولوني العصوى وجدت افات باثولوجيه عباره عن احتقان بالاوعية الدموية مع وجود نخر تخثري بالنبيبات الكلوية. وقد اظهرت المجموعة المصابة بالميكروب القولوني العصوية والمعالجه بالابر اميسين تغيرات باثولوجية طفيفة وانخفاض معدل النفوق الى ٥%و عودة وظائف الكبد والكلى الى المستوى الطبيعي.

نستخلص من تلك الدراسة أن الاصابة بالميكروب القولوني العصوى في بداري تسمين الرومي احدثت بعض التأثيرات العكسية على صورة الدم ووظائف الكبد والكلي وادى استخدام الابرامايسين الى تحسن الحالة الصحية لكتاكيت الرومي بعد العلاج ب٥١ يوم و ينصح باستخدام الابرامايسين بالجرعة العلاجية لعلاج الاصابة بالميكروب القولوني العصوى كما ينصح بعدم تناول لحوم الدواجن المعالجة الا بعد مرور ٢٠ يوم من نهاية العلاج.